Standard Methods for Assessing Biological Integrity of Surface Waters in Kentucky

Commonwealth of Kentucky
Environmental and Public Protection Cabinet
Department for Environmental Protection
Division of Water
February 2008
Revision 3

Kentucky
UNBRIDLED SPIRIT

Methods for Assessing Biological Integrity of Surface Waters in Kentucky

Kentucky Department for Environmental Protection

Division of Water

Frankfort, Kentucky

February 2008, Revision 3

This report has been approved for release:

Sandy Gruzesky, Assistant Director

3/13/08

Date

This document was printed on recycled paper with state funds.

Revision History

The following table reflects revisions and/or changes to the document. The first entry is the most current version. The current revision and all prior versions are kept on file with the Division of Water Quality Assurance Officer.

Document Title and Tracking	Effective Date
Standard Methods for Assessing Biological Integrity of Surface Waters in Kentucky <u>General Content</u> Document was re-formatted for maintaining headers, section titles, etc in a consistent style. All references to detailed water chemistry sampling were removed, and a reference inserted directing the reader to the 'Standard Operating Procedures for Sampling and Monitoring Surface Waters for Kentucky', in draft	March 13, 2008
Methods for Assessing Biological Integrity of Surface Waters in Kentucky	July, 2002

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF APPENDICES

SECTION 1. INTRODUCTION

This manual has been developed by the Kentucky Division of Water (KDOW) as guidance for the uniform collection, analysis, interpretation and quality assurance/quality control of biological samples from surface waters. It represents the third revision of the original manual that was developed in 1993. The procedures defined herein are followed for all biological monitoring and assessment studies conducted by KDOW and are recommended for use in assessments conducted in Kentucky by other entities. Compliance with these procedures is critical to basing sound assessments on water bodies throughout the Commonwealth. Specific uses for these data are:

1. To determine support of waterbody uses as defined by Kentucky Water Quality Standards 401 KAR 5:026 and 5:031.
2. To determine effects of known point or nonpoint sources of pollution on the aquatic biota of the waterbody.
3. To determine background conditions within particular drainages or ecological regions.

Biological integrity is defined as the condition of the aquatic community occurring in natural habitats of unimpaired surface waters as measured by community structure and function. Biological assessments, or bioassessments, are performed to evaluate the biological condition of surface water using biological surveys and other direct measurements of resident biota. The United States Environmental Protection Agency (USEPA) document, "Biological Criteria: National Program Guidance for Surface Waters" (1990), has defined these and several other terms relating to biological assessments, and their definitions have been adopted by KDOW. Definitions for chapter-specific terms are provided in each chapter, where applicable.

Kentucky integrates the collection and analysis of algal, macroinvertebrate, fish, habitat and water chemistry data to determine assessments on the health of all surface waters. Because environmental stressors (e.g., excessive nutrients, organic wastes, industrial toxins and habitat alterations) reveal their impacts on the resident biota, biological communities leave detectable response signatures related to the various types of pollution. Therefore, environmental impacts and pollution abatement success can be directly measured with the standard ecological methods described in this manual.

There are three primary sections within the Division of Water, Water Quality Branch that will utilize this document. The Ecological Support Section is responsible for performing biological assessments using fish, fish tissue, macroinvertebrates and diatoms at targeted stream segments; the Standards and Specification Section performs biological monitoring using macroinvertebrates at probabilistic stream segments; macroinvertebrates and algae are collected by the Total Maximum Daily Load (TMDL) Section at impaired stream segments to develop a screening baseline for the aquatic community.

Macroinvertebrates and fish are collected by the Nonpoint Section of the Watershed Management Branch to determine effectiveness of best management practice implementation.

All contractors working within the Commonwealth should utilize the methods outlined in this manual so that data comparability studies could be done to KDOW data. Any data submitted to

KDOW for review that has not followed the methods outlined in this manual will be scrutinized, flagged with constraints, or not used.

If you have any questions or comments concerning this manual, please contact the Ecological Support Section at the following address:

Kentucky Division of Water
Water Quality Branch
14 Reilly Rd.
Frankfort, KY 40601
(502) 564-3410

SECTION 2. PROJECT PLANNING

2.1 Objectives

Biological surveys will have one or more stated objectives. Examples of objectives for biological surveys include determining support of legitimate waterbody uses as defined under Kentucky Surface Water Standards 401 KAR 5:031, determining effects of point or nonpoint sources of pollution on the aquatic biota of the waterbody and determining background conditions within the drainage

2.2 Study Plans

The following study plan outline is for development of study plans for intensive survey purposes. The study plan should clearly state the objectives of the intensive ecological survey, describe the study area, list known impacts to and uses of the waterbody and list the types of data to be collected and measured. All available historical data are reviewed during design of the survey. The following descriptive information is listed at the beginning of the study plan:

1. Stream Name
2. Major River Basin
3. Stream Order (at mouth)
4. County or Counties Included in the Survey
5. USGS 7.5-min. Quadrangle Names

2.2.1 Survey Dates

Target starting dates for the survey are listed on the study plan. For routine watershed monitoring (e.g., waterbody use-support classifications), appropriate sample index periods are stated in the algal, macroinvertebrate and fish chapters. These dates may not correspond to special situations (e.g., permitting activities and emergency spill or impact studies). Weather, stream flows or workload may affect actual starting dates. Surveys that are to be repeated seasonally are identified as such in the study plan.

2.2.2 Study Area Description

The following elements should be detailed for each study area:

1. Topography
2. Geology
3. Physiographic Region and Ecoregion
4. Stream Length
5. Drainage Basin Area
6. Major Tributaries
7. Flow Characteristics Based on Existing or Extrapolated Data
8. Land Use (Agriculture, Mining, Silviculture, Urban, etc.)
9. Location of Known Point or Nonpoint Sources of Pollution

2.2.3 Parameter Coverage

Parameter coverage varies depending on the objective(s) of the survey. Full coverage includes collection of habitat data and biological, physicochemical and water chemistry samples. Biological samples include algal, macroinvertebrate and fish collections. Sampling of more than one taxonomic group encompasses more than one trophic level (primary producers and secondary and tertiary consumers) and provides a more realistic evaluation of the aquatic ecosystem. Full coverage may also include fish/shellfish contaminant analysis, bacteriological analysis and toxicity testing.

2.3 Station Selection

Stream sites are initially selected from 7.5 min. USGS topographical maps and GIS software. A reconnaissance visit, if necessary, is made to finalize the site selections. A sampling site should include habitats that are typical for the stream reach under study. Locally modified sites with channelized areas, impounded sections, etc. are avoided unless they are the focus of the study. In addition, sampling at or near the mouths of tributaries should be avoided if possible.

The Probabilistic (random) Sampling Program selects stations using a different method. The random survey approach is used to assess aquatic life use support for streams in each watershed management unit. For each basin management unit, U.S. EPA in Corvallis, Oregon is contacted to provide the population of streams to be assessed. This population consists of wadeable streams, orders 1st-4th. Fifty to 75 streams are typically assessed in each basin management unit (BMU). All streams in the defined population are then randomly selected, with the latitude and longitude of the exact location where the assessment is to be conducted. The stream population is weighed so the less numerous, higher order (3rd and 4th) streams will have an equivalent chance of being selected, based on percentages of each stream order in a given basin. Often these sample sites have no public access and landowner permission to gain access must be obtained.

2.3.1 Number and Location of Sampling Sites

The number of sites selected for a survey often depends on the size of the drainage basin and the severity and number of impacts to the stream. However, available personnel and workload, funding or timeline may also limit the magnitude and scope of the study. All point sources, nonpoint sources and inputs from major tributaries are bracketed with an upstream site, a site at the mixing zone of each pollution source and intermediate sites. Control and reference sites (see below) and downstream recovery sites are also established. Downstream sites are selected between the point source and the next major tributary to avoid dilution effects. The number of downstream sites, and distance between these sites, depends on the objectives of the survey, the number of pollution sources, the number of major tributaries and the types of pollutants entering the stream. When the rate of movement of a contaminant or effluent plume is required, several samples are collected along a longitudinal gradient at regular intervals based on flow and travel time of the stream.

2.3.2 Control/Reference Sites

Whenever possible, at least two control sites are sampled for comparative purposes. Control sites are located either upstream of the source(s) of pollution or, when this is not possible, on a nearby, unaffected tributary. Streams within the same drainage basin and ecoregion and with similar physical characteristics and habitats should be used. Control sites from different drainage basins should be used only if no suitable site can be located within the basin of the stream being surveyed. Fixed ecoregional reference sites (streams that are considered the most natural and undisturbed for the particular ecoregion) should be used where possible.

2.3.3 Tributary Sites

When applicable, sites may be selected on each major tributary. Upstream and downstream sites to determine tributary and dilution effects should bracket the tributary.

2.3.4 Recovery Sites

At least one site should be located far enough downstream of the polluted area to detect recovery of the aquatic biota (i.e., when the biota begins to show a similarity to the control/reference site). Location of this site depends on the magnitude and downstream extent of the pollution.

2.3.5 Site Numbering

Existing sampling stations were numbered consecutively from the mouth of the stream to the headwaters in a hierarchical method The station nearest the mouth was assigned the lowest number not previously used. Sites were then numbered consecutively from mouth to headwaters. Next, tributary sites were assigned numbers, starting with the tributary closest to the mouth of the stream being surveyed. All stations on that tributary were numbered consecutively upstream from the confluence. Then, the next tributary was numbered until all sites were assigned numbers. If additional sites were added after the initial study, they were assigned numbers that were not previously used, following the above outlined convention as closely as possible.

When new sites are selected in a watershed that already has stations present, the new site will be assigned the next sequential number for that watershed. The stations table of KDOW's ecological database, Ecological Data Application System (EDAS), should be consulted to determine what the next available site number would be for that watershed. Refer to Appendix A for EDAS site number assignment standard procedures.

2.4 Quality Assurance/Quality Control

Procedures for field and laboratory QA/QC are described in detail in the study plan if they differ from the routine procedures outlined in this manual. Quality Assurance/Quality Control procedures will be discussed in Chapter 11 of this document.

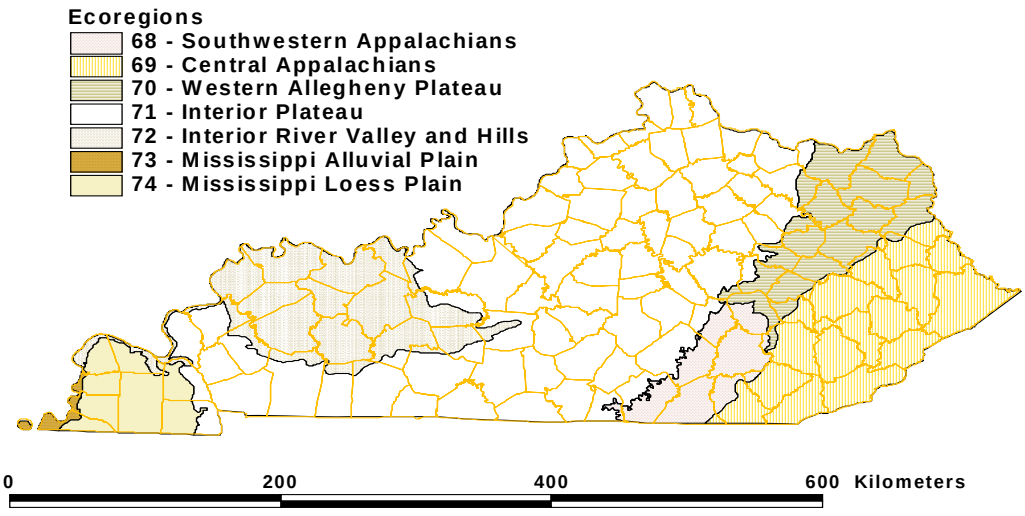
SECTION 3 ECOREGIONS AND REFERENCE CONDITION

3.1 Introduction

The concept of ecological regions, or ecoregions, within physiographic regions is a recent postulation that has received considerable attention. The conceptual framework of aquatic ecoregions is based on definable similarities among streams in a region that are related to the terrestrial characteristics of that region. In effect, streams acquire their characteristics from their watersheds (Likens and Bormann 1974, Hynes 1975). Streams draining watersheds of the same size, with comparable land uses and habitats, and in the same region are more likely to contain similar aquatic communities than those draining watersheds of a different size or in a different region (Hughes et al. 1986). This regional framework facilitates water quality resource management and the development of region-specific biological and chemical criteria.

Omernik (1987) mapped the Level III terrestrial ecoregions of the conterminous United States based on physiography, soil types, potential natural vegetation, geology and land-use types. A revised version of the Level III ecoregions in Kentucky is shown in Figure 3-1. Analysis of the data revealed regional homogeneities, which allowed for the delineation of boundaries between ecoregions and the location of the most typical areas within each ecoregion. Each of the delineated Level III ecoregions is descriptively compared below to adjacent ecoregions (modified after Woods et al. 2002). Level IV sub-ecoregions have been delineated and a map and descriptions are available in Woods et al. (2002).

Figure 3-1 Ecoregions of Kentucky



3.2 Ecoregion Descriptions

Ecoregion 68: Southwestern Appalachians

This region has a mixture of open, low mountains containing a mosaic of forest and woodland with some cropland and pasture. A deeply incised escarpment occurs in the west near the boundary with the Interior Plateau's Eastern Highland Rim (Ecoregion 71). The landscape is underlain by Pennsylvanian and Mississippian rock strata. Ultisols and Inceptisols (soil types) are common and contrast with the Alfisols that dominate the lowlands of the Interior Plateau to the west. Mixed mesophytic forest is generally restricted to the deeper ravines and escarpment slopes, and mixed oaks with shortleaf pine dominate the upland forests. Resource extraction (oil, gas and coal), agriculture and silviculture are the major land uses. Moderate- to high-gradient streams are common and generally have cobble- or boulder-dominated substrates; a few low-gradient streams occur and have gravelly or sandy bottoms. Nutrient and alkalinity levels are typically low compared to adjacent Ecoregion 71. Fish and mussel distributions in Cumberland River tributaries are distinct from the tributaries of the Kentucky River. They are also distinct from areas above Cumberland Falls in Ecoregion 69.

Ecoregion 69: Central Appalachians

Ecoregion 69 is a high, dissected and rugged plateau made up of sandstone, shale, conglomerate and coal of Pennsylvanian age. The plateau is locally punctuated by a few anticlinal ridges. Its rugged terrain, cool climate and nutrient-poor soils sharply limit agricultural potential and result in a mostly forested land cover. The high hills and low mountains are mostly covered by mixed mesophytic forest. Bituminous coal mines are common and have caused the siltation and acidification of streams. Soils have developed from residuum and are mostly Ultisols and Inceptisols; they contrast with the Alfisols that dominate the fertile lowlands of the Interior Plateau Ecoregion of central Kentucky. This ecoregion is bounded to the west by the Western Allegheny Plateau Ecoregion and to the southwest by the Southwestern Appalachian Ecoregion. The region is drained by relatively high-gradient streams, forming the headwaters of the Kentucky, Cumberland,
Licking and Big Sandy river basins. These streams are typically cool, with substrates composed mainly of cobbles and boulders (Omernick, 1987).

Ecoregion 70: Western Allegheny Plateau

The hilly and wooded terrain of Ecoregion 70 was not muted by glaciations. It is more rugged than the agricultural till plains of Ecoregions 55 and 61 in Ohio, but is less rugged than the Central Appalachians (Ecoregion 69). Extensive mixed mesophytic forests originally grew in Ecoregion 70 and contrast with the oak-hickory forest that is found farther west in the lower Interior Plateau (Ecoregion 71). Today, most of the rounded hills of Ecoregion 70 remain in forest; dairy, livestock and general farms, as well as residential developments, are concentrated in the valleys. Resource extraction (oil, gas and coal), silviculture and agriculture remain the major land uses within this ecoregion. Horizontally bedded Pennsylvanian sedimentary rock containing sandstone, siltstone, shale and coal underlies the region. Some areas have eroded down to limestone and may have localized karst development. Moderate to high-gradient

headwater streams of the Kentucky, Licking, Little Sandy and Ohio River basins originate within the Western Allegheny Ecoregion. Upland streams are generally cool with cobble-boulder substrates similar to Ecoregion 69. Nutrient and alkalinity levels are generally higher than the Central Appalachians (Ecoregion 69) but lower than the Interior Plateau (Ecoregion 71).

Ecoregion 71: Interior Plateau

The Interior Plateau Ecoregion is the largest ecoregion in area, encompassing most of the Highland Rim and Blue Grass Sections of the Interior Low Plateau Physiographic Province. Ecoregion 71 is composed of irregular plains, open hills, knobs and large areas of karst topography. Its landforms and soils are the result of the differential weathering of sedimentary strata, stream erosion and deposition, and the solvent action of subterranean water on carbonates. Ecoregion 71 is underlain by Mississippian-through Ordovician-age limestone, chert, sandstone, siltstone and shale. Lithologic and related physiographic boundaries tend to determine the natural subdivisions of the Interior Plateau (71). In areas underlain by cavernous limestone, drainage is primarily underground and only a few entrenched master streams and rivers occur. Rock types are distinct from the unconsolidated coastal plain sands that underlie the Mississippi Valley Loess Plains (74) in western Kentucky and the Pennsylvanian coal strata found in Ecoregions 69, 70 and 72. Maximum elevations and local relief are lower than in the Appalachian and Cumberland Plateau regions to the east (68, 69 and 70) but are greater than in the Interior River Valley and Hills (72). The soils of Ecoregion 71 are highly varied and developed from the underlying sandstone, siltstone, shale and limestone and are not originated from glacial till like those of the Eastern Corn Belt Plains (55) to the north. Lowlands are generally dominated by Alfisols, not Ultisols or Inceptisols that are more characteristic of` Ecoregions 68, 69 and 70.

The natural vegetation is primarily oak-hickory forest, but large areas of bluestem prairie originally occurred in the western karst areas. The potential natural vegetation is distinct from the mixed mesophytic forests of higher, cooler and wetter Ecoregions 68, 69 and 70. Rolling pastures, crop fields and woodlots are common, and agriculture, urban expansion and construction are major land uses. Most of the streams in the Interior Plateau Ecoregion are heavily influenced by human activities. Parts of the Licking, Kentucky, Cumberland, Salt, Green, Tradewater and Ohio River basins are located within the ecoregion. Stream morphology is highly variable across this large ecoregion. Both moderate and high-gradient streams with boulder-cobble substrates and low-gradient streams with sand-gravel bottoms occur. Cool groundwater-fed streams punctuate those areas underlain by extensive and well-developed karst geology. Stream nutrient, alkalinity and hardness levels are higher in most parts of Ecoregion 71 than in Ecoregions 68, 69 and 70.

Ecoregion 72. Interior River Valleys and Hills

This broad, undulating lowland was formed in nonresistant, non-calcareous sedimentary rock of Pennsylvanian age. Often referred to as the "Western Coalfields," it is rich in coal reserves. Large upland areas are veneered by windblown material. Many wide, flat-bottomed, terraced valleys occur and are filled with alluvium, loess and lacustrine deposits. Bottomland hardwood forests and swamp forests once grew on poorly drained, nearly level sites, whereas the upland areas had oak-hickory forests. Patterns of land use are more varied than in the neighboring

ecoregions, and large areas have been strip mined for coal. Drained alluvial soils are farmed for feed grains and soybeans. Undrained valleys are used for forage crops, pasture or woodlots; uplands are used for mixed farming and livestock. Extensive strip mining, as well as crop and livestock production, have impacted stream water quality (acidification, sedimentation, nutrients) and stream habitat (channelization, sedimentation); sheet erosion can be severe on cultivated slopes. Streams feed the Tradewater, Green and Ohio river basins and have relatively low (<5 ft/mile) gradients. Unchannelized upland streams often have gravel bottoms, while sand, silt and mud dominate most lowland channels. Streams typically have lower nutrient, alkalinity and hardness levels than those in Ecoregion 71 do. Wetlands were once common in this region, but many have since been drained for agricultural uses.

Ecoregion 73: Mississippi Alluvial Plain

The Mississippi Alluvial Plain is the smallest ecoregion found in Kentucky, occupying the areas immediately adjacent to the Mississippi River. Rock stratum is almost exclusively composed of alluvial deposits. Mostly flat, broad floodplains with river terraces and levees provide the main elements of relief. Abandoned channels, oxbow lakes, bayous, back swamps and point bars are found in the ecoregion. Fine-grained, poorly drained soils are common, but better drained loamy and sandy soils also occur. Winters are milder and summers are hotter than other Kentucky ecoregions. Bottomland deciduous forest vegetation covered the region before clearance for cultivation. All of the streams within the area drain into the Mississippi River and have been extensively channelized for agriculture. Streams are very low gradient, have sandy to muddy substrates and often contain extensive wetland vegetation.

Ecoregion 74: Mississippi Valley Loess Plains

Ecoregion 74 in far western Kentucky consists of irregular plains, gently rolling hills and, near the Mississippi River, bluffs. It is mostly covered by thick loess and alluvium and underlain by Cretaceous and Tertiary coastal plain sediments. Rock types are distinctly different from the limestone, chert, sandstone, siltstone and shale of the Interior Plateau (71). Ecoregion 74 has less relief than ecoregions farther to the east in Kentucky, and elevations are much lower than in the Appalachian ecoregions. The potential natural vegetation is oak-hickory forest (Kuchler 1966), but agriculture is the dominant land use in Ecoregion 74 in Kentucky. Typically, streams have moderate to low gradients and gravelly to sandy bottoms. Wetlands are common throughout the area. In natural streams, nutrient and alkalinity levels are generally low compared to those of Ecoregion 71.

3.3 Bioregions and Ichthyoregions

Kentucky has a diverse topography, and analysis of the similarity among biological assemblages across geographic scales can help clarify the inherent biological differences in the state. An a posteriori regional classification scheme based on river basins, physiographic regions and ecoregions was developed (Pond et al. 2003, Pond and McMurray 2002, Pond et al. 2000). Bioregions and Ichthyoregions were established to incorporate ecoregion and river basin differences within the state and typically correspond to Level III Ecoregion boundaries (Woods et al. 2002).

3.3.1 Bioregions

For diatoms and macroinvertebrates, four bioregions have been established: Bluegrass (BG), Mountains (MT), Mississippi Valley-Interior River (MVIR) and Pennyroyal (PR). The four bioregions are defined below.

Mountain (MT)

Includes all river systems (Big Sandy, Cumberland, Kentucky, Licking, Little Sandy and minor tributaries of the Ohio River) within the boundaries of the Central and Southwestern Appalachian Ecoregions (69 and 68) and the Western Allegheny Plateau Ecoregion (70).

Bluegrass (BG)

Includes all river systems (Kentucky, Licking, Salt and minor tributaries of the Ohio River) that lie within sub-ecoregions (71d, k, and l) of the Interior Plateau (71).

Pennyroyal (PR)

Includes all river systems (Cumberland, Green, Kentucky, Salt, Tradewater, Tennessee and the minor tributaries of the Ohio River) that lie within sub-ecoregions (71a, b, c, e, f, g, and h) of the Interior Plateau (71).

Mississippi Valley-Interior River (MVIR)

Includes all river systems (lower Cumberland, Green, Tradewater, Tennessee, minor tributaries of the Mississippi River and minor tributaries of the Ohio River) within the boundaries of the Interior River Valleys and Hills (72), Mississippi Alluvial Plain (73), and Mississippi Valley Loess Plain (74).

3.3.2 Ichthyoregions

Due to the strong affinity between fish and river basins the Kentucky Index Biotic Integrity uses six ichthyoregions: Cumberland River above Cumberland Falls (CA), Bluegrass (BG), Upper Green River (GR), Mountains (MT), Mississippi Valley-Interior River (MVIR) and Pennyroyal (PR). The six ichthyoregions are defined below.

Mountain (MT)

Includes all river systems (Big Sandy, Cumberland, Kentucky, Licking, Little Sandy and minor tributaries of the Ohio River) within the boundaries of the Central and Southwestern Appalachian Ecoregions (69 and 68) and the Western Allegheny Plateau (70) Ecoregion, except for the Cumberland River above Cumberland Falls.

Cumberland River above Cumberland Falls (CA)

Encompasses the Cumberland River system above the Cumberland Falls in the Central and Southwestern Appalachian Ecoregions (69 and 68).

Bluegrass (BG)

Includes all river systems (Kentucky, Licking, Salt and minor tributaries of the Ohio River) that lie within sub-ecoregions (71d, k, and l) of the Interior Plateau (71).

Pennyroyal (PR)

Includes all river systems (Cumberland, Green, Kentucky, Salt, Tradewater, Tennessee and the minor tributaries of the Ohio River) that lie within sub-ecoregions (71a, b, c, e, f, g, and h) of the Interior Plateau (71).

Upper Green River (GR)

Includes the Green River system within sub-ecoregion 71g of the Interior Plateau (71).

Mississippi Valley-Interior River (MVIR)

Includes all river systems (lower Cumberland, Green, Tradewater, Tennessee, minor tributaries of the Mississippi River and minor tributaries of the Ohio River) within the boundaries of the Interior River Valleys and Hills (72), Mississippi Alluvial Plain (73), and Mississippi Valley Loess Plain (74).

3.4 Reference Conditions

3.4.1 Defining the Ecological Reference Condition

To address levels of impact on any given stream, a firm understanding of the inherent biological variability and potential of natural streams in a region is necessary. This is accomplished using a regional reference approach (Hughes et al. 1986), which is based on the range of conditions found in a population of sites or streams with similar physical characteristics and minimal human impact. Reference sites are tightly associated with ecoregions, and an effort is made to obtain good coverage of sites within all regions.

Collectively, the reference condition refers to the range of quantifiable ecological elements (chemistry, habitat and biology) that are found in natural environments. In many regions of Kentucky, finding reference streams can be a difficult task, as few regions are without areas of human disturbance. Therefore, reference reaches are more appropriately deemed "least-disturbed." The application of the reference condition involves its comparison to a stream exposed to environmental stress using defined sampling methodology and assessment criteria. Impairment of the test site would be detected if indicator measurements (e.g., species richness,

habitat rating, nutrients) fell outside the range of threshold criteria established by the reference condition.

3.4.2 Selection of Candidate Reference Reach Water bodies

Ecoregional reference reach site selection and evaluation analysis is an ongoing process supported by intensive map and ground reconnaissance. The U.S. Environmental Protection Agency's (USEPA) Biocriteria Program suggests that the selection process for candidate reference reach water bodies should be well documented so that the data defining the reference condition will be scientifically defensible.

In order to comply with USEPA guidelines, the Reference Reach Program follows a step-by-step process for the selection of candidate reference reach water bodies. This process involves the analysis of topographic maps and aerial photography, cross-referencing with other available data sources and field reconnaissance of the candidate watersheds. Because of the diverse land use, topography and physiography across the state, fixed statewide reference criteria are not used. Therefore, reference reach candidates are qualified in a regional context in an effort to find "least-disturbed" conditions among the various ecoregions.

U.S. Geological Survey (USGS) 7.5-min. topographic maps and aerial Digital Orthogonal Quadrangles (DOQs) are analyzed as part of the initial step of the selection process. Each stream is then evaluated based upon the presence or absence of the following:

1) High proportion of forestland and riparian zone quality;
2) Towns or communities along the stream bank;
3) Resource extraction in the watershed (e.g., coal mines, oil wells, gas wells);
4) Hydrologic modification in the watershed (e.g., impoundment, channelization);
5) Major sewage treatment/industrial discharges.

If an adequate riparian zone exists along most of the stream length and minimal land-use activities occur within the watershed, then the stream is recorded as an initial candidate. An initial candidate list is then compiled for each county and Level III ecoregion. Once the initial candidate list is created, it is cross-referenced with other available data sources including the following:

1) Kentucky Rivers Assessment (KDOW and NPS 1992)
2) Kentucky Nonpoint Source Assessment Report (KDOW 1999)
3) Kentucky State Nature Preserves Commission's Fish Collection Catalogue (KSNPC 1983)
4) Kentucky Division of Water's Fish Collection Catalogue (Mills 1988)
5) Aquatic Biota and Water Quality Survey of the Appalachian Province, Vol. 1-3 (Harker et al. 1979)
6) Aquatic Biota and Water Quality Survey of the Upper Cumberland River Basin, Vol. 1-2 (Harker et al. 1980)
7) Aquatic Biota and Water Quality Survey of the Western Kentucky Coal Field, Vol. 1-2 (Harker et al. 1981)

8) Recommendations for Kentucky's Outstanding Resource Water Classifications with Water Quality Criteria for Protection (KNPC 1982)
9) Kentucky Division of Water Intensive Survey Reports (KDOW)
10) Personal communication with Kentucky Division of Water, Kentucky Nature Preserves Commission, Kentucky Department of Fish and Wildlife Resources, and Daniel Boone National Forest personnel, where applicable.

Cross-referencing allows for amendment of the initial candidate list resulting in a smaller, secondary list. Upon completion of cross-referencing, field reconnaissance of the watersheds is conducted. Each site is evaluated on categories and criteria shown in Table 3-1.

Table 3-1. Summary of physical criteria used in the Reference Reach selection process.[1]

Category	Criterion
1) riparian zone condition	well-developed providing some canopy over the stream; presence of adequate aquatic habitats in the form of root mats, coarse woody debris and other allochthonous material
2) bank stability	at least moderately stable with only a few erodible areas within the sampling reach
3) degree of sedimentation	the substrate is 25 percent or less embedded by fine sediment
4) suspended material	the water is relatively free from suspended solids during normal weather conditions
5) evidence of nutrient enrichment	the substrate is relatively free from extensive algal mats that could choke riffle habitats
6) conductivity	conductivity is not highly elevated above what naturally occurs (region-specific)
7) aquatic habitat availability	there is a 50 percent or greater mix of rubble, gravel, boulders, submerged logs, root mats, aquatic vegetation or other stable habitats available for aquatic organisms
8) the presence or absence of trash in the stream	solid waste within the stream and on the stream bank is at a minimum
9) evidence of new land-use activities in the watershed	the land-use conditions remain constant from what is depicted on the most recent USGS topographic or DOQ maps
10) accessibility of the site for collection	Accessible

[1]The reference criteria listed above may vary somewhat among ecoregions.

SECTION 4 SITE CHARACTERIZATION AND MONITORING

4.1 Introduction

Assessing the quality of an area to be sampled is an integral part of any aquatic survey. In intensive impact surveys, every attempt is made to sample water bodies, such as streams and wetlands, with comparable habitat types. Qualitative documentation of habitat quality is recorded on habitat assessment sheets. A more quantitative approach to determine the habitat conditions of a sampling site is found in the Habitat Assessment procedures in Chapter 5.

4.2 Site Characterization

Field observations of site conditions are recorded in the appropriate spaces on the habitat assessment sheet (Appendices A-1 and A-2, depending on high/moderate or low-gradient stream classification). This type of habitat analysis allows the investigator to quickly check off or record observed habitat conditions. Procedures for scoring habitat features with the EPA Rapid Bioassessment Protocol (RBP) (Barbour et al. 1999) Habitat Assessment are presented in Chapter 5.

4.2.1 General Information

Some of the information included on the habitat assessment sheet is gathered in advance, using data from maps and prior reconnaissance visits.

4.2.1.1 Map Information

The following information is gathered from maps, primarily using USGS 7.5 minute topographical maps, county maps, or GIS software program: basin, stream name, location (e.g. highway no., bridge, nearby town), county, latitude and longitude, map name or KDOW map number. Latitude and longitude should be recorded when in the field with a GPS unit.

4.2.1.2 Field Information

The following general information is recorded onsite, prior to biological sampling: weather (e.g. hot, cold, rainy, dry, sunny, etc.), time (beginning) and sample type (e.g., biological, bacteriological, physicochemical, sediment, tissue).

4.2.2 Stream Substrate Quality and Composition

In general, variations in particle size and type are reflected in flowing bodies of water by gradation of habitat types from stream headwaters to mouth. Each longitudinal gradation in substrate type harbors a characteristic biotic community. The absence of characteristic community members in the presence of a favorable substrate type can be a useful indication of stream disturbance.

For visual estimates of substrate size, transects are surveyed in a pool (mid-pool) and riffle (mid-riffle) to estimate the substrate by percent particle size and type of material. Results are expressed as percent of total. Additionally, a Wohlman Pebble Count (or equivalent) can be conducted following procedures found in Harrelson et al. (1994) or Wolman (1954). Sample particles are measured against the particle size chart (Table 4-1) to provide the investigator with a fixed concept of category size. In deep waters, particle size may be determined from dredge grab samples. Results are recorded on the habitat assessment sheet. In addition, the estimated percent riffle, run and pool within the sampling reach are recorded.

Table 4-1: Substrate particle size chart

Categories:	Size (mm):
Boulders	>256 (= 10 in)
Cobble	64 - 256 (= 2.5 - 10 in)
Pebble	16 - 64 (= .63 - 2.5 in)
Gravel	2 - 16 (=.08 - .63 in)
Fines	<2 (= .08 in)
Exposed Bedrock	--
Hardpan Clay	--
Detritus	--

4.2.3 Stream Physical Features

4.2.3.1 Stream Flow Condition

The condition of the stream is evaluated, and one of the following assessments is recorded: perennial, intermittent or interrupted. In addition, the gradient of the stream as either high, medium or low is recorded.

4.2.3.2 Stage and Discharge

The stage of the stream is estimated, and one of the following assessments is recorded: dry, no flow (pooled), low, normal, high or flooded.

Stream discharge may be measured with a flow meter, or estimated using neutral-buoyant objects (e.g., oranges, small sticks, small sponge rubber balls) in areas of laminar flow and along a uniform transect of the channel. Refer to the Kentucky Sampling and Monitoring Water Quality Standard Operating Procedures Manual (2008 in draft) for further detail on measuring stream discharge.

4.2.4 Channel Morphology

4.2.4.1 Stream Depth and Width.

The stream depth range is estimated for the entire reach. The stream width range is estimated for the entire reach.

Additional morphological observations on sinuosity, gradient, slope, etc. may be made on the field sheets.

4.2.5 Canopy

An exposed stream often exhibits increased water temperatures that may be directly or indirectly limiting to some organisms and may be favorable for nuisance algal blooms and decreased dissolved oxygen. Light intensity may be limiting to some organisms and favorable to others. A partially shaded stream generally achieves the greatest diversity. In wadeable streams, sufficient shade to maintain temperatures and habitats that will support indigenous organisms is generally created by a 50% to 75% tree canopy. Natural headwater streams should generally have 75% to 100% tree canopy. Sampling performed in the early spring may be performed prior to full leaf-out, but percent shading recorded should be an estimate of what the canopy will be when fully leafed.

A spherical densiometer should be used to determine actual percentage of cover, when available. The canopy cover will be measured in each of the four cardinal directions from three locations within the stream reach. The value recorded on the habitat assessment sheet will be the average of these values.

The percent canopy shading the stream is recorded on the habitat assessment sheet using these categories.

Fully Exposed	(0 - 25%)
Partially Exposed	(25% - 50%)
Partially Shaded	(50% - 75%)
Fully Shaded	(75% - 100%)

4.2.6 Channel Alterations

Many activities that alter the stream channel require water quality certification by KDOW and Section 404 permits from the U.S. Army Corps of Engineers. Some of these activities include: dredging, channelization, clear and snag, bridge construction and artificial bank stabilization. The occurrence of any channel-altering activities at the site is recorded.

4.2.7 Hydraulic Structures

Hydraulic structures include any natural obstructions or human-made devices that impede or deflect the course of water from its original pathway. The presence and type of hydraulic structures are noted. Some typical examples are dams, bridge abutments, islands, gravel and mud bars and any others to be listed individually.

4.2.8 Watershed Features/Land Use

All land uses occurring within the vicinity of the sampling site are recorded. Examples of land use include silviculture, agriculture, oil exploration, construction, mining, urbanization, onsite

wastewater treatment (e.g. septic tanks), etc. In addition, the intensity of localized watershed erosion as heavy, moderate or none is noted.

4.2.9 Pollution Types

The presence and proximity of point source discharges, such as municipal, private or industrial wastewater treatment plants (WWTP), should be noted. In addition, record any localized nonpoint source impacts near or upstream of the sampling location.

4.2.10 Riparian Vegetation

Indicate the dominant trees, shrubs and herbaceous plants in the riparian zone. Because of its stabilizing effects and its ability to influence water temperatures, a riparian zone of 18 meters or more is preferred. The width of the riparian zone is scored in the RBP Habitat Assessment (Chapter 5). In addition, the number of canopy strata present in the riparian zone as an indication of riparian age and quality (e.g., overstory, understory, herb layer) should be counted.

4.3. Physicochemical Monitoring

4.3.1 Field Physicochemical Sampling

The physicochemical parameters, temperature, dissolved oxygen, pH and specific conductance, will be measured in the field using multi-probe water quality instruments. Information will be recorded on the field data sheet.

4.3.2 Water Chemistry Sampling

Water samples are taken for water chemistry at all biological sampling sites. Water sampling will follow procedures as outlined in the Kentucky Sampling and Monitoring Standard Operating Procedure Manual (2008 in draft). At all sites, sample bottles are collected using the grab sample methods, and analyzed for nutrient and bulk parameters. Specific nutrient parameters are as follows:

- Ammonia
- Nitrate-Nitrite
- Total Kjeldahl Nitrogen
- Total Phosphorus

Specific bulk samples are analyzed for the following parameters:

- Chloride
- Acidity
- Alkalinity
- Sulfate
- Total organic carbon
- Total dissolved solids
- Total suspended solids

Sample handling and storage follow all procedures in the KDOW Sampling and Monitoring SOP (2008 in draft).

In certain circumstances, when additional water chemistry is needed to determine pollution causes and sources, water chemistry may be collected at sites above the selected biological sampling sites. A number of different tools are used to determine which sampling locations should include this additional water sampling. These can include land use assessment, aerial photography, point source locations, mining, urbanization, compromised buffer zones, channelization and past water quality assessments.

Those streams that may indicate an aquatic life use impairment due to habitat and land use assessments while in the field, will be sampled for the additional water quality variables at the first road crossing, or upstream of the first significant tributary.

A significant tributary may be considered as follows in relation to monitored stream order: 1st order at a significant change in land use or half way between primary monitoring station and headwaters; 2nd order = 1st order, 3rd order = 2nd order, 4th order = 3rd order, 5th order = 4th or 3rd order. A 3rd order tributary will be considered significant when there appears to be a significant change in land uses or other parameters, due to the small number of 4th order tributaries of a 5th order stream in the Commonwealth. The name, county and road crossing of the sample location will be recorded. Habitat questions 4-10 will be completed for each of these water sampling locations. Additional notes will be made of observed land uses and specific probable sources of pollutants while traveling the watershed.

SECTION 5. HABITAT ASSESSMENT

5.1 Introduction

A habitat assessment should be conducted at every biological sampling reach. In addition, parameters 4-10 should be assessed at every water quality sampling site located above biological sampling sites. Such assessments will allow investigators to evaluate the quality of in stream and riparian habitat. The availability of quality habitat directly influences the biological integrity of the stream reach. Information obtained from the habitat assessment can be used to supplement biological and physicochemical data when determining the overall health of the stream reach and stream-use designation.

Additionally, habitat assessments can be used to document physical changes that occur at a sampling reach over time. In multi-agency monitoring projects (such as watershed monitoring), habitat assessments provide continuity and consistency between all entities involved in the monitoring effort. Habitat assessment procedures follow those outlined in Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers (Barbour et al. 1999), and as described in the following sections of this manual. Photographs depicting the gradients of habitat parameters can be found in the above-mentioned protocol manual. Other methods of assessing habitat are currently being investigated. If these methods are used, separate habitat criteria will be developed.

5.2 Assessment Procedures

Investigators should conduct a visual-based habitat evaluation of the stream reach by filling out the appropriate Habitat Assessment Field Data Sheet (Barbour et al. 1999). In streams where riffles should naturally be present (e.g., most stream reaches of the Central Appalachian, Western Allegheny, Southwestern Appalachian and Interior Plateau ecoregions would qualify), the High-Gradient Habitat Assessment Field Data Sheet should be used (Appendix B-1). In low-gradient streams where rocky riffles are not naturally present (e.g., most stream reaches in the Mississippi Valley Loess Plain and the Interior River Lowland ecoregions would qualify), the Low-Gradient Habitat Assessment Field Data Sheet should be used (Appendix B-2).

In certain instances, habitat may be preliminarily assessed in the office through the use of topographic maps and aerial surveys. Field reconnaissance will be performed when necessary. When sites are added for water sampling only, habitat characteristics 4-10 will be assessed at each site.

The visual-based habitat evaluation consists of ten parameters that rate in stream habitat, channel morphology, bank stability and riparian vegetation for each sampling reach. A numerical scale of 0 (lowest) to 20 (highest) is used to rate each parameter (Barbour et al. 1999). For each parameter, the investigators will determine which of the following conditions exist at the sampling reach: Optimal, Suboptimal, Marginal or Poor. A parameter score will then be given within the condition category chosen above: Optimal (20-16), Suboptimal (15-11), Marginal (10-6) or Poor (5-0). The investigators will total all of the parameter ratings to obtain a final habitat ranking (Barbour et al. 1999).

5.3 Parameters for Habitat Assessment

These parameters should be evaluated within the sampling reach. All of the areas within the reach should be evaluated together as a composite.

Parameter #1 - Epifaunal Substrate/Available Cover (Both High- and Low-Gradient Sheets)

This metric measures the relative quantity and the variety of stable structures, such as cobble, boulders, fallen trees, logs, branches, root mats, undercut banks, aquatic vegetation, etc., that provide refugia, feeding opportunities and sites for spawning and nursery functions. Assessment is a composite of the entire biological sampling station.

1. Optimal

a. High-Gradient:
>70% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at a stage to allow full colonization potential (i.e., logs/snags that are not new fall and not transient) (20-16).

b. Low-Gradient:
>50% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at a stage to allow full colonization potential (i.e., logs/snags that are not new fall and not transient) (20-16).

2. Suboptimal

a. High-Gradient:
40%-70% mix of stable habitat; well-suited for full colonization potential;
adequate habitat for maintenance of populations; presence of additional substrate in the form of new fall, but not yet prepared for colonization (may rate at the high end of the scale) (15-11).

b. Low-Gradient:
30%-50% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of new fall, but not yet prepared for colonization (may rate at high end of the scale) (15-11).

3. Marginal

a. High-Gradient:
20%-40% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed (10-6).

b. Low-Gradient:
10%-30% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed (10-6).

4. Poor

a. High-Gradient:
<20% stable habitat; lack of habitat is obvious; substrate unstable or lacking (5-0).

b. Low-Gradient:
<10% stable habitat; lack of habitat is obvious; substrate unstable or lacking (5-0).

Parameter #2 Embeddedness - (High-Gradient Sheet)

The extent to which rocks and snags are covered or sunken into the silt, sand, mud or biofilms (algal, fungal or bacterial mats) of the stream bottom. Generally, as rocks become embedded, the surface area available to macroinvertebrates and fish (for shelter, spawning and egg incubation) is decreased; assess in the upstream or central portions of riffles.

1. Optimal

Rocks are 0-25% surrounded by fine sediment. Layering of cobble provides diversity of niche space (20-16).

2. Suboptimal

Rocks are 25%-50% surrounded by fine sediment (15-11).

3. Marginal

Rocks are 50%-75% surrounded by fine sediment (10-6).

4. Poor

Rocks are >75% surrounded by fine sediment (5-0).

Parameter #2 - Pool Substrate Characterization - (Low-Gradient Sheet)

This metric evaluates the type and condition of bottom substrates found in pools of low-gradient streams. Firmer sediment types (e.g., gravel and sand) and rooted aquatic plants support a wider variety of organisms than a pool substrate dominated by mud or bedrock and no plants. In addition, a stream that has a uniform substrate in its pools will support far fewer types of organisms than a stream that has a variety of substrate types.

1. Optimal

Mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation common (20-16).

2. Suboptimal

Mixture of soft sand, mud or clay; mud may be dominant; some root mats and submerged vegetation present (15-11).

3. Marginal

All mud or clay or sand bottom; little or no root mat; no submerged vegetation (10-6).

4. Poor:

Hard-pan clay or bedrock; no root mat or vegetation (5-0).

Parameter #3 - Velocity/Depth Regime - (High-Gradient Sheet)

The best streams in most high-gradient regions will have all of the following patterns of velocity and depth: 1) slow-deep, 2) slow-shallow, 3) fast-deep and 4) fast-shallow; the occurrence of these four patterns relates to the stream's ability to provide and maintain a stable aquatic environment. Investigators may have to scale deep and shallow depending upon the stream size; a general guideline is 0.5 m between shallow and deep.

1. Optimal

All 4 regimes present (20-16).

2. Suboptimal

Only 3 of the 4 regimes present; if fast-shallow is missing, score lower than if missing other regimes (15-11).

3. Marginal

Only 2 of the 4 regimes present; if fast-shallow or slow-shallow are missing, score low (10-6).

4. Poor

Dominated by 1 regime (usually slow-deep) (5-0).

Parameter #3 - Pool Variability - (Low-Gradient Sheet)

This metric rates the overall mixture of pool types found in low-gradient streams according to size and depth. The four basic types of pools are large-shallow, large-deep, small-shallow and small-deep. A stream with many pool types will support a wide variety of aquatic species. Rivers with low sinuosity (few bends) and monotonous pool characteristics do not have sufficient quantities and types of habitat to support a diverse aquatic community. General guidelines are any pool dimension (i.e., length, width, oblique) greater than half the cross-section of the stream for separating large from small and 1 m depth separating shallow and deep.

1. Optimal

Even mix of large-shallow, large-deep, small-shallow and small-deep pools present (20-16).

2. Suboptimal

Majority of pools large-deep; very few shallow (15-11).

3. Marginal

Shallow pools much more prevalent than deep pools (10-6).

4. Poor

Majority of pools small-shallow or pools absent (5-0).

Parameter #4 - Sediment Deposition (Both Sheets)

This metric measures the amount of sediment that has accumulated in pools and changes that have occurred to the stream bottom as a result of deposition. This may cause the formation of islands, point bars (areas of increased deposition usually at the beginning of a meander that increases in size as the channel is diverted toward the outer bank) or shoals or result in the filling of runs and pools. Sediment is often found in areas that are obstructed and areas where the stream flow decreases, such as bends. Deposition is a symptom of an unstable and continually changing environment that becomes unsuitable for many organisms. Examine bars/shoals and pool substrates within the biological monitoring station, when assessing this parameter

1. Optimal

a.High-Gradient
Little or no enlargement of islands or point bars and less than 5%of the bottom affected by sediment deposition (20-16).

b. Low-Gradient
Little or no enlargement of islands or point bars and less than 20% of the bottom affected by sediment deposition (20-16).

2. Suboptimal

a. High-Gradient
Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5%-30% of the bottom affected; slight deposition in pools (15-11).

b. Low-Gradient
Some new increase in bar formation, mostly from gravel, sand or fine sediment; 20%-50% of the bottom affected; slight deposition in pools (15-11).

3. Marginal

a. High-Gradient
Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30%-50% of the bottom affected; moderate sediment deposits apparent at most obstructions and slow areas, bends and pools (10-6).

b. Low-Gradient
Moderate deposition of new gravel, sand or fine sediment on old and new bars; 50%-80% of the bottom affected; sediment deposits at obstruction, constrictions and bends; moderate deposition of pools prevalent (10-6).

4. Poor

a. High-Gradient
Heavy deposits of fine material; increased bar development; more than 50% of the bottom changing frequently; pools almost absent due to substantial sediment deposition (5-0).

b. Low-Gradient
Heavy deposits of fine material; increased bar development; more than 80% of the bottom changing frequently; pools almost absent due to substantial sediment deposition (5-0).

Parameter #5 - Channel Flow Status (Both Sheets)

This metric measures the degree to which the channel is filled with water. The score will change with the seasons. Estimate the percentage of the channel that is wet using the low water mark.

1. Optimal

Water reaches base of both lower banks; minimal amount of channel substrate exposed (20-16).

2. Suboptimal

Water fills >75% of the available channel or <25% of channel substrate exposed (15-11).

3. Marginal

Water fills 25%-75% of the available channel; riffle substrates are mostly exposed (10-6).

4. Poor

Very little water in channel; mostly present in pools (5-0)

The next 5 parameters should evaluate an area from approx. 100-m upstream of the sampling reach through the sampling reach. This whole area should be evaluated as a composite. When determining left and right bank, face downstream.

Parameter #6 - Channel Alteration (Both Sheets)

This metric measures the large-scale, direct changes in the shape of the stream channel. Channel alteration is present when 1) artificial embankments, rip-rap and other forms of bank stabilization or structures are present, 2) the stream is very straight for significant distances because of channelization, 3) dams and bridges are present that obstruct flow and/or 4) dredging or other substrate mining activities are occurring or have occurred.

1. Optimal

Channelization or dredging absent or minimal; stream with normal pattern (20-16).

2. Suboptimal

Some channelization present, usually in areas of bridge abutments; evidence of past channelization (dredging, etc., >20 past years) may be present, but recent channelization not present (15-11).

3. Marginal

Channelization may be extensive; embankments or shoring structures present on both banks; and 40%-80% of the stream reach channelized and disrupted (10-6).

4. Poor

Banks shored with gabion or cement; >80% of the stream disrupted; in stream habitat greatly altered or removed entirely (5-0).

Parameter #7 - Frequency of Riffles (or Bends) - (High-Gradient Sheet)

This metric measures the sequence of riffles and thus the heterogeneity occurring in a stream. Estimate riffle frequency by determining the ratio of distance between riffles divided by the width of the stream. An average of the riffle ratios is determined for biological monitoring stations and the upstream segment.

1. *Optimal*

Occurrence of riffles relatively frequent; ratio of distance between riffles divided by the width of the stream <7:1 (generally 5 to 7); variety of habitat is key; in streams where riffles are continuous, placement of boulders or other large, natural obstruction is important (20-16).

2. *Suboptimal*

Occurrence of riffles infrequent; distance between riffles divided by the width of the stream is between 7 and 15 (15-11).

3. *Marginal*

Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 and 25 (10-6).

4. *Poor*

Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is >25 (5-0).

Parameter #7 - Channel Sinuosity - (Low Gradient Sheet)

This metric evaluates the meandering or sinuosity of the low-gradient stream. A high degree of sinuosity provides for diverse habitat and fauna, and the stream is better able to handle surges when water levels in the stream fluctuate as a result of storms. The absorption of this energy by bends protects the stream from excessive erosion and flooding, and provides refugia for benthic invertebrates and fish during storm events.

To gain an appreciation of this parameter in low-gradient streams, a longer segment or reach than that designated for sampling may be incorporated into the evaluation. In some situations, this parameter may be rated from viewing accurate topographical maps. The "sequencing" pattern of the stream morphology is important in rating this parameter. In "oxbow" streams of coastal areas and deltas, meanders are highly exaggerated and transient. Natural conditions in these streams are shifting channels and bends, and alteration is usually in the form of flow regulation and diversion. A stable channel is one that does not exhibit progressive changes in slope, shape or dimensions, although short-term variations may occur during floods (Gordon et al. 1992).

1. *Optimal*

The bends in the stream increase the stream length 3 to 4 times longer than if it was in a straight line. (Note: channel braiding is considered normal in coastal plains and other low-lying areas. This parameter is not easily rated in these areas.) (20-16).

2. Suboptimal

The bends in the stream increase the stream length 2 to 3 times longer than if it was in a straight line (15-11).

3. Marginal

The bends in the stream increase the stream length 1 to 2 times longer than if it was in a straight line (10-6).

4. Poor

Channel straight; waterway has been channelized for a long distance (5-0).

Parameter #8 - Bank Stability (Both Sheets)

This metric measures whether the stream banks are eroded or have the potential to erode. Each bank is scored independently from 10-0.

1. Optimal

Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems; <5% of bank affected (10-9).

2. Suboptimal

Moderately stable; infrequent, small areas of erosion mostly healed over; 5%-30% of the bank affected (8-6).

3. Marginal

Moderately unstable; 30%-60% of bank in reach has areas of erosion; high erosion potential during floods (5-3).

4. Poor

Unstable; many raw, eroded areas; obvious bank sloughing; >60% of bank has erosional scars (2-0).

Parameter #9 - Bank Vegetative Protection (Both Sheets)

This metric measures the amount of vegetative protection afforded to the stream and the near-stream portion of the riparian zone. Each bank is scored independently from 10-0. Determine what vegetative types (trees, understory shrubs, herbs and non-woody macrophysics) are present on each bank. Those stream banks with different vegetative types provide better erosion protection and provide more of a variety of allochthonous food material. Native vegetation scores higher than invasive or non-native vegetation.

1. Optimal

>90% of the stream bank surfaces and immediate riparian zones covered by natural vegetation, including trees, understory shrubs, herbs and non-woody macrophytes; vegetation disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally (10-9).

2. Suboptimal

70%-90% of the stream bank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one half of the potential plant stubble height remaining (8-6).

3. Marginal

50%-70% of the stream bank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one half of the potential plant stubble height remaining (5-3).

4. Poor

<50% of the stream bank surfaces covered by vegetation; disruption is very high; vegetation has been removed to 5 cm or less in average stubble height (2-0).

Parameter #10 - Riparian Vegetative Zone Width (Both Sheets)

This metric measures the width of the natural vegetation from the edge of the stream bank through the riparian zone. The presence of old fields, paths, walkways, etc., in otherwise undisturbed riparian zones may be judged to be inconsequential to highly destructive to the riparian zone. Each bank is scored independently from 10-0. When determining final scores, the age and density of the riparian vegetation should be evaluated (e.g. A score of 9, instead of 10, should be given to a riparian zone that is over 20 m in width, but is dominated by 5-10 year old hardwood trees).

1. Optimal

Width of riparian zone >18 m; human activities (parking lots, roadbeds, clear-cuts, lawns, pastures or crops) have not impacted the zone (10-9).

2. Suboptimal

Width of riparian zone 13-18 m; human activities have impacted the zone only minimally (8-6).

3. Marginal

Width of riparian zone 6-12 m; human activities have impacted the zone a great deal (5-3).

4. Poor

Width of riparian zone <6 m; little or no riparian zone due to human activities (2-0) (Barbour et al. 1999).

5.4 Habitat Criteria

Habitat evaluations were conducted at biological sampling sites in the Green and Tradewater River basins for 2001, the Big Sandy and Little Sandy River basins for 2002, the Kentucky River basin for 2003, the Licking and Salt River basins for 2004, the Cumberland and the Tennessee and Mississippi River basins for 2005 using the Rapid Bioassessment Protocols format. Historical and current reference reach habitat data were used to produce habitat criteria for the bioregions of Kentucky. The scores for all reference reach stations were ranked and divided into percentiles. The lower quartile (25th percentile) may be considered the dividing line between those habitats fully supporting biotic integrity and those partially supporting biotic integrity. Habitats may be considered poor if they fall below the lowest reference condition score for that area. For assessment purposes, habitat scoring criteria are grouped in the same manner as Bioregions (see Section 3).

For the Bluegrass (BG) Bioregion, reference sites were divided into wadeable and headwater based upon drainage area. Those above five square miles were considered wadeable and below five square miles headwater. Headwater streams in this large ecoregion have a tendency to score higher on certain metrics (i.e. frequency of riffles, bank stability, riparian zone width) than wadeable streams as a result of intensive land use activities (e.g. residential areas, horse farms, etc.). This bias was reflected in final habitat scores. Therefore, separate habitat criteria were developed for wadeable and headwater streams.

Biosurveys conducted within the Pennyroyal (PR) Bioregion use high-gradient habitat assessment sheets. Streams within this bioregion are characterized by having well-developed macrohabitats (riffle, run, pool). Generally, the habitat assessments in this ecoregion score higher than other bioregions, except the Mountains.

Streams sampled within the Mississippi Valley Loess Plains and Interior River Valleys and Hills Ecoregions were generally low-gradient streams with very few riffles. These ecoregions were combined to form the Mississippi Valley-Interior Rivers (MVIR) Bioregion. Low-gradient habitat assessment sheets were used in these ecoregions to rate the available habitat.

Additionally, in low-gradient sections of Interior Plateau and mountainous streams, low-gradient habitat assessment sheets were used.

Mountainous ecoregions of the Commonwealth provided similar habitat opportunities for aquatic community colonization and use. Habitat scores from reference sites in the Central Appalachian, Southwestern Appalachian and Western Allegheny Ecoregions reflected these similarities. Therefore, habitat data from all of these ecoregions were combined to develop habitat criteria for the Mountains (MT).

The following tentative habitat criteria can be used to determine whether the sampling reach is fully supporting, partially supporting or not supporting its designated use:

Bioregion	**Use Support**	**Area Scoring**	
		Headwater (<5.0 mi^2)	**Wadeable (>5.0 mi^2)**
BLUEGRASS	Fully	≥ 156	≥ 130
	Partial	142 - 155	114 - 129
	Non	≤ 141	≤ 113
PENNYROYAL	Fully	≥ 146	≥ 146
	Partial	132 - 145	132 - 145
	Non	≤ 131	≤ 131
MISSISSIPPI VALLEY INTERIOR RIVER	Fully	≥ 135	≥ 135
	Partial	114 - 134	114 - 134
	Non	≤ 113	≤ 113
MOUNTAIN	Fully	≥ 160	≥ 160
	Partial	117 - 159	117 - 159
	Non	≤ 116	≤ 116

Criteria development is an ongoing process. Updates of the criteria will occur as more reference data is collected. We are currently investigating other habitat assessments. Other assessments may not follow these criteria. Separate criteria will be developed when enough information is available. Method and criteria changes will be included in the next SOP update.

SECTION 6. ALGAE

6A. Benthic Algae

6A.1 Introduction

Benthic (attached) algae are sensitive indicators of change in lotic waters and are the primary producers within the stream ecosystem. The benthic algae are usually the dominant component of the periphyton. Because it is attached to the substrate, the benthic algae community integrates physical and chemical disturbances to a stream. Another advantage of using benthic algae in water quality assessments is that the benthic algae community contains a naturally high number of species, making data useful for statistical and numerical applications to assess water quality. Response time of benthic algae is rapid, as is recovery, with re-colonization after a disturbance occurring more rapidly than other organisms.

Diatoms, in particular, are useful indicators of biological integrity because they are ubiquitous; at least a few can be found under almost any conditions. In addition, most can be identified to species by experienced biologists, and tolerances or sensitivities to specific changes in environmental conditions are known for many species (Dixit, et al. 1992, Rott 1991). By using benthic algal data in association with macroinvertebrate and fish data, the biological integrity of the entire ecosystem can be ascertained.

6A.2 Sampling

6A.2.1 Procedures

Conduct a cursory, visual assessment of the reach and record the results on the benthic algae field data sheet (refer to Appendix C-1). Record the following station information in the appropriate spaces at the top of the sheet: KDOW station identification number, stream name, location of the station, collection date, time, county, river basin, KDOW program and name of investigator(s).

After field collection has been accomplished, assess the following conditions and record these assessments, while still in the field, in the proper field data blanks: macrohabitats sampled (e.g., riffle, run, pool), microhabitats sampled (Table 6-1), the collection method used, macroalgae present (e.g., Cladophora, Batrachospermum, Tetraspora, etc.), an estimation of benthic algae coverage (e.g., absent, sparse, moderate, heavy), any comments (e.g., whether scour has recently occurred, the flow status of the stream, etc.) and a qualitative ranking of the algal community. Use this information to supplement more detailed benthic algal community assessments.

6A.2.2 Collection

6A.2.2.1 Natural Substrates (Headwater and Wadeable streams)

Collect benthic algae from all available microhabitats in wadeable streams (Table 6-1). Collect a composite, qualitative sample from microhabitats in roughly the proportion that they occur at the

site. Sample both riffles and pools, or select one major habitat type (usually riffle) if it occurs at all sites to be compared in the study. Collect samples during stable flow conditions. During low-flow periods, pools may be the only habitat available. After extremes of flooding or drought, allow at least a two-week re-colonization period before sampling. Collect samples using the following methods:

- Use a knife blade, micro spatula, toothbrush or similar device to scrape algae from rocks and other hard substrates making every effort to remove all algae from the scraped area. Rinse with distilled water if necessary. Five replicate samples are collected from rocks of similar size whenever possible. Samples from individual rocks can be composited or analyzed separately. Quantitative data can be obtained by measuring the area of substrate sampled.

- Use a suction device to collect algae from bedrock substrates. Press a section of PVC pipe equipped with a neoprene rubber gasket against the bedrock substrate so that the benthic algae within the enclosed area can be dislodged with a stiff-bristled brush. Suction dislodged material into a filter flask using a hand-operated pump. This method can be used to obtain quantitative data from bedrock (modified from Douglas 1958).

- Gently lift algal mats from depositional areas with forceps or by suctioning with a disposable pipette.

Table 6-1: Microhabitats usually found in wadeable streams

Epipelic	Silt and sediment habitats usually in depositional areas with slow current. Algae may form a thin mat that loosely adheres to the surface of the epipelon. To collect epipelic algae, suction material from the mud-water interface using an eye-dropper bulb and a disposable Pasteur pipette or gently lift the algal mat from the surface of the sediment using a knife.
Episammic	Sand habitats. Collection is made in the same manner as above.
Epilithic	Rock or other hard surface habitats including dams, bridge abutments, boat ramps, etc. To sample epilithic algae, scrape or hand pick material from epilithon in riffles, pools and runs.
Epidendric	Woody habitats. To collect epidendric algae, scrape or hand pick material from submerged logs, tree roots, drifts, etc.
Epiphytic	Plant habitats usually associated with aquatic mosses, macrophytes and filamentous algae. To sample epiphytic algae, scrape, wring out, hand pick or collect the entire substrate.
Epizooic	Animal habitats including turtle shells, snail shells and other macroinvertebrates. To sample epizooic algae, scrape, hand pick or collect the entire substrate.

The proportion of sample collected from each site will vary with the relative abundance of each microhabitat at the site.

The following guidelines can be used for a more quantitative collection method; each bulleted statement reflects a microhabitat.

High-Gradient Stream Diatom Collection

- 5 riffle rocks in a transect
- 2 leaf packs (squeeze)
- 2 aquatic plants or roots
- 2 pool rocks
- 2-3 pieces of wood
- Sediment depositional area (skim top layer with cap of jar)
- Sand depositional area (skim top layer with cap of jar)
- Anything that looks different
- Sample microhabitats at relative abundance found at site

Low-Gradient Stream Diatom Collection

- 5 leaf packs
- 5 pieces of wood
- 5 aquatic plants or roots
- 2 sediment depositional areas
- 2 sand depositional areas
- Rocks when available
- Anything that looks different
- Sample microhabitats at relative abundance found at site

6A.2.2.2 Artificial Substrate (Non-wadeable Streams)

Algal data from artificial substrates can be used as a tool to assess water quality, even if the natural assemblage is not exactly duplicated (Patrick 1973, Stevenson and Lowe 1986). In non-wadeable streams, rivers with no riffle areas, wetlands or littoral zones of lakes, surface (floating) or benthic (bottom) periphytometers equipped with glass slides are used for collecting algae. Clay tiles, plexiglass plates or other substrates may be substituted for glass slides. Pre-clean glass slides and rinse with acetone before placing them in the periphytometer. Artificial substrates should be placed in the stream for three to four weeks to allow sufficient time for colonization (Aloi 1990, Weber and Raschke 1970, Weber 1973).

To minimize difficulty or errors in analysis of benthic algae from artificial substrates, this procedure should be followed:

- Set a minimum of three periphytometers at each site, from which individual or composite samples can be obtained.
- Attach periphytometers to trees, snags, bridge pilings or other sturdy anchor sites and away from frequently visited areas (swimming and fishing "holes") to avoid vandalism, theft or other disturbances.
- Reset periphytometers if flooding or desiccation occurs during the exposure period.

- Allow streams to return to pre-scouring or pre-drought conditions before resetting periphytometers.

Samples from replicate periphytometers can be analyzed separately or composited, depending on the goals of the study.

6A.2.2.3 Biomass Samples

For biomass samples:

- Scrape natural substrates in the field using distilled water to rinse the substrates.
- Collect all material and rinse water from the scraped area into an opaque jar and return it, on ice, to the laboratory for filtration of sub samples; filtration may be performed in the field and filters transported on ice to the laboratory.
- Do not add preservatives to chlorophyll-a samples.
- For natural substrates, measure the area sampled by tracing its outline on paper and determining size with a digital planimeter.
- Artificial substrates are wrapped in aluminum foil and transported on ice to the laboratory for sample preparation and analysis.

6A.2.3 Preservation and Labeling

- Collect samples in small, watertight glass vials or screw-top bottles of at least 60 ml in volume.
- Preserve taxonomic samples with 3% to 4% buffered formalin, 2% glutaraldehyde, Lugol's solution or other preservatives listed in the latest edition of Standard Methods (APHA 2007). Chlorophyll a and ash-free dry-weight samples should be placed on ice only.
- Affix a label permanently to the sample container, recording the following information on the label using a pencil or waterproof, indelible ink: waterbody name, location, sample number, date and name of collector.
- Record this information in a laboratory sample logbook (Appendix F-4).
- Refrigerate samples until taxonomic evaluation is completed.
- After taxonomy is completed, archive the processed samples in a cool, dry place and discard the unused portion of the sample.
- Permanent mounts of diatom samples are retained in wooden or plastic slide boxes.

6A.2.4 Sample Analysis

6A.2.4.1 Field Assessment

While in the field, qualitatively rank the benthic algal community at each station. A score of 1 (lowest quality) to 5 (highest quality) is possible. Record scores and a description of the benthic algal community on the benthic algae field data sheet (Appendix C-1). This information may be used to support more detailed assessments of the benthic algal community. The algae are judged using the following ranking criteria:

1. Excellent Quality (5):

The benthic algal community appears diverse with several divisions represented, including chrysophytes, chlorophytes, cyanophytes and rhodophytes. Phytoplankton sub-community is not apparent. Floating algal mats are not present. The algal community is similar to that of reference stations within the same ecoregion.

2. Fair - Good Quality (2-4):

Benthic algae are present in moderate amounts. The benthic algal community may be dominated by one type of growth, such as long filaments of Cladophora. Diversity is low to moderate, and a phytoplankton sub-community is not apparent. Floating algal mats may be present, but are not extensive. Clean water benthic algal taxa (e.g., red algae, Chaetophora, etc.) present in reference reach stations may not be present.

3. Poor Quality (1):

In cases of toxic pollution (acid mine drainage, toxic discharges, etc.), substrates and water column may appear sterile, bleached or rust-colored. Little or no algae are observed. With organic pollution (sewage discharges, etc.), substrates may be covered with thick white, black or gray mats of filamentous bacteria, thick algal mats of cyanophytes (blue-green algae) and/or chlorophytes (green algae). The water column may have a "pea green" appearance as a result of high abundances of euglenophytes, or large floating mats of algae may be present, especially in pools and slow-moving streams. Look for extremes of either characteristic. Diversity is very low. Very few, if any, clean water taxa are present.

6A.2.4.2 Laboratory Analysis

6A.2.4.2.1 Non-Diatom Algae

In this document, the term "non-diatom algae" refers to all taxa that do not belong to the Class Bacillariophyceae.

Slide Preparation and Analysis

Equipment:

- Microscope with 20X and 40X objectives
- Glass microscope slides
- Glass cover slips
- Watch glass
- Disposable pipettes

Procedure:

- Thoroughly shake the sample container to dislodge epiphytes from filamentous taxa and randomly mix all algal organisms.
- Pour the contents into a shallow watch glass so that all filamentous and mat-forming taxa can be separated.
- Using dissecting probes or needle-nosed forceps, place representative filamentous taxa on a pre-cleaned microscope slide or in a settling chamber for inverted microscope use.
- Cover the filaments with approximately 0.5 ml of sample liquid.
- Gently place a cover slip over the sub sample, completing the wet mount.
- Examine each slide at 200x, then at 400x to ensure that smaller organisms are not overlooked.
- Identify all non-diatom algae to the lowest taxonomic level using current taxonomic references. Scan each slide until no new organisms are seen.
- Examine a minimum of three slides for each sample.
- Record observed taxa on the non-diatom bench sheet (Appendix B-2) along with taxonomic division, estimated relative abundance (abundant, common, rare) and any known autecological information.

In some instances, counts of the non-diatom community may be needed in order to obtain specific autecological information.

- Homogenize the sample with a blender.
- Pipette a sub sample into a Palmer counting cell.
- Identify and count 300 algal non-diatom units to the lowest taxonomic level at 400X. Non-diatom algal units are considered instead of cells.

Since colonial, coenobial or filamentous cells normally do not occur singly in a natural environment, counting each cell does not accurately portray relative abundance for that specific taxon. For example, although Pediastrum duplex is composed of several cells, the colony as a whole is counted as one unit. Likewise, coenobia and unicells are each considered one unit. Filaments may be counted either as one unit or counted in units of 10 um lengths (A 100-um filament is 10 units). Record numbers of non-diatom algal units on the non-diatom bench sheet. Enter the data into an appropriate database, such as EDAS (Ecological Data Application System). Taxonomic references are listed at the end of this chapter.

6A.2.4.2.2 Diatoms

After the non-diatom algae have been identified, clear diatom frustules of organic and intercellular material using one of the following oxidation methods (APHA 1998, van der Werff 1955):

<u>Nitric Acid Oxidation</u>

Equipment:

- 2000 ml Erlenmeyer flask
- 1000 ml graduated cylinder

- 20-30 ml of algae sample
- 50 ml of HNO3

Procedure:

- Shake the sample vigorously and immediately pour a portion (about 20-30 ml) of the sample into a large 2000 ml Erlenmeyer flask.
- Under a fume hood, add 50 ml of concentrated HNO3. Allow the sample to oxidize overnight, and then fill the flask with distilled water.
- Let the sample resettle overnight, siphon off the supernatant and pour the remaining diatom solution into a 1000 ml graduated cylinder.
- Fill with distilled water, settle and siphon supernatant at least twice more, until the yellow color changes to clear.
- Remove supernatant and transfer concentrated diatoms into a labeled scintillation vial for permanent storage.
- A small amount of ethanol may be added to the scintillation vial to help prevent fungal growth.

Note: Acid-sample mixture may be boiled for 1 hour or less to assist the burning process.

Hydrogen Peroxide/Potassium Dichromate Oxidation

Equipment:

- 2000 ml Erlenmeyer flask
- 1000 ml graduated cylinder
- 20-30 ml algae sample
- 50 ml of 50% H2O2
- Micro spatula
- K2Cr2O7

Procedure:

- Prepare sample as in nitric acid method, but use 50 ml of 50% H2O2 instead of HNO3.
- Allow to oxidize overnight; then add a micro spatula of K2Cr2O7.
- This will cause a violent exothermic reaction, so be careful to perform this method only under a fume hood.
- When the sample color changes from purple to yellow and boiling stops, fill the flask with distilled water.
- Repeat rinsing steps as outlined for the HNO3 method until the yellow color is gone.

6A.4.2.2.3 Diatom Slide Preparation

Equipment:

- Pre-cleaned microscope slide
- Glass cover slip
- Naphrax mounting medium

- Hot plate

Procedure:

- Place a cover slip on a clean surface, free of vibrations and strong air currents.
- Using a disposable pipette, flood the cover slip with de-ionized water followed by a drop of well-mixed sample.
- Allow to air dry.
- Place a large drop of Naphrax mounting medium onto a pre-cleaned microscope slide.
- Invert the cover slip onto the Naphrax.
- Place the slide on a hot plate on high heat.
- Allow the toluene to boil out of the Naphrax.
- Make sure that this procedure is conducted in or near a laboratory hood where ventilation is good.
- After bubbles of toluene have stopped forming, remove the slide from the hot plate and press cover slip with forceps or toothpicks.
- Allow the slide to cool and harden.
- Remove all excess Naphrax from the slide with a razor blade.
- Affix a slide label to the slide and place in a slide box for storage.

6A.2.4.2.4 Diatom Identification and Enumeration

Equipment:
- Microscope with 100X oil immersion objective
- Immersion oil

Procedure:

- Identify diatoms at 1000X to the lowest possible taxonomic level, preferably to the species or variety level, using current taxonomic references.
- Record all taxa encountered on the diatom bench sheet (Appendix B-3) creating a species list prior to enumeration.
- Scan the slide until five minutes pass without producing any new taxa.
- For quantitative data, count a minimum of 500 valves recording taxa and number counted on the diatom bench sheet.
- Data assessment is based on the completed species list.
- Enter data into an appropriate database, such as EDAS.

6A.2.4.2.5 Biomass - Chlorophyll a and Ash-Free Dry-Weight (AFDW)

Chlorophyll a analyses are performed as described for phytoplankton (Section 6), using U.S. EPA Method 445.0 (USEPA 1992). For Ash-free Dry Weight, replicate samples are analyzed in accordance with Standard Methods (APHA 2007).

6A.3 Data Analysis

An assessment of biological integrity can be made based on the benthic algal data. The goal is to categorize water quality as excellent, good, fair or poor and to determine the degree and cause of aquatic life use impairments in fair or poor streams. A multiple metric index called the Diatom Bioassessment Index (DBI) is used to assess the benthic algal community.

6A.3.1 Diatom Bioassessment Index

Biological indices represent mathematical models of community changes (Perkins 1983). Changes in water quality will affect resident biota, and indices that reflect these changes in a particular community are useful biological indicators of water quality. Several metrics have been used to assess water quality conditions using benthic algae. Some have the diagnostic ability to indicate the type of impact (nutrient enrichment, toxicity, acidity, salinity, sewage and siltation). However, each metric alone could not accurately describe the overall water quality at a site.

In the mid-1980s, phycologists at the KDOW developed a multi-metric index using four benthic diatom metrics. This multi-metric approach was modeled after successfully used indices such as Karr (1981) with fish and Plafkin et al. (1989) with macroinvertebrates. The metrics chosen for the original Diatom Bioassessment Index (DBI) were as follows:

1. Total number of diatom taxa (TNDT),
2. Shannon diversity (H′),
3. Pollution tolerance index (PTI)
4. % sensitive species (%SS)

The DBI was later redefined using box and whisker plots to test metric sensitivity and the Pearson's correlation coefficient index to test for metric redundancy. A new DBI was developed using three of the original metrics (TNDT, H′, and PTI) and the following three new metrics:

5. Cymbella group richness (CGR)
6. Fragilaria group richness (FGR)
7. % Navicula, Nitzschia and Surirella (%NNS). The new DBI provides water resource managers with a very sensitive, community structure-based tool for assessing water quality.

6A.3.2 Diatom Bioassessment Index Metrics

Total Number of Diatom Taxa (TNDT)

Total number of diatom taxa (TNDT) is an estimate of diatom species richness. High species richness is assumed to be the case in an unimpacted site, and species richness is expected to decrease with increasing pollution. Slight levels of nutrient enrichment, however, may increase species richness in naturally unproductive, nutrient-poor streams (Bahls 1992). Low-order,

pristine streams in the Central Appalachian or Western Allegheny ecoregion of eastern Kentucky may fall into this category.

TNDT = total number of diatom taxa identified

TNDT/500 = total number of diatom taxa encountered in a count of 500 individuals

Shannon Diversity

The mean Shannon diversity index is used in diatom assessments. It was chosen primarily because it is commonly used by aquatic biologists, so values will be more readily interpreted and compared with other literature values. Using this index, H′= 0 when only one species is present in the collection, and H′ is at a maximum when all individuals are evenly distributed among the S species.

$$H' = - \sum \frac{n_i}{N} \log_{10} \frac{n_i}{N}$$

where:
ni = number of individuals of species i
N = total number of individuals

H′ can also be expressed using natural logarithms (ln); however, log10 is used for historical comparison with other data collected across the state (Harker et al. 1979). Conversion factors for ln and log2 are available in Weber (1973).

Disadvantages

Diversity is affected by both the number of species in a sample and the distribution of individuals among those species (Klemm et al. 1992). Because species richness and evenness may vary independently, under certain conditions diversity values can be misleading. For example, streams with poor water quality due to toxic discharges may have very low taxa richness, but the individuals present may be very evenly distributed among those few taxa. This often results in high diversity values under stressed water quality conditions (KDOW unpublished data, Pontasch and Brusven 1988, Pontasch et al. 1989).

Archibald (1972) also warns of the dangers of using diatom diversity alone as an indicator of water quality because low diversity values may indicate either heavily polluted water or clean water. Hulbert (1971) goes so far as to state that species diversity is an ecologically meaningless concept and suggests its use be abandoned. Further, according to Perkins (1983), the assumption that individuals in more polluted environments should be less evenly distributed is speculative.

It can be argued that the evenness assumption of the diversity index may be ecologically unsound and that in natural communities individuals are not evenly distributed among species. This is supported by the work of Patrick et al. (1954) on the structure of natural diatom communities.

Advantages

Species diversity, despite the controversy surrounding it, has historically been used with success as an indicator of organic (sewage) pollution (Wilhm and Dorris 1966, Weber 1973, Cooper and Wilhm 1975). Bahls (1992) uses Shannon diversity because of its sensitivity to water quality changes, and Stevenson (1984) suggests that changes in species diversity, rather than the diversity value, may be useful indicators of changes in water quality. It is obvious that successful use of the diversity index depends upon careful application and interpretation. As a metric used in the DBI, it is only one of several metrics and will not be used alone as a water quality indicator.

Pollution Tolerance Index (PTI)

Several recent water quality indices based on pollution tolerance (or sensitivity) of diatom species have been proposed, including the saprobity index of Sladecek (1973), and diatom pollution tolerance indices developed by Lange-Bertalot (1979), Descy (1979), Leclercq and Maquet (1987) and Watanabe et al. (1988). What these indices have in common is that species are differentiated into groups and assigned values relating to pollution tolerance. Calculations involve estimates of the dominance of species and a "subjective assessment of the value of each species as an indicator" (Round 1991).

Ideally, a consensus will be reached on which diatom pollution tolerance index should be used, and a common list of pollution tolerance values will be developed. However, because a species may react differently across different ecoregions and may have variable sensitivities to different types of pollution (e.g., nutrients, metals, pH, salinity), universal values may not be appropriate.

The pollution tolerance index (PTI) used by the Kentucky Division of Water is most similar to that of Lange-Bertalot (1979) and resembles the Hilsenhoff Biotic Index for macroinvertebrates (Hilsenhoff, 1987). Lange-Bertalot distinguished three categories of diatoms according to their tolerance to increased pollution, with species assigned a value of 1 for most tolerant taxa (e.g., Nitzschia palea or Gomphonema parvulum) to 3 for relatively sensitive species. For the PTI, Lange-Bertalot's list has been adapted to four categories to differentiate a large moderately tolerant group of species (similar to his splitting of category 2 diatoms into 2a and 2b); the KDOW diatom pollution tolerance values range from one (most tolerant) to four (most sensitive).

Tolerance values for Kentucky diatoms were generated from a multitude of literature, including Lowe (1974), Patrick and Reimer (1966) Patrick and Reimer (1975), Patrick (1977), Lange-Bertalot (1979), Descy (1979), Sabater, et al. (1988), Bahls (1992), Mississippi Department of Environmental Quality (Stanley Rogers, pers. comm.) and Oklahoma Conservation Commission (Bob Lynch, pers. comm.). The extensive KDOW diatom database collected from 1977 to the present and data collections by the Kentucky Nature Preserves Commission (1979 - 1986) were also instrumental in the determination of tolerance values. The list of tolerance values presently in use (Appendix C-4) will be revised and updated as new autecological data is discovered; however, the tolerances of most common species are fairly well understood. Because the index is based on relative abundances, rare species will have little effect on the final index value.

If no autecological data is known, the species is given a PTI value of 0 and is not used in PTI index calculation.

The formula used to calculate PTI is:

$$PTI = \frac{\sum n_i \; x \; t_i}{N}$$

where:
ni = number of individuals in species i
ti = tolerance value of species i
N = total number of individuals

Siltation Index (%NNS)

The sum of the relative abundances of all Navicula (including Aneumastus, Cavinula, Chamaepinnularia, Cosmioneis, Craticula, Diadesmis, Fallacia, Fistulifera, Geissleria, Hippodonta, Kobayasia, Luticola, Lyrella, Mayamaia, Muellaria, Placoneis and Sellaphora), Nitzschia (including Psammodictyon and Tryblionella) and Surirella taxa reflects the degree of sedimentation at a reach. These three genera are motile, using their raphes to slide through sediment if they become covered. Their abundance expresses the frequency and severity of sedimentation. As sedimentation increases, the %NNS is expected to increase (Bahls et al. 1992).

%NNS = the sum of the relative abundances of all Navicula+Nitzschia+Surirella taxa

Fragilaria Group Richness (FGR)

The total number taxa represented in the sample from the genera Ctenophora, Fragilaria, Fragilariforma, Pseudostaurosira, Punctastriata, Stauroforma, Staurosira, Staurosirella, Tabularia and Synedra reflects high water quality. As water pollution increases, the FGR is expected to decrease.

FGR=Ctenophora+Fragilaria+Fragilariforma+Pseudostaursira+Punctastriata+ Stauroforma+Staurosira+Staurosirella+Synedra+Tabularia

Cymbella Group Richness (CGR)

The total number of taxa represented in the sample from the genera Cymbella, Cymbopleura, Encyonema, Encyonemopsis, Navicella, Pseudoencyonema and Reimeria reflects high water quality. As water pollution increases, the CGR is expected to decrease.

CGR=Cymbella+Cymbopleura+Encyonema+Encyonemopsis+Navicella+ Pseudoencyonema+Reimeria

6A.3.3 Future Diatom Bioassessment Index Metrics

Other metrics may be included in the new DBI as their usefulness is evaluated and more data are collected.

Total Number of All Algal Genera (TNG)

Total number of all algal genera (TNG) may provide a better estimate of diversity than taxa richness. As water pollution increases, TNG is expected to decrease (Barbour et al. 1999).

TNG = total number of benthic algal genera identified (non-diatom + diatom)

Total Number of Divisions Represented (TDiv)

Representatives from several divisions of algae are common from sites with good water quality. The number of divisions represented is reported as an indicator of diversity.

6A.3.4 Calculating the Diatom Bioassessment Index

Each metric is given a calculated score (range 0-100) based on the percent of the standard metric value (i.e., the 95th percentile or 5th percentile) of the entire database (impaired and reference). These percentile thresholds are used to eliminate outliers. The formulae for calculating DBI scores are shown in Table 6-2.

Table 6-2: Metric scoring formulae for the Diatom Bioassessment Index

Metric:	Formula:
TNDT	(TNDT/95th%ile) X 100
H′	(H′/95th%ile) X 100
PTI	(PTI/95th%ile) X 100
FGR	(FGR/95th%ile) X 100
CGR	(CGR/95th%ile) X 100
%NNS	(100 - %NNS)/(100 – 5th%ile) X 100

Metric scores with values greater than 100 receive a score of 100.0. The mean of the six DBI metrics is the final DBI score on a 0-100 scale.

Final DBI scoring criteria are currently being developed for each bioregion in Kentucky using an extensive KDOW database collected from 1986 through 2000. Scoring criteria for the bioregions can be obtained from the Ecological Support Section at (502) 564-3410.

6A.3.5 Other Data Evaluation Methods

Data assessment and interpretation are not restricted to the above metrics. Statistical analyses, when appropriate, may render useful insights into benthic algal data. However, biological data

are often not appropriate for parametric statistical analyses because the basic assumption of normal distribution is not always met. Statistically significant differences are not always ecologically significant (APHA 1998, Elliott 1983). Multivariate analyses are becoming popular in environmental assessments (Ter Braak 1988, Gauch 1982) and as user-friendly software packages become available, these methods will certainly be explored in the future. Such methods are especially useful in determining the tolerances of benthic algal species to specific environmental parameters (Agbeti 1991, del Giorgio et al. 1991, Dixit et al. 1992 and Sabater et al. 1988).

Chlorophyll a

Benthic chlorophyll a values are used as an estimate of algal biomass. Chlorophyll a values can be extremely variable because of the patchiness of benthic algal distribution; therefore, assessments are based on a mean of three or more replicate samples. The values for these replicates are recorded on the chlorophyll a bench sheet (Appendix B-6). These values are used to compare biomass accrual at the same station over time or between stations during the same sampling period. High chlorophyll a values may indicate nutrient enrichment, while low values may either indicate low nutrient availability, toxicity or low-light availability because of shading, sedimentation or high turbidity. Chlorophyll a values are used only in support of other analyses.

Ash-free Dry-weight (AFDW)

Benthic AFDW values are used as an estimate of total organic material accumulated on the artificial substrate. This organic material includes all living organisms (algae, bacteria, fungi, protozoa and macroinvertebrates) as well as non-living detritus. Ash-free dry-weight values have been used in conjunction with chlorophyll a as a means of determining the trophic status (autotrophic vs. heterotrophic) of streams. The Autotrophic Index (AI) is calculated as follows:

$$AI = \frac{AFDW\ (mg/m^2)}{Chlorophyll\ a\ (mg/m^2)}_1$$

High AI values (>200) indicate heterotrophic organisms dominate the community, and extremely high values indicate poor water quality (Weber 1973, Weitzel 1979, Matthews et al. 1980). This index should be used with discretion, as non-living and non-algal organic detritus can artificially inflate the AFDW value. An ash-free dry mass bench sheet should be completed whenever this method is used (Appendix B-7).

6B. Phytoplankton

6B.1 Introduction

Phytoplankton are important primary producers in lakes, impoundments, ponds, wetlands, low-gradient streams and backwater areas. Lotic phytoplankton samples may be collected by the KDOW from biological monitoring stations and from selected intensive survey sites. In addition, phytoplankton samples are collected from streams and reservoirs used as drinking water supplies if taste and odor problems occur. Samples are analyzed for chlorophyll a,

phytoplankton density (cells/ml) and/or phytoplankton community structure, depending upon the nature of the survey. Lentic phytoplankton samples may also be collected to assess water quality and recreation uses.

6B.2 Sampling

6B.2.1 Procedures

- Conduct a cursory visual assessment of the reach and record the results on the phytoplankton field data sheet (Refer to Appendix C-5).
- Record the following station information in the appropriate spaces at the top of the sheet:
    - KDOW station identification number
    - waterbody name
    - location of the station
    - collection date
    - time
    - county
    - river basin
    - purpose of the study (e.g., chlorophyll a, taste and odor, etc.)
    - name of investigator(s).

After sampling has been conducted, record the collection method used, analyses to be conducted (e.g., chlorophyll a, ash-free dry-mass, phytoplankton count), bloom characteristics (e.g., color of surface scum, any odor present, any surface sheen present, thickness of the bloom, etc.) and the surface bloom coverage (See Section 6B.IV.A) in the appropriate spaces on the phytoplankton field data sheet.

6B.2.2 Collection

- Collect samples in one-liter glass or plastic containers.
- Samples from wadeable streams are collected near mid-stream, approximately 0.5 m beneath the surface.
- Composite, euphotic zone samples are collected from unwadeable streams, wetlands, lakes and impoundments using a Kemmerer or Van Dorn sampler.
- Skim surface scums formed by algal blooms for additional algal identification when present.

6B.2.2.1 Chlorophyll a Sample Processing

For chlorophyll a analysis, filter 50 to 100 ml aliquots of the phytoplankton sample through 2.4 cm Whatman GF/C glass microfiber filters. Filter pressure should be maintained at 10 in of mercury or less so as not to collapse the algal walls. Fold filters in half, with algal cells folded to the inside. If filtering is performed in the field, place filters in light-excluding containers, such as empty 35 mm film containers, or wrap filters in aluminum foil and place them in a zip-lock bag. Preserve samples on ice in a cooler until they can be returned to the laboratory. As an alternative to filtering in the field, whole-water samples (1 L or greater) may be preserved on ice and filtered upon return to the laboratory. DO NOT add chemical preservatives to samples to be

analyzed for chlorophyll a. Samples should be filtered within 24 hours of collection and immediately frozen.

6B.2.3 Preservation

Samples for identification and enumeration are preserved with 3% to 4% buffered formalin, Lugols solution, 2% glutaraldehyde, M3 fixative or 6-3-1 preservative. Samples should be labeled with waterbody name, location, site number, date and name of collector. Labels should be written in pencil or indelible ink and attached firmly to the container.

6B.3 Analysis

6B.3.1 Field Assessment

A qualitative field determination of surface algal bloom coverage is recorded on the field data sheet using the following criteria:

Total – 100% (bank to bank)
Dense – 60% to 99%
Moderate – 30% to 59%
Sparse – 1% to 29%
Absent – 0%

6B.3.2 Primary Productivity

If primary productivity studies take place, the procedures followed will be those described in the latest edition of Standard Methods (APHA 2007).

6B.3.3 Chlorophyll a Analysis

Filtration, Extraction and Determination

Replicate samples will be analyzed using a Turner Designs Model 10 fluorometer, using U.S. EPA Method 445.0 (U.S. EPA 1992). The fluorometric method is more sensitive than the spectrophotometric method, and smaller sample volumes can be analyzed.

Filtration

Equipment:

- Hand-operated or electric pump at a vacuum pressure of less than 6 in of mercury.
- 500 ml filter flask
- 50 ml filter funnel
- 2.4 cm Whatman GF/C glass microfibre filters

Procedures:

Phytoplankton

Filter 50 to 100 ml of sample through a 2.4 cm Whatman GF/C filter.

Periphyton

- Scrape a glass microscope slide from periphytometer into a watch glass, rinsing the slide with distilled water.
- Filter the resulting solution, rinsing the watch glass with distilled water.
- Remove the filter from the funnel, fold it in half and place it in a light-excluding container (e.g., 35 mm film container).
- Repeat the process for each replicate sample.
- For natural substrate samples, scrape a known area of substrate into a jar and record the volume of sample.
- Filter a sub sample of 1 to10 ml, depending upon the amount of algae present in the sample. Mechanical blending may be necessary to homogenize the sample if large mats or filaments are present.
- For storage, freeze filters at 4oC for no longer than 30 days.

Extraction

Reagents:
90% aqueous acetone (mix 900 ml reagent grade acetone with 100 ml distilled water)

Equipment:
- Tissue grinder with Teflon pestle
- Glass-grinding vessel with a round bottom that matches the Teflon pestle
- Plastic centrifuge tubes, graduated

Procedure:

- Place one filter in the grinding vessel and add 5 ml of acetone solution and macerate the filter. Pour the resulting solution into a centrifuge tube.
- Rinse the grinder with another 5 ml of the acetone solution and transfer to the centrifuge tube. Steep samples in a dark refrigerator for at least 4 hours, but no more than 24 hours.
- Centrifuge at 1000 g for 20 minutes to clarify the extract.

Analysis

Reagents:
90% aqueous acetone

Equipment:

- Turner Designs model 10 Fluorometer equipped with chlorophyll kit filters and bulb (as
- recommended by the manufacturer)
- Glass cuvette – 13 mm

Procedure:

- Pour aliquot of sample after centrifuged into 13 mm cuvette. Read the concentration directly.
- Record concentration on the chlorophyll a bench sheet (Appendix B-6).
- If necessary, prepare 0.1 or 0.01 dilutions of the sample if the chlorophyll a extract sample is too concentrated and outside of the high range originally calibrated for the Turner Designs Model 10AU. Usually, phytoplankton samples from streams need no dilution. Phytoplankton from enriched streams or eutrophic lakes may need dilutions of 0.1, and periphyton samples may need to be diluted by a factor of 0.01.
- To calculate the final chlorophyll-a concentration, the following formula is used:

CS,c = CE,c (chlorophyll reading) X extract volume (L) X DF(dilution factor) / Sample volume (L)

Identification and Enumeration

- Phytoplankton is concentrated by settling, identified to the lowest possible taxon using appropriate taxonomic references, and if quantitative data are required, enumerated as follows:
- Place an aliquot of concentrated sample into a calibrated counting chamber such as a Sedgewick-Rafter cell or a sedimentation cylinder with a standardized bottom area (inverted microscope technique).
- Allow the sample to settle for an appropriate amount of time. For 1 ml of sample, allow a sedimentation time of one hour; for greater volumes of sample, increase the sedimentation time to ensure settling of all phytoplankton.
- Identify and enumerate phytoplankton with a binocular compound microscope or an inverted phase-contrast microscope equipped with a standard Whipple grid. Examine sedimentation chambers at 400X on an inverted microscope. Examine Sedgwick-Rafter counting chambers at 200X on a compound microscope. A list of taxonomic references follows the text.
- Count a minimum of 200 algal units for each sample. Record on the phytoplankton bench sheet (Appendix B-8). Report results for each taxon as a relative percentage of the total count (relative abundance). Algal density, biovolume, taxa richness, diversity, percent similarity or other metrics will be calculated when needed.

6B.4 Interpretation of Phytoplankton Data

6B.4.1 Chlorophyll a

Chlorophyll a data are compared with other sites in the same drainage, reference reach sites or intensive survey control sites. Chlorophyll a data will also be used for biological trend monitoring. Chlorophyll a is converted into Trophic State Index numbers by formulas determined by Carlson for lakes to add another layer of information to the waterbody. These trophic state index numbers allow the user to interpret the oligotrophic, mesotrophic or eutrophic nature of the water based on the chlorophyll a concentration.

6B.4.2 Community Structure

Phytoplankton abundance will be used to determine the degree and extent of algal blooms. Metrics such as taxa richness, relative abundance, community similarity, diversity or other appropriate measures of phytoplankton community health will be evaluated and compared to other sites in the drainage, reference or control sites, available historical data and appropriate scientific literature.

6B.4.3 Use Impairments

Algal blooms will be evaluated to determine use impairment for Aquatic Life, Recreational and Domestic Water Supply Uses, as well as the minimum criteria applicable to all surface waters. These uses are defined in Kentucky's surface water quality standards (401 KAR 5:031).

Aquatic Life

The autecology of dominant and abundant taxa and associations will be determined when information is available (Anonymous 1967, Bennett 1969, Collins and Weber 1978, Lowe 1974, Palmer 1977, Patrick 1977, Patrick et al. 1975, VanLandingham 1982, Whitford and Schumacher 1963). Presence of the following indicator groups will be recorded:

heterotrophic taxa – indicative of organically enriched conditions
halophilic taxa – indicative of high salinities
acidophilic taxa – indicative of low pH

Domestic Water Supply

Taste and odor algae are identified and enumerated when appropriate. Potentially toxic species will be identified and enumerated.

Recreational Use

Algae will be identified and enumerated. Algal blooms and bloom potential will be evaluated using chlorophyll a, cell density or both types of data.

SECTION 7 MACROINVERTEBRATES

7A. Macroinvertebrates

7A.1 Introduction

Since the early 1900s, aquatic organisms have been used extensively in water quality monitoring and impact assessment (Cairns and Pratt 1993). Macroinvertebrate assemblages have proven to be very useful in detecting even the subtlest changes in habitat and water quality. Today, macroinvertebrates are used throughout the world in water quality assessment, as environmental indicators of biological integrity, to describe water quality conditions or health of aquatic ecosystems and to identify causes of impairment.

The KDOW relies on the analyses of macroinvertebrate communities in use-attainment designations for sections 305b and 303d of the Clean Water Act (CWA) reporting, assessing specific effects of pollutants on water quality and for listing Exceptional Water designations to streams and rivers throughout the Commonwealth.

KDOW defines benthic macroinvertebrates as organisms large enough to be seen by the unaided eye, retained by a U.S. Standard No. 30 sieve (28 mesh/inch, 600 µm openings) and live at least part of their life cycle within or upon available substrates of a waterbody.

7A.2 Type of Collections

The Division of Water (KDOW) typically collects macroinvertebrates either to assess baseline water quality or to make a bioassessment of areas impacted by anthropogenic activities. In addition, a random survey approach is used to assess aquatic life use-support for streams in each watershed management unit (see Section II.E.). Standardized, semi-quantitative collections are made at all sampling locations; however, precise quantitative data may be collected on a case-by-case basis or if biological monitoring requires more rigorous statistical analyses. In general, collection methods used by KDOW are similar to those discussed in Lenat (1988), Plafkin et al. (1989), Klemm et al. (1990), Eaton and Lenat (1991), U.S. EPA (1997b) and Barbour et al. (1999).

7A.3 Sampling

Adherence to sample index periods is very important for accurate bioassessments using macroinvertebrates since biocriteria are calibrated for seasonality. For wadeable and non-wadeable streams, sampling must occur from May through September. For headwater streams (<5 mi2 in drainage area), sampling must take place from February through May. In some cases, sampling outside of these index periods is necessary to assess immediate impacts (e.g., chemical spills) or to adhere to specific guidelines set forth by the U.S. Fish and Wildlife Service or KDOW Standards and Specifications Section for trend monitoring and bioassessments in streams containing federally listed threatened or endangered species. For routine bioassessments or baseline data collection, samples collected outside of these index periods may be considered

unacceptable. Also, biological samples should not be collected during periods of excessively high or low flows or within two weeks of a scouring flow event.

7A.3.1 Sampling Methods

The methods described in this section are modifications of the single and multi-habitat approaches outlined by Barbour et al. (1999). The methods vary somewhat among stream sizes and stream gradients because of differences in habitat types present. KDOW must approve any deviation from the methods described below. For multiple-site intensive surveys of known impacts, and for comparison to regional reference conditions, the 1-m2 kick-net samples should be kept separate from other habitat collections to facilitate data interpretation, provide for better diagnosis of impairment and minimize the influence of uncontrolled variables.

Wadeable-Moderate/High-Gradient

For macroinvertebrates, KDOW generally considers wadeable streams as 3rd order or larger and usually greater than 5 square miles (13 km2) in drainage area. A summary of collection techniques required for these types of streams is shown in Table 7-1. Wadeable low-gradient streams, high-gradient headwater streams and large non-wadeable rivers require different methods and are discussed in Sections B, C and D, respectively. For routine and intensive surveys, it is important that both riffle/run samples and multi-habitat sampling are conducted (see below). As a quick screening tool, a single habitat (riffle/run) sample may be taken to detect obvious impairment from reference conditions. This simplified method may be employed for identifying impacted areas for TMDL development.

Riffle Sample

This technique samples the most important sub habitat (i.e., riffle) found in moderate to high-gradient streams. It requires using a 600 µm mesh, one meter wide net in moderate to fast current in areas with gravel to cobble substrate. Four (4) 0.25 m2 samples are taken from mid-riffle or the thalweg (path of deepest thread of water), dislodging benthos by vigorously disturbing 0.25 m2 (20 x 20 in.) in front of the net. Large rocks should be hand washed into the net. The contents of the net are then washed and all four samples are composited into a 600 µm mesh wash bucket. This sample must be kept separate from all other sub-habitat collections.

Multi-Habitat Sweep Sample

This sample involves sampling a variety of non-riffle habitats with the aid of an 800 x 900 µm mesh triangular or D-frame dip net. Each habitat is sampled in at least three (3) replicates, where possible.

Undercut banks/root mats

These are sampled by placing a large root wad into the triangular or D-frame dip net and shaken vigorously. The contents are removed from the dip net and placed into a mesh wash bucket.

Note: if undercut banks are present in both run and pool areas, each is sampled separately with three replicates.

Marginal emergent vegetation (exclusive of Justicia americana beds)

These are sampled by thrusting (i.e., "jabbing") the dip net into the vegetation for ca. 1 m, and then sweeping through the area to collect dislodged organisms. Material is then rinsed in the wash bucket and any sticks, leaves and vegetation are thoroughly washed and inspected before discarding.

Bedrock or slab-rock habitats

These are sampled by placing the edge of the dip net flush on the substrate, disturbing approximately 0.1 m2 of area to dislodge attached organisms. Material is emptied into a wash bucket.

Justicia americana (water willow) beds

These are sampled by working the net through a 1 m section in a jabbing motion. The material is then emptied into a wash-bucket and any J. americana stems are thoroughly washed, inspected and discarded.

Leaf Packs

Preferably collect from "conditioned" (i.e., not new-fall material) where possible; samples are taken from a variety of locations (i.e., riffles, runs and pools) and placed into the wash bucket. The material is thoroughly rinsed to dislodge organisms and then inspected and discarded.

Silt, sand, and fine gravel

Sieving

A U.S. No. 10 sieve is used to sort out larger invertebrates (e.g., mussels, burrowing mayflies, dragonfly larvae) from silt, sand and fine gravel by scooping the substrate to a depth of ca. 5 cm. A variety of collection sites are sampled in order to obtain three replicates in each substrate type (silt, sand and fine gravel).

Netting (optional)

A fine-mesh Surber sampler or a fine-mesh bag (300 □m) is used to collect sand and silt depositional areas by placing the net on the substrate and vigorously stirring the sediments in front of the net. An area of 0.1 m2 is sampled for each replicate, making sure, where possible, that replicates are taken from different depositional areas. The material is elutriated and sieved in a 300 m nitex sampler constructed of PVC or other fine-meshed net.

Aufwuchs sample

Small invertebrates associated with this habitat are obtained by washing a small amount of rocks, sticks, leaves, filamentous algae and moss into a medium-sized bucket half filled with water. The material is then elutriated and sieved with the nitex sampler.

Rock Picking

Invertebrates are picked from 15 rocks (large cobble-small boulder size; 5 each from riffle, run and pool). Selected rocks are washed in a bucket half filled with water, then carefully inspected to remove invertebrates with fine-tipped forceps.

Wood Sample

Pieces of submerged wood, ranging from roughly 3 to 6 meters (10 to 20 linear feet) and ranging from 5–15 cm (2–6 inches) in diameter, are individually rinsed into the wash-bucket. Pieces of wood are inspected for burrowers and crevice dwellers. Large diameter, well-aged logs should be inspected and handpicked with fine-tipped forceps.

Alternative Methods

The following are methods that may be used for special studies, as approved by KDOW.

Modified Traveling-Kick Method (TKM)

This is an adaptation of Hornig and Pollard's (1978) method. Three TKMs are taken on transects across the stream at mid-riffle in the thalweg. In the event that the TKMs cannot be taken on a transect, they will be collected diagonally in an upstream direction. A triangular or D-frame dip net is placed on small cobble sizes or smaller sized substrate and moved in an upstream direction disturbing an area in front of the dip net of 30 cm (1 ft). The distance covered in the Interior Plateau portion of the state is 1.5 m (5 ft) in 30 seconds, while the distance in the Eastern and Western Coal Fields and Jackson Purchase area is 3 m (10 ft) in one minute.

Surber Sampler

This sampler should be employed only when the riffles are shallow (20 cm or less) and the current is moderate. Three to four Surbers are taken on a transect across the stream at mid-riffle in the thalweg by methods outlined in Needham and Needham (1962) and Klemm et al. (1990). In the event that the Surbers cannot be taken on a transect, they will be collected diagonally in an upstream direction.

Modified Hester-Dendy Multiplate Sampler

Three multiplates, as described by Fullner (1971), are attached by wire or cord to a flotation device and allowed to float 25–30 cm (1 ft) below the surface. The multiplates are collected at

the end of three weeks during the summer in the Interior Plateau or six weeks in the spring or fall in the Interior Plateau and for six weeks in the rest of the state regardless of season.

Basket Sampler

These samplers, described by Mason et al. (1967), may be used in lieu of multiplates. The basket samplers should be filled with limestone rock of approximately 7.5 cm (3 in) in diameter or porcelain spheres of approximately the same diameter. Residence time is the same as the multiplates.

Drift Nets

These should be used in streams that have a current velocity of more than 0.5 m/sec (1.5 ft/sec). A minimum of two drift nets per station should be used. One net should be set 25 cm (10 in) from the surface of the river and one set 10 cm (4 in) below the surface of the water. The collection period should be from one to three hours. Data should be reported in number of organisms per 100 m^3 of flow. Additional information can be found in Klemm et al. (1990).

Dredges

These may be used in lentic-type environments in water depths greater than 1 m and collected on a transect. The number of samples collected on the transect will be dependent upon the goals of the study. All dredge samples are to be washed in a 600 μm mesh bucket.

Table 7-1. Summary of sampling methods for wadeable, moderate/high-gradient streams

Technique:	Sampling Device:	Habitat:	Replicates (composited):
1m2 Kicknet1	Kick Seine/Mesh Bucket	Riffle	4- 0.25m2
Sweep Sample	Dip net/Mesh Bucket	All Applicable	
Undercut Banks/Roots	"	"	3
Emergent Vegetation	"	"	3
Bedrock/Slabrock	"	"	3
Justicia beds	"	"	3
Leaf Packs	Dip net/Mesh Bucket	Riffle-Run-Pool	3
Silt,Sand, Fine Gravel		Margins	
Coarse Seive	US No. 10 Seive		3
Fine Mesh	300 □m nitex net		3
Aufwuchs Sample	300 □m nitex net	Riffle-Run-Pool	3
Rock Pick	Forceps	Riffle-Run-Pool	15 rocks (5-5-5)
Wood Sample	Mesh Bucket	Riffle-Run-Pool	3-6 linear m

1Sample contents kept separate from other habitats

Wadeable Low-Gradient

Low-gradient streams are defined as streams that have velocities less than 0.013 m/sec (0.5 ft/sec) and naturally lack riffle habitat. The most productive habitats of these streams are typically woody snags, undercut banks and aquatic vegetation. A proportional sampling technique is used in most streams of the western parts of the state, particularly in ecoregions 72, 73 and 74. The method follows, in part, the Mid-Atlantic Coastal Plain Streams Workgroup (MACS) protocol (US EPA 1997a), which is also described in Barbour et al. (1999). Essentially, the technique is considered "proportional sampling," in which some predetermined number of sample units (20 in this case) is allocated among distinct and productive mesohabitats in relation to their proportion found within a 100 m stream reach.

A sample unit is called a "jab" in which a D- or A-frame net is thrust into the targeted habitat in a jabbing motion for approximately 0.5 m and then swept with the net two or three times to collect the dislodged organisms. If a jab becomes heavily clogged with debris and sediment, discard and repeat the jab. All material is composited into a wash bucket for further processing. Large leaves and twigs can be washed, inspected and discarded to reduce the volume of the debris in the sample. Sand and sediment can be elutriated using a bucket and 600 µm sieve.

Headwater High-Gradient

In these small streams, riffle habitat predominates and is the primary targeted habitat. Benthic invertebrates should be collected in the spring index period (mid-February to May) as this period offers the highest potential for macroinvertebrate diversity and abundance in these headwater streams. A collection consists of a composited semi-quantitative riffle sample and a composited multi-habitat sample. These two sample types must be kept separate for a more effective diagnosis of impairment. A summary of these collection techniques is shown in Table 7-2.

Riffle Sample

For the semi-quantitative sample, invertebrates are collected from four 0.25 m2 quadrat kick net samples stratified within the thalweg of cobble-boulder riffle habitat. This habitat is targeted to ensure the highest species richness and abundance of macroinvertebrates. The thalweg of a riffle also guarantees the most flow permanence and substrate stability in these often intermittent streams. Two kick net samples are allocated to each of two distinct riffles that are separated by at least one pool or run. This is done to help reduce between-riffle variability. The four samples are composited into a 600 µm mesh wash bucket to yield a 1 m2 semi-quantitative sample. The composited sample is partially field processed using a U.S. #30 sieve (600 µm) and wash bucket. Large stones, leaves and sticks are individually rinsed and inspected for organisms and then discarded. Small stones and sediment are removed by elutriation using the wash bucket and U.S. #30 sieve.

Multi-Habitat Sample

The multi-habitat, qualitative sample consists of a composite of three leaf packs, three jabs in sticks/wood, three jabs in soft sediments, three jabs into undercut banks/submerged roots, handpicking of five small pool boulders and approximately two linear feet of large woody debris. These techniques were described above in the wadeable Moderate/High-Gradient section.

Table 7-2. Summary of sampling methods for headwater, moderate/high-gradient streams.

Technique:	Sampling Device:	Habitat:	Replicates (composited):
1m2 Kicknet[1]	Kick Seine/Mesh Bucket	Riffle	4-0.25m2
Sweep Sample	Dip net/Mesh Bucket	All Applicable	
Undercut Banks/Roots	Dip net/Mesh Bucket		3
Sticks/Wood			3
Leaf Packs	Dip net/Mesh Bucket	Riffle-Run-Pool	3
Silt,Sand, Fine Gravel	Dip net/Mesh Bucket	Margins	3
Rock Pick	Forceps	Pool	5 sm. Boulders
Wood Sample	Forceps/Mesh Bucket	Riffle-Run-Pool	2 linear m

[1]Sample contents kept separate from other habitats

Non-wadeable Streams

Samples taken from non-wadeable (boatable) streams will utilize methods similar to those outlined for wadeable low-gradient streams. A site will consist of a 300-meter stream reach. The proportional sampling technique, utilizing the 20 "jab" method will be augmented with dredge samples, rock picking and a wood sample. Three dredge samples, taken with a petite ponar grab sampler, are taken from each of three transects; one dredge sample is taken from mid-stream and one each from the right and left bank. The sample material is washed thoroughly in a 600 μm mesh wash bucket, then the macroinvertebrates are removed and stored in 70% ethanol. Where available, pick 15 rocks (large cobble-small boulder) and wash and pick 6 m of wood (5–15 cm in diameter).

7A.3.2 Probabilistic (Random) Bioassessments

Probabilistic biosurveys incorporate most of the sampling protocols for macroinvertebrate sample collection described above. The stream reach to be assessed is defined as 40X the wetted-width of the stream. In sampling each stream, the random design restricts the investigator to available habitat offered at each reach. If the stream is a high- to moderate-gradient stream and the particular reach contains no riffle habitat, the macroinvertebrates will be collected using the 20-jab proportional sampling technique.

This method is employed routinely in coastal and low-gradient streams where riffle habitat does not occur. Otherwise, a kick net is used to collect macroinvertebrates from the riffle habitat and an A- or D-frame dip net is employed to collect macroinvertebrates from other habitats that may be available, such as roots/undercut banks, woody debris, aquatic vegetation and leaf packs. Collected benthos are field processed and preserved in 95% ethyl alcohol. Benthic organisms collected from the riffle habitat are "picked" in the field and then preserved (those organisms collected from the riffle habitat are kept separate from those collected in other habitats). This method assures that all uncommon, large and rare invertebrates be identified and enumerated during the sample processing procedure in the laboratory.

Mechanical abrasion of specimens is minimal by sorting and collecting in the field, retaining to a high degree body parts that are often essential for positive generic or specific level identifications.

- First, large rocks, sticks and sizeable substrate are inspected for clinging specimens
- Hand-rinsed into a sieve bucket that has a US #30/600 μm sieve using stream water. To reduce fine material, the bucket is partially submersed and material is washed by swirling and agitating the material until most fines are eliminated.

- Field processing (picking) is accomplished by placing a small amount of material in white plastic or enamel-lined pans.
- Add a small amount of water is filled in the pan to facilitate recognition of macroinvertebrates to be collected.
- A pair of fine-tipped forceps (tweezers) and disposable pipette are tools used to collect specimens from the pan.
- The disposable pipette may be modified to increase the diameter of the opening to accommodate fast, mobile specimens that may otherwise be problematic and time consuming to collect using forceps (e.g. dyticids, Acarina and baetids).

The entire sample is collected from riffle habitat, whenever this is practicable. However, in many streams, especially the productive ones that are nutrient rich, a riffle community may contain 600+ specimens that may be dominated by just a few taxa (e.g. Cheumatopsyche, Hydropsyche, baetids or chironimids). These samples are sorted typically by genus and occasionally by family; a proportional number of each taxon is collected, maintaining the relative abundance of each taxon distributed throughout the habitat (typically at least 200+ specimens are collected). The qualitative samples collected in other habitats (e.g. roots/undercut banks, logs/woody debris, pool and run cobble and boulders, areas of aerated leaf mats, silt/sand and leaf packs) are field sorted and picked similar to the above riffle method.

Only a few relative number of each taxon are retained for laboratory final identification since this component of the stream sample is used to compliment the typically more sensitive, intolerant riffle community that is used in the calculation of the MBI.

Those low-gradient streams that may be found primarily in the Jackson Purchase and Lower Green River Basin (ecoregions 72, 73 and 74), employ the semi-quantitative 20-jab, one-half meter sweep proportional sample method. Macro-invertebrates are collected from the major

habitats of roots/undercut banks, leaf packs (in Kentucky's sample seasons these are primarily restricted to spring sample collections), otherwise aquatic vegetation and logs or woody material. Careful selection of the best available habitat is essential, with the primary consideration given to those substrates in areas of current that are aerated. These samples are then washed of fine sediments and picked in the manner previously described.

7A.3.3 TMDL Monitoring: Rapid Screening Protocols for Macroinvertebrates

For TMDL monitoring purposes, KDOW biologists will implement a single-targeted habitat sub sampling technique combined with family-level taxonomy. Expectation criteria for a 200 organism fixed-count sub sampling procedure have been calculated by rarifying reference reach data to predict metric values within individual bioregions.

As in routine sampling, macroinvertebrates will be collected in riffle habitats using a 600 μm mesh kick net. A composite of four 0.25 m2 samples will be taken at each site. Sample processing will initially consist of field cleaning and sieving to remove coarse and fine sediment, sticks, leaves, etc. A target sample volume should not exceed 1 pint. If the sample volume exceeds 1 pint, place the remainder into a second jar and label as 2 of 2, etc.

Sub sampling will be conducted in a manner as described in this chapter. However, a fixed 200+20% individual sample will be obtained.

7A.4 Sample Processing

7A.4.1 Sorting

- In the field, large sticks and leaves are washed, inspected for organisms and discarded.
- Rocks should be elutriated and hand washed with a bucket and 600 □m sieve.
- This process is repeated until a manageable amount of debris and organisms (relative to size of sample container) can be preserved for laboratory sorting.
- Samples may be partially field picked in the field using a white pan and fine-tipped forceps. Club soda may be used to retard the movement of aquatic organisms when field sorting. Approximately a tablespoon of material to be sorted is placed in a white pan with a small amount of water.
- The material is dispersed evenly throughout the pan, and all macroinvertebrates are removed and placed in 95% ethanol.

Sorting in the laboratory is done with the aid of a circline lamp or dissecting scope against a white background. Staining invertebrates with either rose idwes or phloxine B at a concentration of 100 g/L of ethanol may be done to aid in sorting operations. Sugar floatation techniques (Flannegan 1975) may also be employed to facilitate sorting. While entire picks are routinely done at KDOW, dividing all of the material into fourths may proportionally sub sample unmanageable amounts of organisms and debris. Randomly choose one of the quarters and remove all organisms. If at least 300 organisms are not obtained from the sub sample, pick additional quarters completely until the target number is reached.

After sub sampling, scan the remaining sample under low magnification for rare or large organisms not found in the original sub sample. In addition, certain taxa (e.g., chironomids, hydropsychid caddisflies) may be sub sampled (10%, 20% or 25%) using gridded pans and a random numbers table or following the protocol as detailed below.

7A.4.2 Macroinvertebrate Sub sampling Procedures

For macroinvertebrate collections, semi-quantitative samples (i.e., riffle kick net, or 20-jab low gradient technique) will be sub sampled to a fixed-count of 300+20% individuals. The Macroinvertebrate Bioassessment Index (MBI) criteria listed in Pond et al. (2003) has been modified to account for data generated by the following sub sampling procedure. The qualitative, multi-habitat sampling component will still be conducted, but will not be sub sampled.

- In the laboratory, first drain off preservative from sample.
- Re-fill the container with water and allow sample to sit for several minutes to hydrate the organisms. The drained contents of the sample will be spread evenly in a 22.8 x 34.3 cm glass sub sampling pan.
- Add just enough water to facilitate even spreading.
- A paper grid layout will fit under the pan and contain 24- 5.7 x 5.7 cm grids.
- A random numbers table will be used to generate which grids to pick organisms from.
- Remove four (4) randomly chosen grids (brownies) and place into separate pan.
- A target sub sample size will be 300+20%.

In some streams, densities of macroinvertebrates will be very high and further sub sampling is warranted. If after a cursory glance at the four-grid sub sample there appears to be greater than 300 individuals, place the contents of the four-grid sample into a second gridded pan. Randomly select grids as in the initial sub sample until 300+20% are picked. To get an idea of realized total abundance for interpretive purposes, record the number of grids picked to obtain the desired sample size.

Note: Large clumps of filamentous algae (Cladophora sp.) should be cut into small pieces with scissors to allow for easier spreading in the pan. Large leaf debris should also be removed, washed, inspected and discarded.

7A.4.3 Preservation

Samples are initially preserved in the field in 95% ethanol. Upon returning to the laboratory, all sorted samples are transferred to a fresh 70% ethanol solution.

7A.4.4 Labeling

While at the sampling location, all macroinvertebrate samples will receive a label. The label may be placed in the sample jar or written directly on some portion of the jar. The label will include the site number, if known, stream name, location, county, date sampled and the collector's initials. A permanent label will be placed in the collection jar either when the sample

is returned to the laboratory, or when it is identified. This label will include the site number, stream name, county, date sampled, latitude, longitude, mile point and collector names.

7A.4.5 Taxonomy

All taxonomic identifications are made to the lowest practical level, using the most current taxonomic references available. A partial list of taxonomic references used by KDOW is found at the end of this chapter. A current master taxa list is found in Appendix C-1.

7A.5 Data Analysis

The use of multiple community attributes to assess in stream biological impairment has become widely accepted. This approach was first developed by Karr (1981) for midwest fish communities and now has been refined regionally and used throughout the United States. Karr's methods involved using a variety of community attributes, referred to as "metrics," to assess the condition of biological communities. Each metric is expected to contribute pertinent ecological information about the community under study. Examples of the application of the metric approach to aquatic macroinvertebrate communities can be found in Nuzzo (1986), Ohio Environmental Protection Agency (1987), Bode (1988), Shackleford (1988), Plafkin et al. (1989) and Barbour et al. (1999).

7A.5.1 Core Metrics

The KDOW's metric selection process uses statistical properties of redundancy and sensitivity to evaluate the power of metrics that can discriminate between impaired and unimpaired sites. Metric scoring criteria are established using percentiles of the reference and non-reference data distribution. The following is a list and explanation of each metric commonly used by KDOW. Note that metric combinations and scoring criteria may vary among ecoregions and stream sizes. Many of these metrics (e.g., mHBI, % composition metrics) require quantitative or semi-quantitative sampling (i.e., calculated from riffle kick net sample or 20-jab technique).

Taxa Richness

This refers to the total number of distinct taxa present in the composited sample (both semi-quantitative and qualitative samples combined). In general, increasing taxa richness reflects increasing water quality, habitat diversity and/or habitat suitability.

Ephemeroptera, Plecoptera, Trichoptera Richness (EPT)

This is the total number of distinct taxa (both semi-quantitative and qualitative samples combined) within the generally pollution-sensitive insect orders of Ephemeroptera, Plecoptera and Trichoptera found in the composited sample. This index value will usually increase with increasing water quality, habitat diversity and/or habitat suitability.

Modified Hilsenhoff Biotic Index (mHBI)

The HBI was developed to summarize the overall pollution tolerance of a benthic arthropod community with a single value (Klemm et al. 1990). Hilsenhoff (1977), using a range of 0-5, originally developed the index for Wisconsin riffle/run streams experiencing organic pollution. Hilsenhoff (1982, 1987) later refined the index, expanding the scale to range from 0 to 10. Plafkin et al. (1989) modified the index to include non-arthropod benthic macroinvertebrates. Hilsenhoff (1987) developed tolerance values for a variety of macroinvertebrates from Wisconsin, and Plafkin et al. (1989) added additional tolerance values.

However, KDOW uses tolerance values developed by North Carolina Division of Environmental Management (NCDEM) (Lenat 1993) as well as values developed from KDOW data. These HBI values have been regionally modified for streams of the southeastern United States. Both Hilsenhoff (1988) and NCDEM have developed seasonal correction factors for the HBI. Several states, including Kentucky, have used the mHBI to assess impacts other than organic enrichment and found the mHBI to be a valuable metric. An increasing mHBI value indicates decreasing water quality.

The formula for the HBI is as follows:

$$HBI_m = \frac{\sum n_i \times a_i}{N}$$

where:
ni = number of individuals within a species (maximum of 25),
ai = tolerance value of the species,
N = total number of organisms in the sample.

Modified Percent EPT Abundance (m%EPT)

This metric measures the abundance of the generally pollution-sensitive insect orders of Ephemeroptera, Plecoptera and Trichoptera. The relatively tolerant and ubiquitous caddisfly Cheumatopsyhce is excluded from the calculation. Increasing values indicate increasing water quality and/or habitat conditions.

Percent Ephemeroptera (%Ephem)

The relative abundance of mayflies is calculated to show impacts of metals and high conductivity associated with mining and oil well impacts. Ephemeroptera abundance normally declines in the presence of brine and metal contamination.

Percent Chironomidae+Oligochaeta (%Chir+%Olig)

This metric measures the relative abundance of these generally pollution tolerant organisms. Increasing abundance of these groups suggests decreasing water quality conditions.

Percent Primary Clingers (%Clingers)

This habit metric measures the relative abundance of those organisms that need hard, silt-free substrates to "cling" to. Merritt and Cummins (1996) and Barbour et al. (1999) list habits for most insect genera. Habit information for non-insect taxa can be determined from Pennak (1989), Thorp and Covich (2001) and Barbour et al. (1999).

7A.5.2 Supplemental Metrics

These metrics may be used in special situations such as intensive surveys and nonpoint source studies that evaluate a particular impact. Functional feeding group abundances may also be calculated to provide insight into food/energy relationships among sites.

Jaccard Coefficient of Community Similarity

This similarity index measures the degree of taxonomic similarity between two stations in terms of taxon presence or absence. Coefficient values, ranging from 0 to 1.0, increase as the similarity with the reference station increases. The formula is as follows:

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

where:
a = number of taxa in sample A but not B
b = number of taxa in sample B but not A
c = number of taxa common to both samples
Sample A = reference station
Sample B = station of comparison

Percent Community Similarity (PSc)

The PSc index, discussed by Whittaker (1952), was used by Whittaker and Fairbanks (1958) to compare planktonic copepod communities. It is a good index in bioassessments because it shows community similarities based on relative abundance giving more weight to dominant taxa than rare ones. Percent community similarity values range from 0 (no similarity) to 100 percent. The formula for calculating PSc is:

$$PSc = 100-0.5\Sigma(a-b) = \Sigma\min(a,b)$$

where:
a = percentage of taxa a in sample a
b = percentage of taxa b in sample b

Percent Contribution of Dominant Taxa (PCD5)

This simple measure of redundancy and evenness adds the relative abundance percentages of the five dominant taxa. Highly redundant communities (i.e., communities highly dominated by a few taxa) may reflect a degraded condition.

EPT/Chironomidae (Ratio of EPT to Chironomidae Abundances)

This metric uses the ratio of these indicator groups as a measure of community balance. Communities with a good biotic condition would be expected to have a substantial representation of EPT taxa. Skewed populations having a disproportionate number of generally tolerant chironomids relative to the more sensitive insect groups may indicate environmental stress (Ferrington 1987).

Dominants in Common, Five (DIC5)

This metric, developed by Shackleford (1988), measures the similarity of a reference station and a station of comparison based on the five most abundant taxa at each station. The DIC5 provides a measurement of substitution between the reference community and the downstream station.

Dominants in Common, Ten (DIC10)

This metric, also developed by Shackleford (1988), is the same as the DIC5 metric, but is based on the ten most abundant taxa at each station.

Percentage of Cricotopus+Chironomus Abundance to Total Chironomidae (Cr+Ch /Chironomidae)

This measures the abundance of the pollution-tolerant genera Cricotopus and Chironomus to the total abundance of the family Chironomidae.

Additional information on various metrics may be found in Plafkin et al. (1989), Klemm et al. (1990), Resh and Jackson (1993) and Barbour et al. (1992, 1999). The functional feeding group concept is discussed by Cummins (1973), and genus-level functional feeding group designations for aquatic insects are provided by Merritt and Cummins (1996).

7A.6 Macroinvertebrate Bioassessment Index

Macroinvertebrate data analysis for wadeable or headwater streams is accomplished by using the multimetric approach. Metric criteria are based on reference reach data and may vary from region to region and with stream size (i.e., wadeable or headwater). Core metrics in wadeable streams are Taxa Richness, modified Hilsenhoff Biotic Index, EPT Richness, modified %EPT Abundance and %Chironomidae+Oligochaeta Abundance and % Clinger Abundance.

Additionally, supplemental metrics are used depending on type of impact, stream size, sub-ecoregion, etc. *Each metric is given a calculated score (range 0–100) based on the percent of the standard metric value (i.e., the 95th percentile or 5th percentile). These percentile thresholds are used to eliminate outliers. The formulae for calculating MBI scores are shown in Table 7–3. If an individual metric scores greater than 100, it will automatically be given a score of 100. The individual metric scores are summed and then averaged to produce a Macroinvertebrate Bioassessment Index (MBI) value.

Thresholds are established to assign narrative water-quality ranking of Excellent, Good, Fair, Poor and Very Poor. A separate document detailing the metric selection and evaluation process, bioassessment interpretation and 95th %ile and 5th %ile values has been developed (Pond et al. 2003). This document can be obtained off the Division of Water website at www.water.ky.gov or by contacting the at (502) 564-3410.

An Excel spreadsheet template for calculating a MBI for the standard full, riffle and selective pick collection methodology can also be obtained through the Division of Water. When using this template, two things must be kept in mind: 1) make sure that all metric scores calculated above 100 are given a score of 100 and 2) make sure that when "pulling down" formulae to calculate multiple metric scores that the formulae do not change during the pull down.

In probabilistic (random) sampling, the MBI may not be representative in cases when atypical habitat is sampled. For example, in streams where riffle-run habitat typically occurs but by chance this habitat did not occur in the randomly chosen stream reach, the sampling techniques and the habitat assessment must be considered in making the final aquatic life use determination. Otherwise, the evaluation is made solely using the MBI.

Table 7-3. Examples of metric scoring formulae for the standard Macroinvertebrate Bioassessment Index.

Metric:	Formula:
TR	$\frac{TR}{95th\%ile} X 100$
EPT	$\frac{EPT}{95th\%ile} X 100$
MHBI	$\frac{10 - mHBI}{10 - 5th\%ile} X 100$
m% EPT	$\frac{m\%EPT}{95th\%ile} X 100$
%Clingers	$\frac{\%Clingers}{95th\%ile} X 100$
% Chir+Olig	$\frac{100 - \%Chir + Olig}{100 - 5th\%ile} X 100$

Table 7-4. Calculation of the 300-fixed, rarefied MBI for riffle plus combined select pick or 20-jab				
Metric:	95th or 5th %ile:	Formula:	Example for Kinniconick Creek:	Metric Score:
Genus TR	69	$\frac{TR}{95th\%ile} X100$	$\frac{50}{69} X100$	72.46
Genus EPT	28	$\frac{EPT}{95th\%ile} X100$	$\frac{18}{28} X100$	64.28
MHBI	3.11	$\frac{10 - mHBI}{10 - 5th\%ile} X100$	$\frac{10 - 4.49}{10 - 3.11} X100$	80.03
m%EPT	74	$\frac{m\%EPT}{95th\%ile} X100$	$\frac{79.69}{74} X100$	100.0
%Chir+Olig	1.0	$\frac{100 - \%Chir + Olig}{100 - 5th\%ile} X100$	$\frac{100 - 5.04}{100 - 1.0} X100$	95.92
%Clingers	74	$\frac{\%Clingers}{95th\%ile} X100$	$\frac{60.45}{74} X100$	81.69
			Average Score =	82.39

The Macroinvertebrate Bioassessment Index has been modified to analyze 300-pick, riffle and select pick or 20-jab samples and riffle-only samples identified to the family level. Formulae for calculating metric scores for these modified methodologies are given in Table 7-4 and 7-5, respectively. A set of EDAS queries for calculating metrics and Excel spreadsheets for calculating MBIs from these modified methods are available by contacting the Division of Water.

Table 7-5. Family-level, 200-fixed rarefied riffle-only MBI				
Metric:	95th or 5th %ile:	Formula	Example for UT Flat Creek:	Metric Score:
Family TR	27	$\frac{TR}{95th\%ile} X100$	$\frac{20}{27} X100$	74.1
Family EPT	17	$\frac{EPT}{95th\%ile} X100$	$\frac{10}{17} X100$	58.82
MHBI	2.18	$\frac{10 - mHBI}{10 - 5th\%ile} X100$	$\frac{10 - 4.59}{10 - 2.18} X100$	69.18
m%EPT	86.9	$\frac{m\%EPT}{95th\%ile} X100$	$\frac{62.2}{86.9} X100$	71.57
%Ephem	66.5	$\frac{\%Ephem}{95th\%ile} X100$	$\frac{8.93}{66.5} X100$	13.43
%Chir+Olig	0.68	$\frac{100 - \%Chir + Olig}{100 - 5th\%ile} X100$	$\frac{100 - 4.47}{100 - 0.68} X100$	92.31
%Clingers	75.5	$\frac{\%Clingers}{95th\%ile} X100$	$\frac{25.1}{75.5} X100$	33.01
			Average Score =	58.9

7B. Mussels

7B.1 Introduction

KDOW personnel do not routinely sample freshwater mussels as part of the assessment process. The information gathered during these surveys is used as supplemental information only. Mussel presence may help support a Special Use Water designation and is also used by the Water Quality Certification Section to clarify areas of historical mussel communities and help determine if a new survey should be required when permitting.

Mussels are biologically important as members of the aquatic food web. They serve as food for many aquatic mammals like muskrat and raccoon, as well as fish like the freshwater drum and suckers. They also feed on many small organisms floating in the water such as detritus and microscopic plants and animals (Cicerello and Schuster 2003). Mussels are ecologically important since they improve water quality through filtration and are sensitive to many causes of toxicity (National Native Mussel Conservation Committee [NNMCC] 1998). There have been more than 100 native freshwater mussel species described in Kentucky since 1818 (Cicerello et al. 1991).

Today 55% of the North American mussel fauna are considered extinct or imperiled (Williams et al. 1993). Twenty species have been extirpated or have become extinct from the 104 originally found in Kentucky (Cicerello and Schuster 2003). One goal of the National Strategy for the Conservation of Native Freshwater Mussels is to increase the knowledge of status and trends of native populations through increased sampling in areas needing basic or updated information (NNMCC 1998).

7B.2 Sampling Procedures

7B.2.1 Site Selection

A mussel survey is performed on only a few selected sites every sample year. These sites are generally selected from the KDOW mussel database as historic locations that have not recently been sampled. Sites may also be selected in areas that have been identified during previous sampling years as having a mussel community, but lacking a survey event in the database. One or two large historic beds may be repeatedly sampled every basin cycle to help determine trends in mussel communities (i.e. Green River at Munfordville, Licking River at Butler, Rolling Fork at New Haven).

Sites that have not been selected for an intensive mussel survey, but have mussels present, should have species observed during normal macroinvertebrate sampling recorded and voucher shells returned to KDOW. Surveys will not be limited to 4th order and higher streams since small tributaries may not suffer from as much pollution as the larger streams (Taylor 1980). Surveys are performed from March through October during periods of low water to facilitate location of shells on the stream floor (Payne et al.1993).

7B.2.2 Methodology

Once a site has been selected, a stream reach will be defined that will be used for all biological sampling purposes. Mussel sites will be assessed using all biological methods available with staff on hand including fish, macroinvertebrates, algae and tissue. Standard methods for the other assemblages are outlined in other portions of this manual.
A stream data sheet (Appendix B) will be completed as per the methods described in Section 5. This is important since habitat destruction is the number one cause of mussel decline (Williams et al. 1993). Header information will be filled out for the Unionid Sorting Sheet (Appendix D-2).

Once the stream reach has been selected, a timed search will begin at the downstream edge of the reach working upstream. Timed searches will be used to allow for evaluation of species richness, since they are capable of detecting sparse populations and detect more species per hour than quadrat searches (Vaughn et al. 1997, Strayer et al. 1997). Participants will walk through the shallow water and visually search the bottom of the stream looking for live mussels or mussel shells. View-buckets may be used to help locate live mussels. All shells will be collected in plastic bags. If a live mussel is encountered, the searcher will remove the mussel and identify it to species.

If the searcher is not familiar with mussels, then someone who is familiar with mussel taxonomy should be consulted. In the event that the taxonomist is uncertain, two additional taxonomists should be consulted.

The mussel will be recorded on the Unionid Sorting Sheet as the species agreed upon by two or more of the taxonomists. The mussel will then be replaced in the location from which it was removed to reduce air exposure that might lead to mortality (Waller et al. 1997).

Searching will continue to the end of the stream reach. At the end of the reach searchers will turn around and continue searching on the shoreline, working downstream for shells. Muskrat middens should be searched since they may contain small individuals overlooked in the live search (Dunn 1999). This will continue with all shells collected and placed in plastic bags. After a total of five person-hours, shells will be sorted along the shore with representative shells of every species taken back to KDOW for vouchering. Those of questionable identity will have all individual shells returned. Shells not kept at KDOW due to space constraints will be offered to KSNPC, EKU and other repositories in the area.

7B.2.2.1 Alternative Method

Nylon rope ladder - This method was developed specifically to observe mussel populations or individual mussels in wadeable streams. It is used for long-term monitoring purposes. A rope ladder is constructed to form successive square-meter blocks, using ½ inch nylon rope and ¼ inch metal bars as rungs. Concrete rebar (½ x 36 in.) stakes driven downward into the stream banks serve as a permanent reference and attachment site for the ladder. The square meter blocks are numbered from left to right facing upstream. Mussel(s) located within each block are charted on paper to represent their location in the streambed.

SECTION 8 FISH COMMUNITY STRUCTURE

8.1 Introduction

The evaluation of fish community structure is an important component of biological monitoring, intensive surveys, Exceptional Water determinations and reference reach surveys providing reliable assessments for Section 305b of the Clean Water Act (CWA). A multi-metric index, the Kentucky Index of Biotic Integrity (KIBI), is used to assess stream health by examining fish community structure.

Advantages of using fish as biological indicators include their 1) widespread distribution from small streams to all but the most polluted waters; 2) utilization of a variety of trophic levels; 3) stable populations during summer months; and 4) the availability of extensive life history information (Karr et al. 1986). The methods used for collecting, analyzing and reporting of fish community structure data are outlined in this section.

8.2 Sampling Methods

Standardization of methods is essential since it will allow streams to be assessed and compared, establishing trends and long-term monitoring records (Bonar and Hubert 2001). To ensure collection of standardized fish community data, stream size (i.e., drainage area) has been used to designate streams into two classes, headwater and wadeable, and a set methodology is outlined for each classification. Headwater streams are streams with a drainage area < 5 mi.2. Wadeable streams are streams with a drainage area greater > 10 mi.2. However, streams with the drainages of 5-10 mi.2 fall within a "gray" area of stream classification and best professional judgment should be used to determine if the stream is headwater or wadeable.

The sampling index period is mid-March through October. Prior to sampling, the field crew should research the distributional records and familiarize themselves with the possible ichthyofauna for a particular basin and compile a species list for that basin. Special awareness and precautions should be made for the possibility of encountering any Federal Threatened/Endangered species.

8.2.1 Headwaters Streams

- The sample reach must range between 100-125 meters in length, which should consist of riffles, runs and pools, if they are present.
- The reach must not be associated within the immediate area (<100 meters) of major tributary confluences or human structural influences, such as bridges, road crossings (fords), low head dams or any other similar structure, unless the purpose of obtaining the fish community data is related to these influences.
- Sampling in headwater streams must consist of using a backpack electrofisher unit working in an upstream manner (collectors must be familiar with and follow all safety procedures as suggested by the manufacturer). The electrofishing duration within the sample reach should be a minimum of 600 "shocking" seconds and a maximum of 1000 "shocking" seconds.

- The lower and upper ends of the reach should be associated with a natural in stream barrier such as a riffle.
- The sampling crew should consist of a minimum of two members with at least one member having prior electrofishing experience.
- The crew should work upstream shocking in a side-to-side/bank-to-bank sweeping technique. The crew collects the stunned fish with dip nets and places them into a live well or bucket for preservation or identification after the reach has been sampled.
- One pass of the reach is sampled from the downstream end to the upstream end, with all recognizable habitats thoroughly sampled, following the sweeping technique (Barbour et al. 1999).
- If deemed necessary, a seine can be used to sample pool habitat more efficiently.

The experience of the crew and their ability to see and net the fish improves the effectiveness of sampling the reach. Polarized sunglasses are recommended, since they will cut down on the glare of the water. In addition, features such as water clarity, flow, depth and time of day need to be considered to obtain optimal success in sampling. If these conditions are not adequate or practical, sampling needs to be postponed until an efficient sampling effort can be obtained.

8.2.2 Wadeable Streams

Wadeable streams are larger bodies of water and provide more variability of habitat than headwater streams so the reach sampled will be determined by a greater time designation within the given length designation. Because of the habitat variability, a combination of seining and electrofishing is used, since both methods have advantages and disadvantages in different habitat types and species groups. The combination of seining and electrofishing yields better results than one technique independently (Onorato et al. 1998; Yoder and Smith 1999). The collectors should be aware of the advantages and shortcomings of each technique. KDOW has observed electrofishing to be more effective in streams that have numerous boulders, undercut banks and woody debris. KDOW also has observed that electrofishing tends to be biased toward catostomid and centrarchid members while not fully representing the schools of cyprinids (i.e., Lythrurus and Notropis spp.) in large pools. However, cyprinids can be effectively sampled with a seine in large pool habitats to yield a better representation of their presence in the community (Onorato et al. 1998).

- The reach length should be between 100 and 200 meters.
- Sample reach length will be recorded on the fish data sheet.
- The sample reach should consist of at least two riffles, runs and pools each. In cases where two riffles, runs, and pools cannot be sampled, either one riffle, run and pool is sampled or the recommended reach length of the stream is sampled.
- Sampling must done using a seine (at least 3.4×1.8 m with 0.3 cm mesh) and an electrofisher (any suitable generator-powered electrofisher may be used).
- A seine is used for approximately 30-90 minutes from start to finish.
- The electrofisher (backpack, tote barge or similar unit) is typically then used in areas not efficiently sampled with the seine (e.g., root masses, undercut banks, rock slabs, boulder/cobble substrates, fallen trees, etc).

- The electrofisher should be used for at least 600 "shocking" seconds to a maximum of 2000 "shocking" seconds of effort.
- Also, like headwater streams, the electrofishing method should be done in a sweeping manner and the reach must not be associated within the immediate area (<100 meters) of major tributary confluences or human structural influences.

Best professional judgment by the collector is used to determine the appropriate gear and effort for a particular habitat, but a combination of the two methods (seine and electrofisher) must be used. Typically a reach is thoroughly sampled when no new species are being collected and all habitats have been thoroughly sampled.

Documentation of methodology is required to help provide insight into the results of the sampling effort, particularly if only one technique is used. Again the efficiency of sampling depends on the experience of the crew and their ability to see and net the fish. Polarized sunglasses are recommended. The water clarity, flow and depth and the time of day need to be considered to obtain optimal success in sampling. If these conditions are not adequate or practical, sampling needs to be postponed until an efficient sampling effort can be obtained.

8.2.3 Preservation

Fish collections are preserved in the field with a 10%-15% buffered formalin solution and placed in Nalogene fish jars. Nalogene fish jars should be inspected for soundness prior to the collection trip to ensure that formalin does not leak into the transport vehicle. Large specimens are to be identified in the field, recorded and released, unless the specimen(s) represent a significant ichthyological find (e.g., state or drainage record), then they are to be preserved as voucher specimens. Easily identified fish that are collected in large numbers (i.e., Campostoma spp.) are also recorded in the field and released.

Photographs of large specimens and vouchers of all released specimens need to be made for verification. If possible, at least five specimens of each species released should be kept as vouchers from the sample event. If a species or genus is viewed but not collected, and if positively identified, these records should be noted (i.e., Hypentelium nigricans, Micropterus spp. or Lepomis spp.).

8.3 Laboratory Processing

To process fish in the laboratory, follow these procedures:

- Fish need to be stored in the formalin solution for at least 2 weeks (larger fish should be fixed for at least 3–4 weeks unless injected with a formalin solution in the field).
- Samples are then triple rinsed and soaked for 1-3 days in tap water.
- The fish are then transferred into 70% ethanol for long-term preservation and storage in glass fish jars.
- Identify and count fish using all available taxonomic keys and distribution records. In a given sampling season, 10% of the samples collected should be re-identified by another qualified fish taxonomist for the purpose of quality assurance/quality control.

- Independent taxonomic verifications of selected species are obtained as needed from recognized experts (e.g., Ecological Support Section KDOW or Kentucky State Nature Preserves Commission).
- Species and number of individuals collected are recorded on data sheets (Appendix D-1) and used for evaluating species composition, relative abundance, species richness, presence/absence of indicator species and calculation of the Kentucky Index of Biotic Integrity (KIBI).
- The occurrence of species that are listed as rare, endangered or threatened by federal or state agencies is also documented, and the appropriate agencies are notified with the Threatened/Endangered Species Report Form (Appendix D-2).
- Other characteristics of fish communities that are evaluated include the presence of hybrids, size/age distribution of populations, incidence of disease and occurrence of parasites. These and other non-routine assessments of fish communities are included in specific study plans.

8.4 Kentucky Index of Biotic Integrity

8.4.1 Introduction

The Index of Biotic Integrity (IBI) as described by Karr (1981) was used to assess the fish community structure and biotic integrity of Midwestern streams. This IBI was composed of 12 equally weighted metrics that were grouped into three general categories: Species Richness and Composition (Category I), Trophic Composition (Category II) and Fish Abundance and Condition (Category III). Each metric was assigned a 5, 3 or 1 value depending upon whether the obtained value strongly approximates the expected value (5), somewhat approximates the expected value (3) or does not approximate the expected value (1).

The individual metric scores were summed and a total IBI score ranging from 12-60 was achieved. Metrics in Category I often vary with region and stream size, while less variation was usually found among those in Categories II and III (Karr et al. 1986). Five classifications based on total IBI scores were assigned by Karr (1981) to describe the quality of the fish community at each site. Scores that fall between categories were evaluated based on professional experience.

While the practice of using a multimetric fish index to assess stream health has persisted, numerous modifications and testing of the original IBI have been made to provide more accuracy and precision within different regions (Ohio EPA 1987, Barbour et al. 1999, Hughes and Oberdorff 1999, Maret 1999, Smogor and Angermeier 2001), ecosystems (Minns et al. 1994, Emery et al. 2003) and fauna (Lenat 1993, Deshon 1995, Barbour et al. 1996).

In the early 1990s KDOW started a Reference Reach Program to develop biocriteria and to identify trends in the Commonwealth's lotic systems. Therefore, Karr's IBI was modified by KDOW (1997) and followed the framework of Karr (1981) and Karr et al. (1986). However, no metric evaluation process was performed, criteria were not established for all ecoregions and scoring of individual metrics was a visual interpretation of a point on a graph, which lead to inconsistencies in scoring by users. Therefore, following the approaches detailed by Barbour et al. (1999), Simon (1999), McCormick et al. (2001) and Smogor and Angermeier (2001), the

Kentucky Index of Biotic Integrity (KIBI) was developed (Compton et al. 2003). The objectives of the new index were to provide reliable and consistent analysis and application among users and to cover all regions and wadeable streams in a uniform approach. Notable changes in the IBI were the metrics selected and the scoring criteria scale.

8.4.2 Metrics

Fish assemblages in the state show strong correlation with ecoregions, basins, physiographic regions and stream size. Development of criteria for an IBI must be region and stream-size specific to correspond with the differences within the ecoregion/basin framework (Fausch et al. 1984 and Angermeier et al. 2000). Therefore, several candidate metrics were tested and validated for their sensitivity and variation among each region and stream size following Smogor and Angermeier (1999) and McCormick et al. (2001). Metrics were selected to demonstrate attributes of a fish community that show responsiveness to disturbances, predictability and uniformity throughout the state. A list of candidate metrics is provided in Table 8-1.

After testing the candidate metrics, seven metrics were selected to structure the multimetric KIBI (Table 8-2). The metric selection process uses statistical properties of redundancy and sensitivity to evaluate the discriminating power of a metric between impaired and relatively unimpaired sites (Reference sites). The following is a list and explanation of each metric used in the Kentucky IBI. A master list of Kentucky fishes with their trophic and tolerance classifications is provided in Appendix D-3. This list has been compiled using scientific literature, historic data, consultation with fisheries biologists and professional experience (Ohio EPA 1987, Etnier and Starnes 1993, KDOW 1997, Goldstein and Simon 1999, McCormick et al. 2001). Any necessary modifications to the list will be made as additional information becomes available.

The seven metrics retained for the KIBI were Native Richness (NAT); Darter, Madtom and Sculpin Richness (DMS); Intolerant Richness (INT); Simple Lithophilic Spawners (SL); Relative Abundance of Insectivorous Individuals, excluding Tolerant Individuals (%INSCT); Relative Abundance of Tolerant Individuals (%TOL); and Relative Abundance of Facultative Headwater Individuals (%FHW). NAT was used only in wadeable streams and was replaced by %FHW in headwater streams. Environmental parameters that were significantly correlated ($r>0.2$, $p<0.01$) to metrics are noted.

Native Species Richness (NAT)

This is the total number of native species present in a sample. Non-native species were excluded since they were a direct indication of anthropogenic impairment. This is a modification from Karr's (1981) total number of species and was used in several other indices (Robinson and Minshall 1992, Barbour et al. 1999, Smogor and Angermeier, 1999). NAT was found to have poor sensitivity in headwater streams and will be used only in wadeable streams. A moderate amount of impairment (e.g., increased nutrients or increased temperature) slightly alters the typical habitat, which allows for the presence of species that usually do not inhabit small streams (e.g., Lepomis spp.). A replacement metric (%FHW) for headwater streams is described below. NAT correlated positively with the following USEPA Rapid Bioassessment Protocol habitat parameters: epifaunal substrate, riparian vegetative zone width, channel alteration, pool

variability, pool substrate characterization and total habitat score. NAT correlated negatively with conductivity, NH3, TN and nitrate (N).

Darter, Madtom and Sculpin Richness (DMS)

This is the total number of the species present in a sample within the tribe Etheostomatini (darters), the genus Noturus (madtoms) and the genus Cottus (sculpins). These groups, relatively, are intolerant of or sensitive to pollution. This metric was a modification of Karr's (1981) Darter Richness metric. DMS correlated positively with embeddedness, epifaunal substrate, bank vegetative protection, sediment deposition, riparian vegetative zone width and frequency of riffles, pool variability, pool substrate characterization, channel sinuosity, total habitat score and channel alteration. DMS correlated negatively with conductivity, NH3 and TN.

Intolerant Species Richness (INT)

This is the total number of intolerant species present in a sample, and was originally used by Karr (1981). Members of this metric were believed to represent the first species to disappear after impairment and the last to reestablish after restoration. The metric initially failed the variability evaluation, but after examination of the metric regionally, it was found to be less variable and have good discriminatory power. INT correlated positively with all habitat parameters except for channel flow status and correlated negatively with all chemical parameters except for N.

Simple Lithophilic Spawning Species Richness (SL)

This metric is the total number of simple lithophilic spawning species and represents species that require relatively clean gravel and exhibit simple spawning behavior (Ohio EPA 1987, Simon 1991). The metric was considered a habitat metric and was expected to decline with impairment and be particularly sensitive to siltation (Berkman and Rabeni 1987). SL correlated positively with all habitat parameters except channel flow status, embeddedness and velocity depth regime and correlated negatively with all chemical parameters except conductivity and N.

Relative Abundance of Insectivorous Individuals (%INSCT)

This metric is the relative abundance of insectivorous individuals excluding tolerant individuals. The metric is a modification of Karr's (1981) relative abundance of insectivorous cyprinids and Ohio EPA's (1987) relative abundance of insectivorous individuals. %INSCT correlated positively with embeddedness, epifaunal substrate, sediment deposition, riparian vegetative zone width, channel alteration, velocity depth regime, pool substrate characterization, pool variability, channel sinuosity and total habitat, and correlated negatively with conductivity and NH3.

Relative Abundance of Tolerant Individuals (%TOL)

This metric was originally used by Karr (1981) and represents a proportion of individuals that are pollution tolerant and increase in abundance with impairment (negative response). For scoring, actual %TOL values were inversed to respond like prior positive response metrics.

%TOL correlated positively with embeddedness, epifaunal substrate, sediment deposition, channel alteration, velocity depth regime, pool substrate characterization, pool variability, channel sinuosity and total habitat score and correlated negatively with conductivity, NH3 and TKN.

Relative Abundance of Facultative Headwater Individuals (%FHW)

The metric was designed to detect the abundance of species that were atypical of headwater streams (e.g., Lepomis spp.) or typically exhibit low abundance in small streams (e.g., Campostoma spp.), but tend to increase in abundance with impairment (negative response). Semotilus atromaculatus was not considered a member since reference and test averages were roughly the same (30%). The metric replaced NAT in headwater streams. For scoring, actual %FHW values were inverted to respond like prior positive response metrics. %FHW correlated positively with embeddedness, epifaunal substrate, bank stability, bank vegetative protection, sediment deposition, riparian vegetative zone width, channel alteration, frequency of riffles, velocity depth regime and total habitat score and correlated negatively with conductivity and NH3.

Table 8-1: Metrics used in the KIBI		
KIBI Metrics:		Response to Disturbance:
1.	Native Species Richness (NAT)1	Negative
2.	Darter, Madtom, Sculpin Species Richness (DMS)	Negative
3.	Intolerant Species Richness (INT)	Negative
4.	Simple Lithophilic Spawning Species Richness (SL)	Negative
5.	Relative Abundance of Insectivorous Individuals (%INSCT)	Positive
6.	Relative Abundance of Tolerant Individuals (%TOL)	Negative
7.	Relative Abundance of Facultative Headwater Individuals (%FHW)1	Negative

1 NAT is used in wadeable stream assessment; %FHW is used in headwater stream assessments.

8.4.3 KIBI Scoring

Fish community structure is affected by stream size. Adjusting for drainage area enhances the metrics' performance to detect disturbances instead of stream-size effects (Smogor and Angermeier 1999). Therefore, regression equations were used to calibrate for catchment area size and to serve as a substitute for the maximum species richness lines described by Fausch et al. (1984) and Karr et al. (1986). The calibration process is outlined in Compton et al. (2003). The calibration process provides a normalized distribution of values from which a rank and percentile can be determined regardless of catchment area. This allows for uniform scoring across all stream sizes.

Scores for the metric values were divided by the 95th %ile of the reference dataset for the respective metric and multiplied by 100 to score the metric on a 0-100 point scale. A continuous

scale was used since it was believed to be more responsive with the continuous scale of various environmental parameters than prior categorical scoring (5, 3, 1), as used in Karr's (1981) IBI (Hughes et al. 1998, McCormick et al. 2001). Metrics from sites that performed exceptionally well and scored above 100 were set at 100. Metrics from sites that performed poorly and had negative values were set at 0. If collections had 50 or fewer fish individuals, the relative abundance metrics were set at 0. If collections had 51-99 fish individuals, relative abundance metrics were set at 50, unless the metric score was already below 50, in which case the value was not changed.

This automatic scoring of proportional metrics was based on the scoring modification principle used by Ohio EPA (1987) and Simon (1999). The final KIBI score, on a 0-100 point scale, was the average of the remaining metric scores. A summary outline of the calculation process is found in Table 8-2. KIBI application users can find the Reference Regression Equation (RRE), Catchment Area Constant (CAC) and metric value 95th %ile value for each retained metric. Example metrics RRE, CAC and 95th %ile are shown in Table 8-3.

Table 8-2: Metric scoring calculation process

Convert site catchment area (sq. miles) to Log10. This value will represent 'x' in the Reference Regression Equation (RRE) (see Table 8-3). Inverse negative response relative abundance metrics, 100 minus metric's actual/raw value. Solve for the Expected Value of metric using the Log10 of a site's catchment area as 'x' in the RRE (Table 9-3) of the respected metric. Subtract Actual Value (raw data) from the Expected Value (Step 3) to obtain a Residual Value. This number will be positive or negative based on site quality. To normalize Residual Value data for all catchment areas, a Catchment Area Constant (CAC) (Table 9-3) is used for each metric. CAC is added to the Residual Value (Step 4) to obtain Metric Value. Metric Value is divided by 95th %ile (Table 8-3) of the reference dataset for the respective metric and multiplied by 100 to equal Metric Score. The average of the retained Metric Scores equals the final KIBI score; KIBI score is a whole integer.
Scoring rules:
If Metric Score >100 then score as 100. If Metric Score < 0 then score as 0. If total number of individuals (TNI) < 50 then set % metrics at 0. If TNI 51-99, then set % metrics at 50, unless metric value is already fewer than 50; then do not modify. If TNI > 99, then % metric scores are not modified.

Table 8-3. KIBI metrics with reference regression equations (RRE), catchment area constant (CAC) and metric value 95th %tile			
KIBI Metrics:	Reference Regression Equations:	CAC:	95th %:
NAT1	y = 10.123x + 4.4279	20.49	28.2
DMS	y = 2.967x + 1.5037	6.21	9.3
INT	y = 2.6679x - 0.1395	4.09	7.7
SL	y = 4.4162x + 0.9526	7.96	12.5
%INSCT	y = -10.326x2 + 44.989x + 17.575	58.88	87.8
%TOL	y = -5.4568x2 + 31.379x + 41.6	77.65	101.5
%FHW1	y = 8.9128x2 - 59.151x + 98.557	27.14	61.4

Note: NAT for wadeable streams and %FHW for headwater streams

Final KIBI scoring criteria have been developed for each ichthyoregion in Kentucky using the extensive DOW database. See Compton et al (2005) for KIBI criteria. A copy can be obtained from the Kentucky Division of Water by contacting the Ecological Support Section at (502) 564-3410.

An Excel spreadsheet template for calculating a KIBI can also be obtained through the Division of Water. When using this template, two things must be kept in mind: 1) make sure that all metric scores calculated above 100 are given a score of 100 and 2) make sure that when "pulling down" formulae to calculate multiple metric scores that the formulae do not change during the pull down.

8.5 Reporting Fish Data

8.5.1 Community Structure Data

The condition of the fish community and the related stream health are determined by the calculated KIBI for the site. Relative abundance, species composition, richness, the evaluation of species tolerances to environmental perturbations and the condition of fishes are all attributes that are factored into the KIBI score in the form of metrics. Shifts in these respective attributes within the community should indicate a positive or negative change in the stream condition.

In addition, the presence or absence of indicator species and the presence of rare or endangered species should provide insight into the stream condition and causes for the respected changes or current condition. Correlations with habitat, land use, water quality and any other pertinent information should be made to enhance the KIBI score and to provide intuitive information to the stream ecosystem function and condition. The age and size class distribution of species populations should be noted and assessed in relation to recruitment potential; spawning and

nursery area availability should also be considered. Any limiting factors, either natural or man-induced, are reported.

8.5.2 Distribution Data

Monitoring of fish community structure is useful for stream health and fish populations as a whole but may be too broad for the monitoring of a particular species. Therefore, reliable records from collections can provide useful status information for a given species in the state. Considerable data are available on fish distribution in Kentucky (e.g. Burr and Warren 1986, KDOW data) and the environmental requirements of many of the more common species.

Distribution is affected by many natural factors, including evolution of drainage patterns, stream order, substrate, temperature, cover availability, gradient, current velocity, seasonal flow variability and presence and abundance of food organisms (Maret 1999 and Strange 1999). Fish have inherent ranges of tolerances for many of these natural factors. A variation outside these ranges of tolerance may be the result of detrimental conditions that are reflected in the presence, structure and relative abundance of fish populations. Therefore, a compilation of all existing ichthyological data is necessary for each collection, using both historic and present fish population data and field survey data to assist in the management of a species or species groups.

Also, physical and chemical characteristics of the stream are assessed in order to determine habitat availability and suitability for a species. All of these aspects help provide insight into the status and condition of a given species population.

SECTION 9. FISH AND MACROINVERTEBRATE CONTAMINANT ANALYSIS

9A. Fish Contaminant Analysis

9A.1 Introduction

Pollutants enter the watershed through permitted point source discharges (e.g., industrial and municipal facilities) and nonpoint sources (e.g., agricultural practices, urban runoff and atmospheric deposition). The pollutants may bioaccumulate in organisms living in these waters. These organisms provide a good method to monitor for potential pollution problems in the system. Contaminant analyses are performed on fish in these watersheds to provide background information on contaminant concentrations and to indicate potentially harmful concentrations.

When harmful concentrations of contaminants are found, the data are compared to the respective consumption concentration guidelines for issuing risk-based advisories. The Food and Drug Administration is responsible for monitoring contamination of market fish and shellfish. The responsibility for issuing fish consumption advisories, which are designed to protect citizens from eating contaminated fish, falls upon the state. The following information includes the standardized methods for obtaining fish samples that can be used for issuing the advisories.

9A.2 Site Selection

Fish tissue sample sites are selected and prioritized based on spill response, consumption advisories, Biological Monitoring Program (BMP), and fishing pressure on Kentucky water bodies. Sampling due to a spill response will take priority when fish tissue samples are requested and sites are determined based on characteristics of the spill.

Water bodies with consumption advisories are sampled a minimum of every other year. Sample sites for consumption advisories are chosen based on accessibility and resources. The Fish Tissue Program works within the 5-year basin cycle. In each basin, sample sites are chosen in a manner to reflect known fishing pressure. When the amount of fishing pressure is unknown in an area of the basin, sites are chosen by conditions that represent a potential fishing area such as water depth, habitat, and structure. Fish tissue samples are also collected at BMP sites in each basin.

9A.3 Field Sampling Procedures

Fish specimens for contaminant analysis are collected at various sites within Kentucky's streams, rivers and lakes/reservoirs. An attempt is made to collect composite samples of target species at each sampling site to facilitate comparisons between sites.

Target Species

For all biological sampling events, an initial list of target species may be developed on the basis of prior sampling, types of fish known to occur in the area, stream size, type of collecting

equipment required and the purpose of the study. Although the actual species collected varies, two trophic groups are preferentially sought: predators and bottom feeders. The following fish are used for contaminant analysis whenever possible:

Bottom feeders

- Carp - Cyprinus carpio
- Channel Catfish - Ictalurus punctatus or other ictalurids
- Redhorse suckers - Moxostoma spp.
- Other sucker species - Ictiobus spp., Carpiodes spp., Catostomus commersonii, Minytrema melanops

Predators

- Black Basses - Micropterus spp.
- Temperate Basses – Morone spp.
- Rock Bass - Ambloplites rupestris
- Crappie - Pomoxis spp.
- Sunfish - Lepomis spp.
- Sauger - Stizostedion canadense
- Walleye - Stizostedion vitreum

In the event that target species are not collected at the site, other species that are consumed locally and are of harvestable size may be collected. If no harvestable sized fish are collected, a large sample of small fish of the same species may be collected for a whole body composite sample to determine if more intensive sampling needs to be performed.

9A.3.2 Sampling

Typically, samples should be collected from late summer to early fall (August - October) (Phillips 1980). This is when the lipid content that contains many organic pollutants is highest. However, there are many exceptions coinciding with target species such as spawning period, budget constraints or availability of monitoring personnel in the summer months.

Various sampling techniques are used to collect fish. The most common methods of collection use:

- Electrofishing units
- Seines
- Fishing poles
- Gill nets
- Trammel nets
- Hoop nets
- Trotlines
- Jug lines
- Bank lines
- Rotenone (lock chambers only)

When compiling a composite sample, the KDOW attempts to collect fish within 90% of each other in total length. The size difference of individuals making up a composite sample shall not have a size difference (largest to smallest) greater than 75% (GLSFATF 1993).

- Composite samples should contain three to ten individuals of the same species. Replicate composite samples are collected approximately 10% of the time.
- When possible, the right fillet is taken from an individual and the left fillet and body are retained for a whole-body sample.
- Whole-body samples are utilized if fish species or individuals are too small to fillet.
- Fish are identified, weighed and measured in the field to the nearest gram and mm total length and recorded on the fish tissue field data sheet (Appendix E-1).
- The total body length is determined by measuring from the anterior most part of the fish to the tip of the longest caudle fin ray (when the lobes of the caudle fin are compressed dorsoventrally) (Anderson and Gutreuter 1983).
- Any additional information, such as abnormalities (fin erosion, skin ulcers, skeletal deformities, tumors), should also be noted.

9A.3.3 Fillet Procedure

Fillets are the primary sample to be analyzed for contaminants. Fish samples are usually filleted in the field. However, if this cannot be done, they should be stored on ice immediately and returned to the lab within 24 hours for processing. Periodic wipe tests should be conducted in the work area to monitor for significant levels of metal and organic concentrations (U.S. EPA 1999).

- The work surface for filleting is covered with clean aluminum foil or Teflon.
- The fish should be scaled, except for scale less fish such as catfish and bullheads where the skin is removed.
- The fish are then filleted so as to include all flesh and fatty deposits from the back of the head to the tail and from the top of the back down to and including the belly flap area of the fish.
- Carefully remove the fillet from the body cavity to avoid puncturing it and internal organs; the rupture of internal organs will contaminate edible tissue samples (Stober 1991, U.S. EPA 1986). If the rupture of internal organs contaminates the fillet tissue, rinse it in contaminant-free water.
- Remove all fins, the tail, head, viscera and major bones. Only fillets, typically removed from the right side of each fish, are used for the composite edible portion sample for consumption advisories, not whole bodies (Federal Register 1979).
- If only a small sample could be obtained, both right and left fillets may be used.
- The remainder is reserved for the whole-body composite sample (right fillet + whole body right fillet was taken from) if needed.
- When sampling and filleting, caution should be taken to not contaminate the samples (e.g., handling gasoline containers then the fish samples or exposing fish samples to engine exhaust, etc.).
- The fillet knife is cleaned and rinsed with 10% nitric acid and then acetone after each sample. The aluminum foil covering on the workstation is replaced between samples.

9A.3.4 Packaging

Fillet samples are rinsed with ambient water, wrapped in extra heavy-duty aluminum foil, and placed in a waterproof plastic bag. Composite samples of the same species can be wrapped together in aluminum foil and placed in one bag. If the remaining carcass is to be used for whole-body analysis (fish with one fillet removed), it is also rinsed with ambient water, wrapped and labeled. If more than one species is collected from a site, these packaged composite samples should be kept together in one large waterproof plastic bag if possible. Once packaged, the samples are stored in ice for transport to the laboratory freezer. If samples are not to be transported to the laboratory that day, they should be frozen, preferably with dry ice.

9A.3.5 Labeling and Chain-of-Custody

Labels for each sample will contain the following information: stream name and sampling location, date, county, latitude and longitude, collectors, collection method (be specific as to type of electrofisher, net, etc.), type of fish, individual lengths (inches) and weights (ounces), and type of fillet. Types of fillets include whole body (WB), left fillet (LF), right fillet (RF) or left plus right fillet (BF). These variables are needed to enter the data into the EDAS database. Other information that is needed can be obtained at a later date. All samples should be properly labeled and returned with a fish tissue data sheet (Appendix E-1). Proper chain of custody procedures should be followed (KDOW 1986).

9A.4 Laboratory Procedures

Tissue samples are frozen prior to processing. The frozen samples are cut into small pieces with a meat saw, blended with dry ice and homogenized in a stainless steel industrial blender or a meat grinder, depending on the size of the fish being processed. Equipment for processing fish tissue samples will be cleaned between samples as follows:

- Wash with mild detergent
- Rinse with hot tap water
- Rinse with distilled water
- Rinse with 10% nitric acid
- Rinse with acetone

9A.4.1 Composite Samples

Approximately one pint (500 ml) of ground, homogenized composite fillet tissue is placed in a pre-cleaned glass jar with a teflon-lined lid. The sample is then labeled and kept frozen until analysis by the DES analytical laboratory. The remainder of the fillet sample is combined with the whole-body fish tissue and ground together to produce the final composite whole-body sample. Approximately one pint (500 ml) of the whole-body sample will be placed in a pre-cleaned glass jar with a Teflon-lined lid, labeled and kept frozen until analysis.

9A.4.2 Individual Fish Samples

When individual fish are processed, procedures similar to those outlined above are followed for both fillet and whole-body samples if at least one-half pint (250 ml) of fillet tissue can be obtained from the sample.

9A.5 Interpretation of Fish Tissue Data

Fish tissue data can often be difficult to interpret accurately. But as we become more aware of harmful side effects of contaminants and the guidelines are adjusted accordingly, the advisories provide the most up-to-date guidelines for interpreting and comparing the data obtained from the tissue samples. For example, advances in technology and research have allowed us to better determine the effects of specific contaminants at specific concentrations. This allows us to issue levels of contaminant advisories for individuals based on the amount of risk it poses.

The Commonwealth of Kentucky uses risk-based advisories. These advisories help to establish a guideline for comparing the data obtained from the tissue samples. Consumption guidelines are based on the edible portion (e.g., fillet) of the fish, which is checked for an array of contaminants (Table 9-1). When the sample contaminant concentrations are obtained, they are compared to consumption concentration guidelines that range from unrestricted consumption to no consumption.

Consumption guidelines for children, pregnant and nursing women and potential childbearing women are one group higher than for the general population (GLSFATF 1993). This separate guideline was provided because of concern for developmental effects in children and fetuses whose reduced body weight causes sensitivity to and builds up contaminant concentrations.

An example of risk-based protocols with associated contaminant concentration guidelines for mercury and PCBs are presented at the end of this section (Tables 9-2 and 9-3, respectively). Based on the results, an advisory is implemented until future studies indicate a change in advisory levels. Once an advisory is issued for a specific waterbody, residual levels of the contaminant must fall below the state criterion for at least two consecutive years before the waterbody is removed from the advisory list. If the contaminant does not yet have an associated risk-based protocol, one should be implemented on an as-needed basis. FDA action levels could be used as a threshold concentration to determine that there is a need for researching and implementing a risk-based protocol for those substances (U.S. FDA 1980). Whole-body fish tissue data cannot be used for consumption advisories. Whole bodies can be used, however, as an indicator of areas where more extensive sampling needs to be performed as a result of inflated contaminant concentrations.

9B. Macroinvertebrate Tissue Analysis

9B.1 Introduction

Macroinvertebrate tissue samples can be used to assess the bioaccumulation of pollutants in aquatic food chains and to provide background, control or reference tissue data for streams and rivers. Collection and analysis of these types of samples are performed in biological surveys on a case-by-case basis.

9B.2 Target Species - Sample Preparation - Laboratory Procedures

Composite macroinvertebrate samples consist of individuals of the same or similar species. Target macroinvertebrate species are crayfish, mussels and helgrammites. Species and sample size should be recorded for all samples. Additionally, length/weight and age measurements should be recorded for mussels. Only the mussel body, not the shell, is used for analysis.

All samples are either wrapped in clean aluminum foil or placed in pre-cleaned glass jars with Teflon lined lids and held on ice until return to the laboratory. The samples are frozen until processing. Samples are labeled, stored and processed in a manner similar to that used for fish tissue preparation.

At least 5 grams of homogenized macroinvertebrate tissue must be obtained for analysis by the DES laboratory. The chemical analyses performed are the same as for fish tissue analysis (Table 9-1).

Table 9-1 Parameters for tissue analysis

% Lipids	Methoxychlor
Hexachlorocyclopentadiene	Mirex
Etridiazole	Endosulfan I
Hexachlorobenzene	Endosulfan II
Propachlor	Endosulfan sulfate
Trifluralin	Endrin aldehyde
alpha-BHC	Endrin ketone
beta-BHC	Permethrins (cis & trans)
gamma-BHC (Lindane)	4,4-Dichlorobiphenyl (Surrogate)
delta-BHC	Decachlorobiphenyl (Surrogate)
Aldrin	Aroclor 1016
DCPA	Aroclor 1221
Chlorothalonil	Aroclor 1232
Heptachlor	Aroclor 1242
Chlorpyrifos	Aroclor 1248
Heptachlor epoxide	Aroclor 1254
Oxychlordane	Aroclor 1260

trans-Chlordane	Aroclor 1262
cis-Chlordane	Aroclor 1268
trans-Nonachlor	Toxaphene
Chlordene	Decachlorobiphenyl (Surrogate)
cis-Nonachlor	Aluminum
Technical Chlordane	Manganese
Dieldrin	Zinc
Endrin	Beryllium (GFAA)-Tissue
2,4'-DDE	Nickel (GFAA)-Tissue
4,4'-DDE	Mercury (AA)-Tissue
2,4'-DDD	Arsenic
4,4'-DDD	Cadmium
2,4'-DDT	Chromium
4,4'-DDT	Copper
Total DDT	Lead

Table 9-2. Monthly risk-based fish consumption limits for methylmercury[1]

Fish Meals/Month:	Methylmercury EPA fish tissue concentrations:
16	> 0.03–0.06
12	> 0.06–0.08
8	> 0.08–0.12
4	> 0.12–0.24
3	> 0.24–0.32
2	> 0.32–0.48
1	> 0.48–0.97
0.5	> 0.97–1.9
No consumption	> 1.9

[1]Pregnant and nursing women, potential childbearing women and children would be one group higher.

Table 9-3. Monthly risk-based fish consumption limits for PCBs[1]

Fish Meals/Month:	PCBs GLP fish tissue concentrations: (ppm, wet weight)
Unrestricted	0.00-0.05
4	> 0.05-0.20

1	> 0.20-1.00
0.5	> 1.00-1.90
No consumption	>1.90

[1]Pregnant and nursing women, potential childbearing women and children would be one group higher.

Table 9-4. Short list of action levels for poisonous or deleterious substances in human food and animal feed[1]

Substance:	Level established in:	Action level[2]:
Alfatoxin	Food & Feeds	20 ug/kg
Aldrin, Dieldrin	Fish & Seafoods	0.3 ppm
BHC	Frog Legs	0.5 ppm
Chlordane	Fish	0.3 ppm
DDT, DDE, TDE	Fish	5.0 ppm
Dioxin	Fish	50 ppt / 25 ppt[3]
Endrin	Fish & Shellfish Fishmeal, Fish soluble, Fish oil	0.3 ppm
Heptachlor, Heptachlor Epoxide	Fish & Shellfish	0.3 ppm
Chlordecone (formerly Kepone)	Fish	0.3 ppm
Mercury	Fish, Shellfish, Crustaceans, Aquatic Animals (Edible portions only)	1.0 ppm
Mirex	Fish	0.1 ppm
Paralytic Shellfish Toxin	Clam, Mussels, Oysters	80 ug/100g meat
Toxaphene	Fish	5.0 ppm
PCB	Fish	2 ppm

[1]Action Levels are established and revised according to criteria specified in Title 21 Code of Federal Regulations, parts 109 & 509 and are revoked when a regulation established a tolerance for the same substance and use becomes effective.http://vm.cfsan.fda.gov/~lrd/fdaact.html

[2]Represent limits at or above which FDA will take legal action to remove adulterated products from the market.
[3]Represents limit at which FDA issues advisory to limit consumption.

SECTION 10 INTEGRATING MULTIPLE ASSEMBLAGES

10.1 Introduction

KDOW biologists typically use more than one biotic assemblage to assess aquatic life use support for wadeable streams. A weight-of-evidence approach is utilized when more than one biotic assemblage will be used for determination of use support. The steps used to integrate the data in the weight-of-evidence approach are outlined below**.**

10.2 Process

Step 1: Determine the number of assemblages used for use support assessment.

Step 2: Derive a narrative classification rating for each index score (DBI, MBI, KIBI) used. The narrative ratings (Excellent to Very Poor) are derived from the tables presented above for each assemblage.

Step 3: Compare the narrative classification ratings.

Step 4: If all three narrative classification ratings are in agreement, then an overall rating that is identical with the narrative classification ratings of each community is given for the site (i.e. if algae = good, macroinvertebrates = good, and fish = good, then the site is assessed as being good). If only two assemblages are assessed and both are in agreement, then an overall rating that is identical with the narrative classification ratings of each community is given for the site (i.e. if algae and macroinvertebrates = good, then the site is assessed as being good).

Step 5: If two of the three narrative classification ratings are in agreement and the other is close to the first two, then an overall rating that matches the two in agreement will be given for the site (i.e. if algae = good, macroinvertebrates = fair, and fish = fair, then the site is assessed as being fair).

Step 6: If two of the three narrative classification ratings are in agreement and the other is drastically different than the first two, then land use, geology, hydrologic conditions, habitat conditions, sampling conditions, sampling effort, seasonality, and physicochemical properties are examined to identify why the one community so drastically differed from the others.

If some logical explanation can be made for the discrepancy, then by using the weight-of-evidence approach, the narrative rating in question may be overlooked in favor of the two agreeing ratings (i.e. if algae = good, macroinvertebrates = good, and fish = very poor, because water levels were very low making collection difficult, then the site would be assessed as being good).

If a logical explanation for the disparity cannot be deducted, then the site may need to be revisited so that an adequate assessment can be made.

If only two assemblages are being assessed and the narrative classification ratings do not agree, then land use, geology, hydrologic conditions, habitat conditions, sampling conditions, sampling

effort, seasonality, and physicochemical properties are examined to identify the reason for the disagreement.

If some logical explanation can be made for the discrepancy, then by using a weight of evidence approach, an overall rating is given to the site (i.e. if algae = good and macroinvertebrates = fair, because the water chemistry was good, but there was not enough habitat available for the macroinvertebrates to thrive, an overall rating of fair may be given to the site).

If a logical explanation for the disparity cannot be deducted, then the site may need to be revisited and another assemblage sampled so that an adequate assessment can be made.

Step 7: If none of the three narrative classification ratings agree, then land use, geology, hydrologic conditions, habitat conditions, sampling conditions, sampling effort, seasonality, and physicochemical properties are examined to identify why the communities differ in their assessments. Based upon the weight-of-evidence approach, an overall assessment of the site may be given. It may be that the narrative rating that is in the middle of the other two may be used (i.e. if algae = excellent, macroinvertebrates = fair, and fish = poor, then the site may be assessed as being fair, if supplemental evidence supports that decision). If a consensus cannot be reached, then re-sampling may be needed so that an adequate bioassessment can be made.

Step 8: Determine Aquatic Life Use Support.
If an overall rating is excellent or good, then the site fully supports its designated use. If an overall rating is fair, poor or very poor, then the site does not support its designated uses.

Example of bioassessment integration. In the Kinniconick Creek example, diatom, macroinvertebrate, and fish assemblages were used. The DBI and MBI scores were 82 and the KIBI score was 73, each representing a narrative rating of excellent. Since all three narrative ratings agreed, then an overall narrative classification of excellent was given to Kinniconick Creek. Therefore, it was determined that Kinniconick Creek fully supports in designated use.

SECTION 11. QUALITY ASSURANCE

11.1 Habitat

A habitat assessment will be performed at all sites (Appendices A-1 and A-2). The habitat assessment sheet will be completed jointly by all members of the field crew. Each habitat assessment will be logged into the habitat assessment logbook (Appendix F-1) and filed in the habitat folder for the basin management unit being sampled that year.

All field personnel will be trained annually at locations of known habitat value to help ensure uniformity among KDOW personnel. Personnel will independently evaluate habitat and turn in a habitat assessment sheet to the designated habitat experts for evaluation. The median overall score of the expert group will be deemed the correct score. Personnel must score within 10 points of the median to pass. Personnel will continue to train and perform habitat evaluations until they attain the 10 point accuracy. Habitat training records will be kept in the master logbook (Appendix F-2).

11.2 Algae

11.2.1 Field

Sample labels shall contain complete and accurate information. Before leaving a sampling site, all sampling equipment will be checked for residual algae material, rubbed clean and thoroughly rinsed with distilled water. One duplicate sample from 10% of the reaches sampled within a season will be collected and analyzed to ensure precision and repeatability of the sampling technique. Phycologists shall be trained in the sampling techniques on a regular basis.

11.2.2 Laboratory

Samples shall be recorded in the sample logbook (Appendix F-5). Sample logbooks will contain date collected, preserved, date sample was burned, number of rinses used in processing, date microscope slide made, date slide identified and date data entered into EDAS to facilitate tracking of samples. A voucher collection will be maintained of all samples and diatom slides. Label information must be accurate and complete. Vials containing processed diatoms and diatom microscope slides will be stored indefinitely in KDOW. Diatom samples will be discarded in one year, after the presence of useable slides has been verified.

11.2.3 Identification

Phycologists shall discuss any problem identifications with other phycologists. Any problem taxa shall be sent to an outside taxonomist to assist in identification. Duplicate samples will be processed and analyzed in the same procedures as regular samples. Duplicate samples collected from one site will be identified by the same phycologist to account for in-field variability at the site. Ten percent of the microscope slides will be randomly chosen for identification by all in-house phycologists. Identification of replicate samples between in-house phycologists should exceed 75% similarity. If there is not another phycologist present, an outside phycologist should

be utilized to periodically check identifications. The most recent taxonomic references shall be used for identification. Phycologists will participate in algal identification workshops, seminars and training sessions to keep updated on taxonomy and ecology of diatoms and other algal taxa when available.

11.3 Macroinvertebrates

11.3.1 Field

All samples will be clearly and correctly labeled in the field. Five percent of samples collected will be duplicated to evaluate precision and repeatability of the technique and the sampling crew. The field crew will select a stream reach that has sufficient habitat to accommodate a duplicate sample. After the samples have been collected, with the appropriate sampling methods for that stream reach, it will be labeled as the duplicate and processed with the rest of the samples. After any sampling has been completed, all sampling gear will be thoroughly cleaned to remove all macroinvertebrates so that specimens are not carried to the next site. The equipment shall be examined prior to sampling at the next site to ensure that no macroinvertebrates are present.

11.3.2 Laboratory

Sample information will be recorded in the sample logbook (Appendix F-4). Initial entries include stream name, site location/county, date collected and collectors' initials. Additional entries include date the sample is sorted, sorters' initials, QA/QC sorters' initials, date identified, date QA/QC identified, date entered into EDAS and date QA/QC is entered into EDAS. Samples will be kept in a storage area for five years. Samples will be maintained at museum quality making sure that they are fully immersed in preservative.

All individuals sorting macroinvertebrate samples shall be certified by a macroinvertebrate specialist each year. Ten percent of the pans sorted will be examined by a second qualified biologist to ensure that all organisms are removed from the sorting pan or grid. If fewer than ten organisms are found, the sample is considered valid. If more than ten organisms are found, then the sample fails and another successive pan will be checked. This will continue until the sorter passes the procedure. The sorting pans will be thoroughly rinsed after each pan is picked.

11.3.3 Identification

Five percent of samples collected will be re-identified by a second taxonomist to evaluate precision and accuracy. A target of 90% similarity in taxonomic composition is acceptable. A third taxonomist will reconcile differences in identifications between the first two taxonomists if necessary. A voucher collection will be maintained. At least one and, if possible, three to five specimens of the vouchered taxon will be placed in the vial. These specimens will be properly labeled, preserved and stored in the laboratory for future reference. Rare and difficult taxa will be verified by other branch biologists or noted taxonomic experts. Invertebrate samples will be kept for 5 years.

A bench sheet shall be produced during the sample identification process (Appendix C-2). After the sample has been identified, the data will be entered into the database. The bench sheet will be filed in the designated file cabinet for that years sampling. When available and as resources allow, biologists will take part in macroinvertebrate identification training to ensure consistent and accurate identification. In addition, the KDOW will maintain a library and list of taxonomic references.

11.4 Fish

11.4.1 Field

A field crew will consist of at least one trained ichthyologist who is knowledgeable of the current identification and nomenclature of Kentucky fishes. The ichthyologist is to make sure voucher representatives (5 specimens, if possible) of all fish are taken and all specimens that are in question are preserved correctly for laboratory examination. All voucher collections will be labeled clearly and correctly. All released specimens will be noted. Collection labels are to be accurate and complete with all pertinent information included.

Five percent of samples taken in a season will be duplicated/replicated by a field crew. These samples will be taken either at the exact site within a two-week period or taken on the same day within the sample reach by either the same field crew or an alternate crew to ensure precision and repeatability of the sampling technique.

11.4.2 Laboratory

Sample information will be recorded in the sample logbook. Initial entries include date collected, county, stream name, site location, collectors and preservative. Additional entries include date when sample is transferred into water, date when sample is switched to 70% ethanol, date sample is identified and by whom, and date when samples are entered into EDAS and recorded in the fish community collection logbook (Appendix F-3).

11.4.3 Identification

During identification of specimens, the use of all pertinent keys and distributional records is required and final identification will be cross-referenced with the KDOW voucher collection as needed. Ichthyologists should discuss any problematic identification with other KDOW ichthyologists. Recognized taxonomic experts shall assist in difficult specimens and confirmation of specimens. Ichthyologists will participate in fish identification workshops, seminars and training sessions, when available.

Ten percent of the collections in a season will be re-identified and enumerated by another ichthyologist. The re-identification and enumeration of a collection should exceed 90% accuracy. Duplicate samples should exceed 75% similarity (Whittaker 1952). Duplicate/replicate samples will be processed and analyzed in the same procedures as regular samples.

Fish are kept at DOW until all the samples from that year are identified and assessments completed. Samples are then transported to Southern Illinois University at Carbondale for curation. Samples from enforcement cases or spill response are maintained as long as needed for resolution of the case.

11.5 Fish Tissue

To assure that samples are being processed and analyzed properly, 10% of the ground tissue samples will be submitted as duplicate samples for comparison. Duplicate sample results within 20% relative standard deviation will be accepted as accurate data. Duplicate sample results that fall outside 20% will be considered suspect and re-sampling will be considered.

Fish tissue samples are stored in a locked freezer until processing is complete, and samples are transferred to the chemistry lab for analysis. Fish tissue sample information, such as station name and number, location, type and size of fillets, collectors, date processed, processor(s) and date delivered to the laboratory, is recorded in a logbook (Appendix F-6). Fish tissue sample remnants will not be returned to KDOW after processing

.

11.6 Data Entry

A minimum of 10% of the sites will be chosen for data entry quality assurance. Selection will be done by a random numbers table and numbers assigned to each site on the biological monitoring stations logbook. With each chosen site, all data entry for that site will be checked (water chemistry, habitat, algae, etc.). The quality assurance check will be performed by someone who did not enter any of the data initially.

Data entry errors will be corrected as they are encountered. Error rate will be determined by dividing the number of incorrect entries by the number of entry fields for that sheet. Sheets should be 95% correct to pass quality assurance. If a sheet is less than 95% correct, then another sheet by the same entry individual will be checked. This will be continued until the entering individual has achieved a 95% correct level. Quality assurance will be recorded on the individual logbooks for each type of information collected.

11.7 Equipment Calibration and Maintenance

Equipment will be maintained to the highest extent possible. Calibrations will be performed as per directions in the Owner's Manual for that piece of equipment. A logbook (Appendix F-7) will be kept for tracking calibrations for each parameter of each meter. An additional logbook will be maintained (Appendix F-8) for tracking all maintenance or repairs that are performed for each meter. Training on the care, maintenance, storage, calibration and use of the equipment will be held annually. Training records will be entered into the master logbook (Appendix F-2).

Microscopes and dissecting scopes will be cleaned and checked once every three years, or as identified as necessary. Field equipment will checked periodically during the field season and at the end of the season to verify that all probes are working correctly. Repairs will be done as necessary and maintenance will be done as needed.

11.8 Overall Quality Assurance

All bench sheets, logbooks, field data sheets, training records, chemical analysis reports and other hard copies will be kept in a file cabinet organized by stream and year. Paper records will be housed in designated file cabinets for a period of time as deemed appropriate by the department QA officer, or as defined by Division of Water record retention policy.

The biological monitoring stations logbook (Appendix F-10) will be used to help track completion of all parts of a biological assessment. It contains information about what assemblages are collected and when all of them are completed and in EDAS.

SECTION 12 HEALTH and SAFETY

12.1 Vehicle Usage

All employees will adhere to traffic laws and safety practices as outlined in the Commonwealth of Kentucky's, "Policy Statement on the Operation and Use of State Vehicles, Policy Statement #01-001."

Specific points listed in the policy statement include obeying speed limits, using safety restraints and prohibiting the use of cell phones, alcohol and drugs/medications that might cause impairment while driving.

Vehicles will be winterized each year according to the guidelines in Policy Statement #01-001. Water Quality Branch and Watershed Management Branch staff are responsible for the maintenance of state vehicles. These vehicles should be periodically checked for safety and mechanical problems. When safety and mechanical problems arise, the vehicle should be taken to a certified mechanic for repair in a timely manner.

Vehicle sign-out sheets need to be filled out with users, destination and cell phone number. In case of an emergency, central office personnel should know which vehicle is being used, where the vehicle was going and the cell phone number associated with that vehicle.

Cell phones need to be charged and turned on when the vehicle in out in the field.

All Water Quality Branch and Watershed Management Branch vehicles should have the following items: gasoline card, insurance card, usage and maintenance logbook, fire extinguisher, cell phone, spare tire, jack, crowbar and a first aid kit.

Off-road tires are recommended for those vehicles that go onto non-paved roads or areas.

All staff should be trained in the proper usage of fire extinguishers.

Staff should attend defensive driving and fire extinguisher classes.

12.2 Watercraft Safety

The watercraft trailer should be inspected before each trip to ensure that lights, wheels/tires, tie-down straps, hitch, spare tire and safety chains are working properly. Although trailer lights are not required by law to be operational during daylight hours, it is highly recommended that trailer lights be functioning when Trailering a watercraft.

If any piece of safety equipment is not working properly, it should be repaired or replaced before leaving for the sampling trip.

An inspection of the weldings, bolts/nuts and frame of the trailer should be conducted to identify any possible problems before leaving the central office complex.

The electrical plug for trailer lights should always be unplugged before backing the watercraft into the water.

Wheel bearings should be well greased for all sampling trips and tires should be properly inflated.

The watercraft should be inspected before leaving for a sampling trip to ensure that the motor, propeller and bilge pump, if equipped, are in working order. Additionally, the frame and integrity of the boat hull should be inspected to identify any possible problems.

If the watercraft is to be used for electrofishing, the electrical unit, generator, work lights and safety railing should be inspected before each trip for proper working order.

If any piece of equipment is not working properly, it should be replaced or repaired before the sampling trip.

All employees will follow safe boating practices as described in the 2006 Kentucky Sport Fishing and Boating Guide. Many of the rules outlined for vehicle usage apply to watercraft safety. Watercraft operators will observe all right-of-way procedures, restricted zones, idle speed zones and other buoyed areas.

Staff will attend trailering classes when they become available.

Watercraft should not be operated during severe weather, such as thunderstorms and snowstorms, and when the water levels are such that the watercraft cannot be operated safely.

All employees will wear a personal floatation device (PFD) while the boat is underway. Each watercraft shall have a Type IV PFD that can be thrown to someone in case of an emergency. It is recommended that survival suits be worn when water temperatures are below 60 ^{o}F.

In addition to PFDs, all watercraft are required to have the following equipment: a fire extinguisher, navigation lights (from sunset to sunrise), first aid kit and a signaling device.

Additionally, it is recommended that each watercraft carry a tow rope, paddle, cell phone, GPS unit, anchor, map of the waterbody, bailing bucket and tool set. An inventory of the above-mentioned equipment should be conducted before leaving the central office complex to ensure proper working order.

If any piece of equipment is not working properly, it should be repaired or replaced before leaving for the sampling trip. Staff will attend boating classes when they become available.

All field personnel are required to attend CPR training on a yearly basis. Every 3 years, field personnel are required to take a first aid refresher course.

12.3 Safety in the Field

12.3.1 Pre-Field Preparation

For general safety purposes, field crews should consist of more than one field person, especially if it is necessary for field personnel to get into a stream.

Members of a field crew should familiarize themselves with the nearest hospital, doctor's office or instant medical care provider.

Each field crew should use the following personal protective equipment (PPEs) for each sampling trip: waders, boots and long pants. It is also recommended that each field crew use gloves (when taking water chemistry samples), bug repellent and hand sanitizer.

Each field crew shall take an inventory/checklist of the required PPEs before each sampling trip making sure that all equipment is working properly. If any PPE is found to be inadequately working, such as leaking, ripped, etc, it should be repaired or replaced before leaving for the sampling trip.

Field crew allergies, such as bee stings, should be identified before the sampling trip.

Field crews should be properly dressed for the weather conditions. Coats, gloves and head coverings should be used during the late fall, winter and early spring to reduce the threat of hypothermia. Shorts can be worn under waders during the summer to reduce the threat of heat exposure.

Drinking water and other liquids should be available to field crews during sampling trips. Water coolers with ice can assist in reducing dehydration and heat exposure illnesses.

12.3.2 In Field Procedures

Field crews should survey the sampling location first before getting into the stream to identify any possible safety hazards.

Areas that seem to be unsafe should be avoided.

Each field crew member should be aware of his/her surroundings so to recognize any change in safety conditions, such as oncoming severe weather, possible dangerous animals and illegal human activities.

During high flow or runoff events, biological sampling should be postponed until baseline conditions exist. When high flow or runoff events are targeted for TMDL, NPS or other water quality sampling, field crews shall not put themselves in a dangerous situation. If at all possible, water samples and flow measurements should be collected from a bridge or from the bank. During high flow events, a PFD should be used by all field crew personnel if it is determined that in-stream sample collection and/or flow measurement is necessary and not dangerous.

If the field crew has any doubts concerning the safety of a location, sampling should be postponed or terminated. A field hazard assessment sheet should be filled out by each field crew for each sampling site.

While water chemistry sampling gloves should be used to reduce exposure of in-stream contaminants and reduce the likelihood of contaminating the water sample, gloves are not really practical for biological sampling. Hands and arms are often extended deeper into the water than the gloves can protect. Biological sampling often requires finger dexterity that gloves cannot provide.

While conducting in-stream sampling of water chemistry, flow or biology, waders and boots should be utilized. These PPEs provide a barrier for in-stream contaminants and from electricity that is used while electrofishing. Waders also provide some protection from biting insects, ticks, spiders, snakes, poison ivy, and stinging nettle. It is recommended that a wading belt be used to reduce the chances of water filling the waders during a fall. Boots should have felt or studded soles to reduce the chances of slipping or falling. Care should be taken around stream bank mud, boulders, bedrock or large woody debris to reduce the threat of a falling injury.

When transporting, a formaldehyde container it should be transported in a secondary leak proof container of sufficient volume to hold the amount in the storage container. When pouring formaldehyde into fish collection jars, gloves should be worn to prevent skin exposure.

Unless placing a specimen into a collection jar, they should be closed to prevent the splashing of ethanol or formalin out of the jar. Jars should be kept away from the facial area to reduce splashing and inhalation exposure. Collection jars should be inspected before use to check for leakage of fluids. If leaks are found, the jar needs to be discarded. Plastic collection jars should be utilized, if possible, to reduce the chances of breaking glass. Plastic field pipettes should also be used instead of glass ones.

Gasoline cans should have tight seals to eliminate the escape of fumes. Backpack electrofishers should be refueled in an open area. Care should be taken when pouring gasoline into the electrofisher so that spillage and inhalation and skin exposure can be reduced. Collection jar and gasoline lids should be tightened upon completion of the sampling event to reduce spillage and release of fumes.

When processing fish tissue samples, field crew should be extremely careful when filleting fish, running nets and handling fish. Cuts and puncture wounds can occur even when crew members are being careful. First aid kits should be readily available during these sampling activities.

If serious injury or exposure occurs while in the field, then proper first aid attention will be administered by other field crew members and the victim will be transported to a medical facility as quickly as possible.

For chemical exposure refer to the appropriate MSDS sheets for proper first aid treatment. MSDS sheets for chemicals, transported in a vehicle or boat, shall be maintained in a readily accessible location in that vehicle. If any exposure occurs while out in the field (this includes

insect and tick bites and exposure to poison ivy), a 1A1 exposure or injury form needs to be submitted to the Division of Workman's Compensation within 24 hours of exposure or injury.

12.3.3 After the Sampling Trip

Preserved samples and gasoline cans should be removed from the vehicle after the sampling trip so that the threat of breakage or spillage in the vehicle is reduced.

The gasoline cans and gasoline backpack electrofishers should be stored in the hazardous materials building.

Samples shall be stored in a well-ventilated area until processing can be conducted.

All safety equipment should be inspected to identify any possible future problems (e.g. tears or rips in waders).

12.4 Safety in the Laboratory

Laboratory safety procedures outlined in the Water Quality Branch Health and Safety Manual (KDOW in draft) and the Division for Environmental Services (DES) Chemical Hygiene Plan should be followed by staff employees while working in the laboratories.

Proper PPE shall be worn by all DOW personnel while processing samples and handling chemicals.

Refer to the appropriate MSDS sheet for the correct PPE while handling chemicals. It is recommended that lab coats also be worn to protect clothing from spillage.

To prevent the burning of skin, oven mitts shall be utilized when boiling diatom samples or using other exothermic reactions.

Forceps should be used to remove specimens from collecting jars.

Eye and ear protection and lab coat should be used when processing fish tissue samples.

Fume hoods are located in the algae and fish processing laboratories. When working with chemicals that cause harmful fumes, KDOW personnel shall use a fume hood to reduce the threat of inhalation exposure. In the algae processing laboratory, when preserving algal samples, boiling algal samples, preparing chlorophyll a samples and preparing diatom slides, a fume hood shall be utilized. In the fish processing laboratory, when changing preservatives in collection jars, cleaning fish tissue equipment and processing fish tissue samples, a fume hood shall be utilized.

Toxic or caustic materials that are currently in use should be stored in a fume hood. Excess toxic or caustic materials and gasoline should be stored in the hazardous materials building. Two or more personnel should be involved in retrieving materials from the hazardous materials building.

It is recommended that the door of the hazardous materials building be propped open when entering so that ventilation can occur.

Extreme care should be taken when processing fish tissue samples. When cutting frozen fish samples with a ban saw, fingers should be kept away from the blade at all times. Gloves should be used when handling dry ice. Dry ice should also be used in well-ventilated environments. Care should be taken when handling and cleaning blenders so to reduce the chance of cutting fingers by the blades. Fans and the fume hood should be on, when cleaning fish tissue processing equipment with acetone and nitric acid.

Mercury thermometers are used in several of the KDOW laboratories. If a mercury thermometer should break, all individuals within that lab must be quarantined. All other personnel near the lab should be evacuated. Entrance into the lab will be restricted until clean-up crews arrive. Mercury clean-up crews will be called on-site to decontaminate the laboratory.

When any chemical spill occurs, the first line supervisor will be notified. The first line supervisor will notify the second line supervisor and the division safety officer. The division safety officer will then notify the department safety officer. Do not attempt to clean-up a chemical spill, if inhalation exposure or skin, throat or eye irritation is a threat.

If injury or exposure occurs within the laboratory facilities, then proper first aid attention will be administered by other lab personnel as soon as possible. If the condition is serious, the victim should be transported to a medical facility as soon as possible. For chemical exposures refer to the appropriate MSDS sheet for first aid treatment. MSDS sheets shall be maintained in a readily accessible location in the lab for each chemical stored or used in the lab. If any exposure occurs while in the laboratory, a 1A1 exposure or injury form needs to be submitted to the Division of Workman's Compensation within 24 hours of exposure or injury.

SECTION 13. LITERATURE CITED

Agbeti, M.O. 1991. Relationship between diatom assemblages and trophic variables: a comparison of old and new approaches. Can. J. Fish. Aquat. Sci. 49:1171-1175.

Allen, R.K., and G.F. Edmunds. 1959. A revision of the genus Ephemerella (Ephemeroptera: Ephemerellidae). I. The subgenus Timpanoga. The Canadian Entomologist 91:51-58.

Allen, R.K., and G.F. Edmunds. 1961. A revision of the genus Ephemerella (Ephemeroptera: Ephemerellidae). III. The subgenus Attenuatella. Journal of the Kansas Entomological Society 34:161-173.

Allen, R.K., and G.F. Edmunds. 1962. A revision of the genus Ephemerella (Ephemeroptera: Ephemerellidae). V. The subgenus Drunella in North America. Miscellaneous Publications of the Entomological Society of America 3:583-600.

Allen, R.K., and G.F. Edmunds. 1963. A revision of the genus Ephemerella (Ephemeroptera: Ephemerellidae). VI. The subgenus Seratella in North America. Annals of the Entomological Society of America 56:583-600.

Allen, R.K., and G.F. Edmunds. 1965. A revision of the genus Ephemerella (Ephemeroptera: Ephemerellidae). VIII. The subgenus Ephemerella in North America. Miscellaneous Publications of the Entomological Society of America 4:243-282.

Aloi, J.E. 1990. A critical review of recent freshwater periphyton field methods. Can. J. Fish. Aquat. Sci. 47:656-670.

American Public Health Association. 1998. Standard methods for the examination of water and wastewater.
Amer. Publ. Heal. Assoc., Amer. Water Works Assoc. and Water Poll. Contr. Fed. 20th edition. Washington, D.C.

Anderson, R.O., and S.J. Gutreuter. 1983. Length, weight and associated structural indices. pp.283-300.
in Fisheries Techniques. L.A. Nielson and D.L. Johnson (eds.). American Fisheries Society, Bethesda, MD.

Angermeier, P.L., R.A. Smogor, and J.R. Stauffer. 2000. Regional frameworks and candidate metrics for
assessing biotic integrity in the Mid-Atlantic Highland streams. Tran. Am. Fish. Soc. 129: 962-981.

Anonymous. 1967. Environmental requirements of blue-green algae. Proceedings of a symposium,
1966 September 23-24. Univ. of Wash. And Fed. Water Poll. Contr. Admin., Pac. NW Water Lab., Corvallis, OR.

Archibald, R.G.M. 1972. Diversity in some South African diatom associations and its relation to water quality. Wat. Res. 6:1229-1238.

Bahls, L.L. 1992. Periphyton bioassessment methods for Montana streams. Mont. Dept. of Health and
Env. Services. Water Qual. Bur. Helena, MT.

Bahls, L.L., R. Burkantis, and S. Tralles. 1992. Benchmark biology of Montana reference streams. Dept. of Health and Envir. Sci., MT Water Qual. Bur., Helena, MT.

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J. B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates, and fish, second edition.
EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water, Washington, D.C.

Barbour, M.T., J.L. Plafkin, B.P. Bradley, C.G. Graves, and R.W. Wisseman. 1992. Evaluation of EPA's rapid bioassessment benthic metrics: metric redundancy and variability among reference stream sites. Environ. Toxicol. Chem., 11:437-449.

Bastian. 1996. A framework for biological criteria for Florida streams using benthic macroinvertebrates. J. North Am. Benthol. Soc. 15(2):185-211.

Bednarik, A.F., and W.P. McCafferty. 1979. Biosystematic revision of the genus Stenonema (Ephemeroptera:Heptageniidae). Canadian Bulletin of Fisheries and Aquatic Sciences 201:1-73.

Bennett, H.D. 1969. Algae in relation to mine water. Castanea 34:306-327.

Berkman, H.E., and C.F. Rabeni. 1987. Effect of siltation on stream fish communities. Env. Bio. of fishes. 18: 285-294.

Bjorn, T.C., M.A. Brusven, M.P. Moinau, J.H. Milligan, R.A. Klamt, E. Chacho, and C. Schaye. 1977. Transport of granitic sediment in streams and its effects on insects and fish. Univ. of Idaho, For. Wildl. Range Exp. Sta., Bull. No. 17.

Bode, R.W. 1988. Quality assurance work plan for biological stream monitoring in New York State. New York State Dept. Environ. Conserv., Albany, NY.

Bonar, S.A., and W. A. Hubert. 2001. Standard sampling of inland fish: Benefits, challenges, and a call for action. Fisheries. 27: (3) 10-16.

Brigham, A.R., W.U. Brigham, and A. Gnilka (eds.). 1982. Aquatic insects and Oligochaetes of North and South Carolina. Midwest Aquatic Enterprises, Mahomet, IL.

Brinkhurst, R.O. 1986. Guide to the freshwater microdrile Oligochaetes of North America. Canada Special Publications Fisheries Aquatic Science 84:1-259.

Brown, H.P. 1972. Aquatic dryopoid beetles (Coleoptera) of the United States. Biota of freshwater ecosystems identification manual no. 6. Water Pollution Control Research Series, EPA, Washington, D.C.

Brown, H.P., and D.S. White. 1978. Notes on Separation and Identification of North American Riffle
Beetles (Coleoptera:Dryopoidea:Elmidae). Entomological News 89:1-13.

Burch, J.B. 1972. Freshwater sphaeriacean clams (Mollusca: Pelecypoda) of North America.

Burns, R.G., and J.D. Hewlett. 1983. A decision model to predict sediment yield from forest practices. Water Res. Bull., 19: 9-14.

Burr, B.M., and M.L. Warren Jr. 1986. A distributional atlas of Kentucky fishes. Kentucky State Nature Preserves Comm. Sci. Tech. Ser. 4.

Cairns, J., Jr., and J.R. Pratt. 1993. A history of biological monitoring using benthic macroinvertebrates. Pages 10--27 in D.M. Rosenberg and V.H. Resh (eds). Freshwater biomonitoring and benthic macroinvertebrates. Chapman and Hall, New York, NY.

Camburn, K.E., R.L. Lowe, and D.L. Stoneburner. 1978. The haptobenthic diatom flora of Long Branch Creek, South Carolina. Nova Hed. 30:149-279.

Carle, F.L., and P.A. Lewis. 1978. A new species of Stenonema (Ephemeroptera:Heptageniidae) from Eastern North America. Annals of the Entomological Society of America 71:285-288.

Ceas, P.A., and L.M. Page. 1997. Systematic studies of the Etheostoma spectabile complex (Percidae: Subgenus Oligocephalus), with description of four new species. Copeia 3: 496-522.

Cicerello, R.R., and G.A.Schuster. 2003. A guide to the freshwater mussels of Kentucky. KSNPC Scientific and Technical Series 7:1-62.

Cicerello, R.R., M.L. Warren, Jr., and G.A. Schuster. 1991. A distributional checklist of the freshwater unionids (Bivalvia: Unionoidea) of Kentucky. Am Malac. Bull. 8: 113-129.

Clay, W.M. 1975. The fishes of Kentucky. Kentucky Department of Fish and Wildlife Resources, Frankfort, KY.

Collins, G.B., and C.I. Weber. 1978. Phycoperiphyton (algae) as indicators of water quality. Trans. Amer. Micros. Soc., 97:36-43.

Collins, G.B., and R.G. Kalinsky. 1977. Studies on Ohio diatoms: I. Diatoms of the Scioto River Basin. Bull. Ohio Biol. Sur. 5(3):1-45.

Compton, M.C., G.J. Pond, and J.F. Brumley. 2003. Development and application of the

Kentucky Index of Biotic Integrity (KIBI). Kentucky Department for Environmental Protection, Division of Water, Frankfort, KY.

Cooper, J.M., and J.L. Wilhm. 1975. Spatial and temporal variability in productivity, species diversity, and pigment diversity of periphyton in a stream receiving domestic and oil refinery effluents. SW Nat. 19:413-428.

Cummings, K.S., and C.A. Meyer. 1992. Field guide to freshwater mussels of the Midwest. INHS Manual 5. 194 pp.

Cummins, K.W. 1973. Trophic relations of aquatic insects. Ann. Rev. Entomol., 18: 183-206.

Cummins, K.W. 1962. An evaluation of some techniques for the collection and analysis of benthic samples with special emphasis on lotic waters. Amer. Midl. Nat., 67: 477-504.

Czarnecki, D.B., and D.W. Blinn. 1978. Diatoms of the Colorado River in Grand Canyon National Park and vicinity. (Diatoms of Southwestern USA II). Biblio. Phyc. 38. J. Cramer. Berlin, Germany.

Del Giorgio, P.A., A.L. Vinocur, R.J. Lombardo, and H.G. Tell. 1991. Progressive changes in the structure and dynamics of the phytoplankton community along a pollution gradient in a lowland river – a multivariate approach. Hydrobiol. 224:129-154.

Descy, J.P. 1979. A new approach to water quality estimation using diatoms. Nova Hedwigia 64: 305-323.

Deshon, J.E. 1995. Development and application of the invertebrate community index (ICI). Pages 217-243 in W.S. Davis and T.P. Simon (eds.). Biological assessment and criteria: Tools for water resource planning and decision making. Lewis Publishers, Boca Raton, FL.

Dillard, G.E. 1989b. Freshwater algae of the Southeastern United States. Part 2. Chlorophyceae: Ulotrichales, Microsporales, Cylindrocapsales, Sphaeropleales, Chaetophorales, Cladophorales, Schizogoniales, Siphonales, and Oedogoniales. Biblio. Phyc. 83. J. C

Dillard, G.E. 1990. Freshwater algae of the Southeastern United States. Part 3. Chlorophyceae: Zygnematales: Zygenmataceae, Mesotaeniaceae, and Desmidaceae (Section 1). Biblio. Phyc. 85. J. Cramer. Berlin, Germany.

Dillard, G.E. 1991. Freshwater algae of the Southeastern United States. Part 4. Chlorophyceae: Zygnemateles: Desmidaceae (Section 2). Biblio. Phyc. 89. J. Cramer. Berlin, Germany.

Dillard, G.E. 1989a. Freshwater algae of the Southeastern United States. Part 1. Chlorophyceae: Volvocales, Testrasporales, and Chlorococcales. Biblio. Phyc. 81. J. Cramer. Berlin, Germany

Dillard, G.E. 1993. Freshwater algae of the Southeastern United States. Part 6. Chlorophyceae: Zygnematales: Desmidiaceae (Section4). Biblio. Phyc. 93. J. Cramer. Berlin, Germany

Dixit, S.S., J.P. Smol, J.C. Kingston, and D.F. Charles. 1992. Diatoms: Powerful indicators of environmental change. Environ. Sci. Tech. 26(1):23-33.

Douglas, B. 1958. The ecology of the attached diatoms and other algae in a small stony stream. J. Ecology 46:295-322.

Dunn, H.L. 1999. Development of strategies for sampling freshwater mussels (Bivalvia:Unionidae). Proceedings of the First Freshwater Mollusk Conservation Society Symposium, pages 161-167.

Eaton, L.E., and D.R. Lenat. 1991. Comparison of a rapid bioassessment method with North Carolina's qualitative macroinvertebrate collection method. J.N. Am. Benthol. Soc., 10:335-338.

Elliott, J.M. 1983. Statistical analysis of samples of benthic invertebrates. Second Ed. Freshwater Bio. Assoc. Sci. Pub. No. 25.

Emery, E. B., T. P. Simon, F. H. McCormick, P. A. Angermeier, J. E. DeShon, C. O. Yoder, R. E. Sanders, W. D. Pearson, G. D. Hickman, R. J. Reash, and J. A. Thomas. 2003. Development of a multimetric index for assessing the biological condition of the Ohio River. Trans. Am. Fish. Soc. 132: 791-808.

Entwisle, T.J., J.A. Sonneman, and S.H. Lewis. 1997. Freshwater algae in Australia: a guide to conspicuous genera. Sainty and Assoc. Pty. Ltd., Potts Point, Australia.

Epler, J. H. 1995. Identification manual for the larval Chironomidae (Diptera) of Florida. Revised edition. FL Dept. Environ. Protection, Tallahassee, FL. 317 pp.

Epler, J.H. 1996. Identification manual for the water beetles of Florida (Coleoptera: Dryopidae, Dytiscidae, Elmidae, Gyrinidae, Haliplidae, Hydraenidae, Hydrophilidae, Noteridae, Psephenidae, Ptilodactylidae, Scirtidae). FL Dept. Environ. Protection, Tallahassee, FL.

Epler, J.H. 2001. Identification manual for the larval Chironomidae (Diptera) of North and South Carolina. NC Dept. of Environ. And Nat. Res., Raleigh, NC.

Etnier, D.A., and W.C. Starnes. 1993. The fishes of Tennessee. University Tennessee Press, Knoxville, TN.

Etnier, D.A., and W.C. Starnes. 1993. The fishes of Tennessee. University Tennessee Press, Knoxville, TN.

Fausch, K.D., J.R. Karr, and P.R. Yant. 1984. Regional application of an index of biotic integrity based on stream fish communities. Tran. Am. Fish. Soc. 113: 39-55.

Federal Register. 1979. Polychlorinated biphenyls (PCBs): reduction of tolerances. Volume 44

(127): 38330-38340 Friday, June 29, 1979 Rules.

Ferrington, L.C. 1987. Collection and identification of floating exuviae of chronomidae for use in studies of surface water quality. SOP No. FW130A, U.S. Environmental Protection Agency, Region VII, Kansas City, KS.

Flannegan, J.F. 1975. Sorting techniques for the estimation of epiphytic invertebrate populations. Fresh.Biol. 16(2): 161-173.

Flint, O.S. 1984. The genus Brachycentrus in North America, with a proposed phylogeny of the genera of Brachycentridae (Trichoptera). Smithsonian Contributions to Zoology.

Flint, O.S., Jr. 1960. Taxonomy and biology of nearctic limnephilid larvae (Trichoptera), with special reference to species in Eastern United States. Entomologicia Americana XL:1-117.

Flint, O.S., Jr.. 1962. Larvae of the caddis fly genus Rhyacophila in Eastern North America (Trichoptera: Rhyacophilidae). Proceedings of the United States National Museum 113:465-493.

Floyd, M.A. 1995. Larvae of the caddisfly genus Oecetis (Trichoptera: Leptocerida) in North America. Ohio Biol. Surv. Bull. Vol. 10 No. 3.

Fullington, K.E., and K.W. Stewart. 1980. Nymphs of the stonefly genus Taeniopteryx (Plecoptera: Taeniopterygidae) of North America. Journal of the Kansas Entomological Society 53(2):237-259.

Fullner, R.W. 1971. A comparison of macroinvertebrates collected by basket and modified multiple-plate samplers. J. Water Poll. Contr. Fed. 43: 494-499.

Funk, D.H., and B.W. Sweeney. 1994. The larvae of eastern North American Eurylophella Tiensuu (Ephemeroptera: Ephemerellidae). Trans. of the American Entomological Society 120:209-286.

Gauch, H.G. 1982. Multivariate analysis in community ecology. Cambridge Univ. Press. 298 pp.

Glover, J.B. 1996. Larvae of the caddisfly genera Triaenodes and Ylodes (Trichoptera: Leptoceridae) in North America. Ohio Biol. Surv. Bull. Vol. 11 No. 2

Goldstein, R.M., and T.P. Simon. 1999. Toward a united definition of guild structure for feeding ecology of North American freshwater fishes. Pages 123-202 in T.P Simon (editor). Assessing the sustainability and biological integrity of water resources using fish communities. CRC Press, Boca Raton, FL.

Gordon, N.D., T.A. McMahon, and B.L. Finlayson. 1992. Stream hydrology: an introduction for Great Lakes Sport Fish Advisory Task Force (GLSFATF). [1993]. Protocol for a uniform Great Lakes Sport Fish Consumption Advisory, September 1993 Draft. Great Lakes Sport Fish

Advisory Task Force, Council of Great Lakes Governors, Chicago, IL. 81p. MESB-FP 9/16/94.

Harker, D.F., Jr., M.L. Warren, Jr., K.E. Camburn, and R.R. Cicerello. 1981. Aquatic biota and water quality survey of the western Kentucky coal field, Vol. 1-2. Tech. Report. Kentucky Nature Preserves Commission, Frankfort, KY, USA.

Harker, D.F., Jr., M.L. Warren, Jr., K.E. Camburn, S.M. Call, G.F. Fallo, and P. Wigley. 1980. Aquatic biota and water quality survey of the upper Cumberland River basin, Vol. 1-3. Tech. Report. Kentucky Nature Preserves Commission, Frankfort, KY, USA.

Harker, D.F., Jr., S.M. Call, M.L. Warren, Jr., K.E. Camburn, and P. Wigley. 1979. Aquatic biota and water quality survey of the Appalachian Province, eastern Kentucky. KNPC; Frankfort, KY, Tech. Rept.

Harrelson, C.C., C.L. Rawkins, and J.P. Potyondy. 1994. Stream Channel Reference Sites: An llustrated Guide to Field Technique. Gen. Tech. Rep. RM-245. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station. 61p.

Hawkins, C. P., M.L. Murphy, and N.H. Anderson. 1982. Effects of canopy, substrate composition and gradient on the structure of macroinvertebrate communities in Cascade Range streams of Oregon. Ecology, 63: 1840-1856.

Hewlett, J.D. 1978. Forest water quality: an experiment in harvesting and regenerating Piedmont forest. Univ. of Georgia, Athens, GA.

Hilsenhoff, W.L. 1977. Use of arthropods to evaluate water quality of streams. Tech. Bull. No. 100, Dept. Nat. Res., Madison, WI.

Hilsenhoff, W.L. 1982. Using a biotic index to evaluate water quality in streams. Tech. Bull. 132. Dept. Nat. Res., Madison, WI.

Hilsenhoff, W.L. 1987. An improved biotic index of organic stream pollution. Great Lakes Entomol. 20: 31-39.

Hilsenhoff, W.L. 1988. Seasonal correction factors for the biotic index. Great Lakes Entomol. 21: 9-13.

Hilsenhoff, W.L. 1973. Notes on Dubiraphia (Coleoptera: Elmidae) with descriptions of five new species. Annals of the Entomological Society of America 66:55-61.

Hitchcock, S.W. 1974. Guide to the insects of Connecticut: Part VII. The Plecoptera or stoneflies of Connecticut. State Geological and Natural History Survey of Connecticut Bulletin 107:191-211.

Hobbs, H.H., Jr. 1972. Crayfishes (Astacidae) of North and Middle America. Biota of freshwater

ecosystems identification manual no. 9. Water Pollution Control Research Series, E.P.A., Washington, D.C.

Hohn, M.H., and J. Hellerman. 1963. The taxonomy and structure of diatom populations from three North American rivers using three sampling methods. Trans. Am. Microsc. Soc. 82:250-329.

Holsinger, J.R. 1972. The freshwater amphipod crustaceans (Gammaridae) of North America. Biota of freshwater ecosystems identification manual no. 5. Water Pollution Control Research Series, E.P.A., Washington, D.C.

Hornig, C.E., and J.E. Pollard. 1978. Macroinvertebrate sampling techniques for streams in semi-arid regions. Comparison of surber method and unit-effort traveling kick method. Off. Res. Devel., Environ. Monit. Sup. Lab., EPA 600/4-78-040. U.S. Environmental Protection Agency, Las Vegas, NV.

Hudson, P.A., D.R. Lenat, B.A. Caldwell, and D. Smith. 1990. Chironomidae of the southeastern United States: a checklist of species and notes on biology, distribution, and habitat. Fish and Wildlife Research. 7:1-46.

Hughes R.M., D.P. Larsen, and J.M. Omernik. 1986. Regional reference sites: a method for assessing stream potentials. Environmental Management 10(5): 629-635.

Hughes, R.M., and T. Oberdorff. 1999. Applications of IBI concepts and metrics to waters outside the United States and Canada. Pages 79-93 in T.P Simon (editor). Assessing the sustainability and biological integrity of water resources using fish communities. CRC Press, Boca Raton, FL.

Hughes, R.M., P.R. Kaufmann, A.T. Herlihy, T.M. Kincaid, L. Reynolds, and D.P. Larsen. 1998. A process for developing and evaluating indices of fish assemblage integrity. Can. J. Fish. and Aqu. Sci.. 55: 1618-1631.

Hulbert, S.H. 1971. The nonconcept of species diversity: a critique and alternative parameters. Ecol. 52(4):577-586.

Hustedt, F. 1927-1966. Die kieselalgen in Rabenhorst's Kryptogamen-flora von Deutsch. Ost. Und der Schw. VII. Leipzig, W. Germany.

Hustedt, F. 1930. Bacillariophyta (Diatomeae) in Pascher, A. (ed). Die suswasser flora Mitteleuropas. (The freshwater flora of middle Europe). Gustav Fischer Verlag, Jena, Germany.

Hynes, H.B.N. 1975. The stream and its valley. Internationale Vereinigung fur Theoretische und Angewandte Limnologie Verhandlungen 19: 1-15.

Jarrett, G.L., and J.M. King. 1989. The diatom flora (Bacillariphyceae) of Lake Barkley. U.S. Army Corps of Engineers; Nashville Dist.; #DACW62-84-C-0085.

Jenkins, R.E., and N.M. Burkhead. 1993. Freshwater fishes of Virginia. American Fisheries Society, Bethesda, MD.

Johnson, R.R., and J.F. McCormick. 1978. Strategies for protection and management of floodplain wetlands and other riparian ecosystems, Proceedings of a Symposium. U.S. Dept. of Ag., For. Serv., Gen. Tech. Rep. WO-12, Washington, D.C.

Karr, J.R. 1981. Assessment of biotic integrity using fish communities. Fisheries 6: 21-27.

Karr, J.R. and I.J. Schlosser. 1978. Water resources and the land-water interface. Science, 201: 229-234.

Karr, J.R., and I.J. Schlosser. 1977. Impact of nearstream vegetation and stream morphology on water quality and stream biota. U.S. Environmental Protection Agency, Off. of Res. and Devel., Athens, GA. EPA 600/3-77-097.

Karr, J.R., K.D. Fausch, P.L. Angermeier, P.R. Yant, and I.J. Schlosser. 1986. Assessing biological integrity in running waters: a method and its rationale. Illinois Natural History Survey, Champaign, IL, Special Publication 5.

Karr, J.R., L.A. Toth, and G.D. Garman. 1981. Habitat preservation for Midwest stream fishes, principles and guidelines. U.S. EPA, Off. of Res. and Devel., Corvallis, OR.

Kenk, R. 1972. Freshwater planarians (Turbellaria) of North America. Biota of freshwater ecosystems identification manual no. 1. Water Pollution Control Research Series, U.S. Environmental Protection Agency, Washington, D.C.

Kentucky, Commonwealth of. 2005. Water quality standards (401 KAR 5:031). Frankfort, KY

Kentucky Department for Environmental Protection (KDEP). 2005. Quality Management Plan. Environmental and Public Protection Cabinet, Department for Environmental Protection, Frankfort, KY.

Kentucky Division of Water (KDOW). 1997. Reference reach fish community report. Tech. Rep. 52. Water Quality Branch, Frankfort, KY.

Kentucky Division of Water (KDOW) 2008 (in draft). Standard Operating Procedures for Sampling and Monitoring Surface Waters in Kentucky. Environmental and Public Protection Cabinet, Department for Environmental Protection, Frankfort, KY

Kentucky Division of Water (KDOW). 2005. Kentucky Ambient/Watershed Water Quality Monitoring Standard Operating Procedure Manual. Environmental and Public Protection Cabinet, Department for Environmental Protection, Frankfort, KY.

Kentucky Division of Water and National Park Service. 1992. Kentucky Rivers Assessment. General Services Administration, Printing and Distribution Branch. Atlanta, GA. 264 pp.

Kentucky Division of Water. 1986. Quality assurance guidelines. Kentucky Dept. Env. Prot., Division of Water, Frankfort, KY.

Kentucky Division of Water. 1999. Kentucky Nonpoint Source Assessment Report. Kentucky Department for Environmental Protection, Division of Water. Frankfort, KY.

Kentucky Division of Water. 2005. Quality Management Plan. Environmental and Public Protection Cabinet, Department for Environmental Protection, Division of Water. Frankfort, KY.

Kentucky Nature Preserves Commission. 1982. Recommendations for Kentucky's Outstanding Resource Water Classifications with Water Quality Criteria for Protection. Kentucky Nature Preserves Commission. Frankfort, KY. 459 pp.

Kentucky Nature Preserves Commission. 1983. Fish collection catalogue. Kentucky Nature Preserves Commission. Frankfort, KY.

Kirchner, R.F., and B.C. Kondratieff. 1985. The nymph of Hansonoperla appalachia Nelson (Plecoptera: Perlidae). Proceedings of the Entomological Society of Washington. 87(3):593-596.

Klemm, D.J. 1972. Freshwater leeches (Annelida: Hirudinea) of North America. Biota of freshwater ecosystems identification manual no. 8. Water Pollution Control Research Series,

Klemm, D.J. 1982. Leeches (Annelida:Hirudinea) of North America. EPA-600/3-82-025. Office of Research and Development, Cincinnati, OH.

Klemm, D.J., P.A. Lesis, F. Fulk, and J.M. Lazorchak. 1992. Macroinvertebrate field and laboratory methods for evaluating the biological integrity of surface waters. U.S. Environmental Protection Agency; Off. Res. Devel.; Cincinnati, OH. EPA/600/4-90-030.

Klemm, D.J., P.A. Lewis, F. Fulk, and T.M. Lazorchak. 1990. Macroinvertebrate field and laboratory methods for evaluating the biological integrity of surface waters. U.S. Environmental Protection Agency, Aquat. Biol. Branch and Devel. Eval. Branch, Qual. Assur. Res. Div., Environ. Syst. Lab., EPA/600/4-901/030, Cincinnati, OH.

Kondratieff, B.C., R.F. Kirchner, and J.R. Voshell Jr. 1981. Nymphs of Diploperla. Annals of the Entomological Society of America 74:428-430.

Krammer, K., and H. Lange-Bertalot. 1986-1991. Susswasserflora von Mitteleuropa. Band 2. Parts 1-5. Bacillariophyceae. Gustav Fischer Verlag. Stuttgart, Germany.
Kuchler, A.W. 1966. Potential natural vegetation. Map, Scale-1:7,500,000. USDA Forest Service. Washington, D.C.

Kuehne, R.A., and R.W. Barbour. 1983. The American darters. University of Kentucky Press, Lexington, KY.

Lago, P.K., and S.C. Harris. 1987. The Chimarra (Trichoptera: Philopotamidae) of eastern North America with descriptions of three new species. Journal of the New York Entomological Society 95:225-251.

Lange-Bertalot, H. 1979. Pollution tolerance as a criterion for water quality estimation. Nova Hed. 64:283-304.

Lange-Bertalot, H. 1980. New species, combinations and synonyms in the genus Nitzschia. Bacillaria 3:41-77.

Lange-Bertalot, H., and R. Simonsen. 1978. A taxonomic revision of the Nitzschia lanceolatae Grunow: 2. European and related extra-European freshwater and brackish water taxa. Bacillaria 1:11-111.

Lazorchak, J.M., D.J. Klemm, and D.V. Peck (editors). 1998. Environmental Monitoring and Assessment Program-Surface Waters: Field Operations and Methods for Measuring Ecological Condition of Wadeable Streams. EPA/620/R-94/004F. U.S. Environmental Protection Agency, Washington, D.C.

Leclercq, L., and B. Maquet. 1987. Deux nouveaux indices diatomique et de qualite chimique des eaux courantes. Comparison avec differents indices existants. Cah. Biol. Mar. 28:303-310.

Lenat, D.R. 1988. Water quality assessment of streams using a qualitative collection method for benthic macroinvertebrates. J.N. Am. Benthol. Soc. 7(3): 222-233.

Lenat, D.R. 1993. A biotic index for the southeastern United States: derivation and list of tolerance values, with criteria for assigning water-quality rating. J.N. Am. Benthol. Soc., 12(3): 279-290.

Likens, G.E., and F.H. Bormann. 1974. Linkages between aquatic and terrestrial ecosystems. Bioscience 21: 447-456.

Lowe, R.L. 1974. Environmental requirements and pollution tolerance of freshwater diatoms. U.S. Environmental Protection Agency; Nat. Environ. Res. Center; Off. Res. Devel.; Cincinnati, OH. EPA-670/4-74-005.

Maret, T.R. 1999. Characteristics of fish assemblages and environmental conditions in streams of the Upper Snake River basin, in eastern Idaho and western Wyoming. Pages 273-300 in T.P

Mason, W.T., J.B. Anderson, and G.E. Morrison. 1967. Limestone-filled, artificial substrate sampler-float unit for collecting macroinvertebrates in large streams. Prog. Fish. Cult., 29:74.

Matthews, R.A., P.F. Kondratieff, and A.L. Buikema, Jr. 1980. A field verification of the use of the autotrophic index in monitoring stress effects. Bull. Environ. Contam. Tox. 25:226-233.

McAlpine, J.F., B.V. Peterson, G.E. Shewell, H.J. Teskey, J.R. Vockeroth, and D.M. Wood (coords.). 1981. Manual of nearctic Diptera, Vol. 1. Research Branch of Agriculture Canada, Monograph 27.

McAlpine, J.F., B.V. Peterson, G.E. Shewell, H.J. Teskey, J.R. Vockeroth, and D.M. Wood (coords.). 1987. Manual of nearctic Diptera, Vol. 2. Research Branch of Agriculture Canada, Monograph 28.

McAlpine, J.F., B.V. Peterson, G.E. Shewell, H.J. Teskey, J.R. Vockeroth, and D.M. Wood (coords.). 1989. Manual of nearctic Diptera, Vol. 3. Research Branch of Agriculture Canada, Monograph 28.

McCafferty, W.P. 1975. The burrowing mayflies (Ephemeroptera: Ephemeroidea) of the United States. Trans. of the American Entomological Society 101:447-504.

McCafferty, W.P., and Y.J. Bae. 1990. Anthopotamus, a new genus for North American species previously known as Potamanthus (Ephemeroptera: Potamanthidae). Entomological News 101(4):200-202.

McCafferty, W.P., M.J. Wigle, and R.D. Waltz. 1994. Contributions to the taxonomy and biology of Acentrella turbida (McDunnough) (Ephemeroptera: Baetidae). Pan-Pacific Insects 70:301-308.

McCormick, F.H., R.H. Hughes, P.R. Kaufmann, D.V. Peck, J.L. Stoddard, and A.T. Herlihy. 2001. Development of an index of biotic integrity for the Mid-Atlantic Highlands Region. Tran. Am. Fish. Soc. 130: 857-877.

Merritt, R.W., and K.W. Cummins (editors). 1996. An introduction to the aquatic insects of North America, 3rd ed. Kendall/Hunt Publishing Company, Dubuque, IA.

Mettee, M.F., P.E. O'Neil, and J. M. Pierson. 1996. Fishes of Alabama and the Mobile basin. Oxmoor House Inc., Birmingham, AL.

Mills, M.R. compiler. 1988. Fish collection catalogue of the Kentucky Division of Water (1976-1987). Technical Report No. 30. Kentucky Department for Environmental Protection, Kentucky Division of Water, Frankfort, KY.

Minns, C.K., V.W. Cairns, R.G. Randall, and J.E. Moore. 1994. An index of biotic integrity (IBI) for fish assemblages in the littoral zone of Great Lakes' areas of concern. Can. J. Fish. and Aqu. Sci.. 51: 1804-1822.

Morihara, D.K., and W.P. McCafferty. 1979. The Baetis larvae of North America (Ephemeroptera: Baetidae). Trans. of the American Entomological Society 105:139-221.

National Native Mussel Conservation Committee (NNMCC). 1998. National strategy for the conservation of native freshwater mussels. J. of Shellfish Research, Vol 17, No 5: 1419-1428.

Needham, J.G., and P.R. Needham. 1962. A guide to the study of fresh-water biology. 5th ed. Holden-Day, Inc., San Francisco, CA.

Needham, J.G., M.J. Westfall, and M.L. May. 2000. Dragonflies of North America. Scientific Publishers. Gainesville, FL.

Newbold, J.D., D.C. Erman, and K.B. Roby. 1980. Effects of logging on macroinvertebrates in streams with and without buffer strips. Can. J. Fish. Aquat. Sci., 37: 1076-1085.

Nuzzo, R.M. 1986. Macroinvertebrate rapid assessment methodology. Massachusetts Div. Water Poll. Contr., Westboro, MA.

Ohio Environmental Protection Agency. 1987. Biological criteria for the protection of aquatic life. Vol. II. Users manual for biological assessment of Ohio surface waters. Division of Water Quality Monitoring and Assessment, Surface Waters Section, Columbus, OH.

Ohio Environmental Protection Agency. 1987. Biological criteria for the protection of aquatic life: Vol. III. Standardized biological field sampling and laboratory methods for assessing fish and macroinvertebrate communities. Div. Water Qual. Monit. Assess., Columbus, OH.

Omernik, J.M. 1987. Ecoregions of the conterminous United States. Ann. Assoc. Amer. Geog. 77:118-125

Onorato, D. P., R. A. Angus, and K. R. Marion. 1998. Comparison of a small-mesh seine and a backpack electroshocker for evaluating fish populations in a North-Central Alabama stream. N. Am. J. Fish. Mang. 18: 361-373.

Page, L.M. 1983. Handbook of darters. Tropical Fish Hobbyist Publications, Neptune City, NJ.

Page, L.M., and B.M. Burr. 1991. A field guide to freshwater fishes, North America north of Mexico. Houghton Mifflin Company, Boston, MA.

Palmer, C.M. 1977. Algae and water pollution. An illustrated manual on the identification, significance, and control of algae in water supplies and polluted water. U.S. Environmental Protection Agency; Municip. Environ, Res. Lab.; Off. Res. Devel.; Cincinnati, OH. EPA-600/9-77-036.

Parmalee, P.W., and A.E. Bogan. 1998. The freshwater mussels of Tennessee. The University of Tennessee Press, Knoxville, TN.

Patrick, R. 1973. Use of algae, especially diatoms, in the assessment of water quality. In Biological methods for the assessment of water quality. Amer. Soc. Test. And Mat. Pub. 528:76-

95.

Patrick, R. 1975. The diatoms of the United States, exclusive of Alaska and Hawaii. Monograph No. 13. Volume 2, part 1. Acad. Nat. Sci. Phila., Philadelphia, PA.

Patrick, R. 1977. Ecology of freshwater diatoms and diatom communities. In D. Werner, ed. The biology of diatoms. Botan. Mono. Vol. 13, Blackwell Sci. Pub.

Patrick, R., and C. W. Reimer. 1966. The Diatoms of the United States, exclusive of Alaska and Hawaii. Monograph No. 13. Acad. Nat. Sci. Phila., Philadelphia, PA.

Patrick, R., and C. W. Reimer. 1975. The Diatoms of the United States. Vol. 2, Part 1. Monograph No. 13. Acad. Nat. Sci. Phila., Philadelphia, PA.

Patrick, R., and C. W. Reimer. 1978. How to know the freshwater algae. 3rd Ed. Wm. C. Brown Co., Dubuque, IA.

Patrick, R., M.H. Hohn, and J.H. Wallace. 1954. A new method for determining the pattern of the diatom flora. Notulae Naturae. Acad. Nat. Sci. Philadelphia 259:1-12.

Payne, B.S., A. C. Miller, and R. Whiting. 1993. Designing a riverine mussel survey. Pages 150-156 in K.S. Cummings, A.C. Buchanan, and L.M. Koch, eds. Conservation and Management of Freshwater Mussels. Proceedings of a UMRCC Symposium, 12-14 October 1992, St. Louis, Missouri. Upper Mississippi River Conservation committee, Rock Island, IL.

Pennak, R. W. 1989. Freshwater invertebrates of the United States, 3rd edition. J. Wiley & Sons, New York.

Perkins, J.L. 1983. Bioassay evaluation of diversity and community comparison indexes. J. Water Poll. Contr. Fed. 55(5):522-530.

Pescador, M. L., and L. Berner. 1980. The mayfly family Baetiscidae (Ephemeroptera). Part II Biosystematics of the Genus Baetisca. Trans. American Entomological Society 107:163-228.

Pfankuch, D.J. 1978. Stream reach inventory and channel stability evaluation. U.S. Dept. Ag., for. Serv., Northern Reg., Missoula, MT.

Pflieger, W.L. 1975. The fishes of Missouri. Missouri Department of Conservation, Columbia, MO.

Phillips, D.J.H. 1980. Quantitative Aquatic Biological Indicators. Pollution Monitoring Series. Applied Science Publishers Ltd., London, England.

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross, and R.M. Hughes. 1989. Rapid bioassessment protocols for use in streams and rivers: benthic macroinvertebrates and fish. U.S. Environmental Protection Agency, Off. of Water, EPA/444/4-89-001, Washington, D.C.

Platts, W.S. 1978. Livestock grazing and riparian/stream ecosystems - an overview. in O.B. Cope ed. Proceedings of the forum-grazing and riparian/stream ecosystems. 34-39. Trout Unlimited, Inc. Vienna, VA.

Platts, W.S. 1981a. Sheep and cattle grazing strategies on riparian stream environments. In: Proceeding of the wildlife livestock relationships symposium pp. 251-270. U.S. Dept. Ag., For.Serv., Inter-mountain Exp. Sta., Boise, ID.

Platts, W.S. 1981b. Streamside management to protect bank-channel stability and aquatic life, pages 245-555 In: Interior west watershed management, proceedings of a symposium. WA State Univ., Coop, Ext., Pullman, WA.

Platts, W.S. 1982. Livestock and riparian-fishery interactions: what are the facts? Trans. 47th N. American Wild. and Nat. Res. Conf., pp. 507-515.

Platts, W.S., W.F. Megahan, and G. W. Minshall. 1983. Methods for evaluating stream riparian and biotic conditions, U.S. Dept. Ag., For. Serv., Intermountain For. and Range Exp. Sta.,

Pond, G.J., and S.E. McMurray. 2002. A macroinvertebrate bioassessment index for headwater streams of the Eastern Coalfield Region, Kentucky. Kentucky Department for Environmental Protection, Kentucky Division of Water. Frankfort, KY.

Pond, G.J., J.F. Brumley, and R.E. Houp. 2000. Preliminary ordination of stream organisms in Kentucky. Abstract in Bull. North Am. Benthol. Soc. 17(1):448.

Pond, G.J., S.M. Call, J.F. Brumley, and M.C. Compton. 2003. The Kentucky macroinvertebrate bioassessment index: derivation of regional narrative ratings for assessing wadeable and headwater streams. Kentucky Dept. for Env. Prot., Kentucky Div. of Water, Frankfort, KY.

Pond, G.J., S.M. Call, J.F. Brumley, and M.C. Compton. 2003. Preliminary ordination of stream organisms in Kentucky. Abstract in Bull. North Am. Benthol.Soc. 17(1): 448.

Pontasch, K.W., and M.A. Brusven. 1988. Diversity and community comparison indices: assessing macroinvertebrate recovery following a gasoline spill. Water Res. 22(5):619-626.

Pontasch, K.W., E.P. Smith, and J. Cairns, Jr. 1989. Diversity indices, community comparison indices, and canonical discriminant analysis: interpreting the results of multispecies toxicity tests. Water Res. Vol. 23(10):1229-1238.

Prescott, G. W. 1962. The algae of the western Great Lakes area. Wm. C. Brown co., Dubuque, IA.

Prescott, G.W., H.T. Crousdale, and W.C. Vinyard. 1975. A Synopsis of North American desmids. Part II. Desmidaceae: Placodermae. Section 1. Univ. NE Press, Lincoln, NE.

Prescott, G.W., H.T. Crousdale, and W.C. Vinyard. 1977. A synopsis of North American desmids. Part II. Desmidaceae: Placodermae. Section 2. Univ.Nebraska Press, Lincoln, NE.

Provonsha, A.V. 1991. A revision of the genus Caenis in North America (Ephemeroptera: Caenidae). Trans. of the American Entomological Society 116:801-884.

Resh, V. H., and J. K. Jackson. 1993. Rapid assessment approaches to biomonitoring using benthic macroinvertebrates. Pages 195-223 in D. M. Rosenberg and V. H. Resh, Editors. Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman and Hall, NY.

Resh, V.H. 1976. The biology and immature stages of the caddisfly genus Ceraclea in eastern North America (Trichoptera: Leptoceridae). Annals of the Entomological Society of America 69:1039-1061.

Robinson, C.T., and G.W. Minshall. 1992. Refinement of biological metrics in the development of biological criteria for regional biomonitoring and assessment of small streams in Idaho, 1991-1992. Idaho State University, Department of Biological Sciences, Pocatello, ID.

Robison, H.W., and T.M. Buchanan. 1988. Fishes of Arkansas. University Press, Fayetteville, AR.

Rosgen, David L. 1996. Applied River Morphology, Widland Hydrology, Pagosa Springs, CO.

Rott, E. 1991. Methodological aspects and perspectives in the use of periphyton for monitoring and protecting rivers. In Whitton, B.A., E. Rott, and G. Friedrich (eds). Use of algae for monitoring rivers. Institute fur Botanik, University of Innsbruck, Austria.

Round, F.E. 1991. Diatoms in water-monitoring studies. J. Appl. Phy. 3:129-145.

Round, F.E., R.M. Crawford, and D.G. Mann. 1990. The diatoms: the biology and morphology of the genera. Cambridge Univ. Press, Cambridge, England.

Sabater, S., F. Sabater, and J. Armengol. 1988. Relationships between diatom assemblages and physico-chemical variables in the River Ter (NE Spain). Int. Rev. Ges. Hydrobiol. 73:171-179.

Schefter, P.W., and G.B. Wiggins. 1986. A systematic study of the nearctic larvae of the Hydropsyche morosa group (Trichoptera: Hydropsychidae). Miscellaneous Publications of the Royal Ontario Museum, Toronto, Canada.

Schlosser, I.J., and J.R. Karr. 1981a. Riparian vegetation and channel morphology impact on spatial patterns of water quality in agricultural watersheds. Env. Mngt., 5: 233-243.

Schlosser, I.J., and J.R. Karr. 1981b. Water quality in agricultural watersheds: impact of riparian vegetation during base flow. Water Res. Bull., 17: 233-240.

Schuster, G.A., and D.A. Etnier. 1978. A manual for the identification of the larvae of the caddisfly genera Hydropsyche Pictet and Symphitopsyche Ulmer in eastern and central North America (Trichoptera: Hydropsychidae). EPA-600/4-78-060.

Shackleford, B. 1988. Rapid bioassessments of lotic macroinvertebrate communities: biocriteria development. Arkansas Dept. Poll. Contr. and Ecol., Little Rock, AR.

Simon, T.P. 1991. Development of index of biotic integrity expectations for the ecoregions of Indiana. I. Central Corn Belt Plain. U.S. Environmental Protection Agency, Region V, Environmental Sciences Division, Monitoring and Quality Assurance Branch: Ambient Monitoring Section, Chicago, IL. EPA 905/9-91/025.

Simon, T.P. (editor). 1999. Assessing the sustainability and biological integrity of water resources using fish communities. CRC Press, Boca Raton, FL.

Simonsen, R. 1987. Atlas and catalogue of the diatom types of Friedrich Hustedt. Vol. 1-3. J. Cramer. Berlin, Germany.

Simpson, K. W., and R.W. Bode. 1980. Common larvae of Chironomidae (Diptera) from New York State streams and rivers. New York State Museum Bulletin 439:1-105.

Simpson, P. W., J. R. Newman, M.A. Keirn, R.M. Matter, and P.A. Guthrie. 1982. Manual of stream channelization impacts on fish and wildlife. U.S. Dept. of the Int., Fish and Wild. Serv., Washington, D.C. FWS/OBS-82/24.

Sladecek, V. 1973. System of water quality from the biological point of view. Arch. Hydrobiol./Ergebn. Limnol. 7:1-218.

Smith, P.W. 1979. The fishes of Illinois. University of Illinois Press, Urbana, IL.

Smogor, R.A., and P.L. Angermeier. 1999. Effects of drainage basin and anthropogenic disturbance on relations between stream size and IBI metrics in Virginia. Pages 249-272 in T.P Simon (editor). Assessing the sustainability and biological integrity of water resources using fish communities. CRC Press, Boca Raton, FL.

Smogor, R.A., and P.L. Angermeier. 2001. Determining a regional framework for assessing biotic integrity of Virginia streams. Tran. Am. Fish. Soc. 130: 18-35.

Stark, B.P. 1986. The nearctic species of Agnetina (Plecoptera: Perlidae). Journal of the Kansas Entomological Society. 59(3):437-445.

Stevenson, R.J. 1984. Epilithic and epipelic diatoms in the Sandusky River, with emphasis on species diversity and water pollution. Hydrobiol. 114:161-175.

Stevenson, R.J., and R.L. Lowe. 1986. Sampling and interpretation of algal patterns for water quality assessments. In B.G. Isom, ed. Rationale for sampling and interpretation of ecological

data in the assessment of freshwater ecosystems. Amer. Soc. Test. And Mat. ASTM STP 894:118-149.

Stevenson, R.J., M.L. Bothwell, and R.L. Lowe (eds). 1996. Algal Ecology: freshwater benthic ecosystems. Academic Press, San Diego, CA.

Stewart, K.W., and B.P. Stark. 1984. Nymphs of North American Perlodinae genera (Plecoptera: Perlodidae). The Great Basin Naturalist 44(3):373-415.

Stewart, K.W., and B.P. Stark. 1988. Nymphs of North American stonefly genera (Plecoptera). Thomas Say Foundation Series, Entomological Society of America 12:1-460.

Stober, Q.J. 1991. Guidelines for fish sampling and tissue preparation for bioaccumulative

Strange, R. M. 1999. Historical biogeography, ecology, and fish distribution: conceptual issues for establishing IBI criteria. Pages 65-78 in T.P Simon (editor). Assessing the sustainability and biological integrity of water resources using fish communities. CRC Press, Boca Raton, FL.

Strayer, D.L., S. Claypool, and S. Sprague. 1997. Assessing Unionid populations with quadrat and timed searches. Pages 163-169 in K.S. Cummings, A.C. Buchanan and L.M. Koch (eds). Conservation and Management of Freshwater Mussels II. Proceedings of a UMRCC Symposium, 16-18 Oct 1995, St. Louis, Missouri. Upper Mississippi River Conservation Committee, Rock Island, IL.

Taylor, C.A., and G.A. Schuster. 2004. The Crayfishes of Kentucky. INHS Special Publication No. 28 viii-219 pp.

Taylor, R.W. 1980. Mussels of Floyd's Fork, a small northcentral Kentucky stream (Unionidae). Nautilus 94: 13-15.

Ter Braak, Cajo J.F. 1988. CANOCO – A FORTRAN program for canonical community ordination by (partial), (detrended), (canonical) correspondence analysis, principal component analysis, and redundancy analysis (version 2.1). Ag. Math. Gr. Tech. Rep.: LWA-88-02. Wageningen, The Netherlands.

Thorp, J.H., and A.P. Covich (editors). 1991. Ecology and Classification of North American Freshwater Invertebrates. Academic Press, New York.

Thorp, J.H., and A.P. Covich (eds). 2001. Ecology and classification of North American freshwater invertebrates. 2nd ed. Academic Press, New York.

Trautman, M.B. 1981. The fishes of Ohio with illustrated keys, revised edition. Ohio State University Press, Columbus, OH.

U.S. Environmental Protection Agency. 1986. Pesticide Fact Sheet-Diazinon. Office of Pesticides and Toxic Substances, Office of Pesticide Programs, Washington, D.C.

U.S. Environmental Protection Agency. 1992. Methods for the determination of chemical substances in marine and estuarine environmental samples. Version 1.1. U.S. Environmental Protection Agency; Off. Res. Devel.; Cincinnati, OH. EPA-600/R-92-121.

U.S. Environmental Protection Agency. 1996. The Volunteer Monitor's Guide to Quality Assurance Project Plans. U.S. Environmental Protection Agency, Office of Wetlands, Washington D.C. EPA 841-B-96-003.

U.S. Environmental Protection Agency. 1999a. Guidance for assessing chemical contaminant data for use in fish advisories: Fish sampling and analysis, vol. 1. EPA 823-R-99-007.

U.S. Environmental Protection Agency. 1999b. Guidance for assessing chemical contaminant data for use in fish advisories: Risk assessment and fish consumption limits, vol. 2. EPA 823-R-99-008.

U.S. Environmental Protection Agency. 1999c. Guidance for assessing chemical contaminant data for use in fish advisories: Overview of risk management, vol. 3. EPA 823-B-96-006.

U.S. Environmental Protection Agency. 1999d. Guidance for assessing chemical contaminant data for use in fish advisories: Risk Communication, vol. 4. EPA 823-R-99-5-001.

U.S. Environmental Protection Agency. 2001. EPA Requirements for Quality Assurance Project Plans (EPA QA/R-5). U.S. Environmental Protection Agency, Office of Environmental Information, Washington D.C. EPA/240/B-01/003.

U.S. Environmental Protection Agency. 2002. Guidance for Quality Assurance Project Plans (EPA QA/G-5). U.S. Environmental Protection Agency, Office of Environmental Information, Washington D.C. EPA/240/R-02/009.

U.S. Environmental Protection Agency. 1997a. Field and laboratory methods for macroinvertebrate and habitat assessment of low gradient nontidal streams. Mid-Atlantic Coastal Streams Workgroup, Environmental Services Division, Region 3, Wheeling, WV.

U.S. Environmental Protection Agency. 1997b. Monitoring guidance for determining the effectiveness of nonpoint source controls. U.S. Environmental Protection Agency, Office of Water, Washington D.C. EPA 841-B-96-004.

U.S. Environmental Protection Agency. 1982. Freshwater snails (Mollusca: Gastropoda) of North America. EPA-600/3-82-026. USEPA, Office of Research and Development, Cincinnati, OH.

Van der Werff, A. 1955. A new method of concentrating and cleaning diatoms and other organisms. Int. Ver. Theor. Angew. Limnol. Verh., 12:276-277.

VanLandingham, S.L. 1982. Guide to the identification, environmental requirements, and

pollution tolerance of blue-green algae. U.S. Environmental Protection Agency; Off. Res. Devel.; Cincinnati, OH. EPA-600/3-82-073.

Vaughn, C.C., C.M. Taylor, and K.J. Eberhard. 1997. Comparison of the effectiveness of timed searches vs quadrat sampling in mussel surveys. Pages 157-162 in K.S. Cummings, A.C. Buchanan and L.M. Koch (eds). Conservation and Management of Freshwater Mussels II. Proceedings of a UMRCC Symposium, 16-18 Oct 1995, St. Louis, Missouri. Upper Mississippi River Conservation Committee, Rock Island, IL.

Waller, D.L. , J.J. Rach, and W.G. Cope. 1995. Effects of handling and aerial exposure on the survival of unionid mussels. Journal of Freshwater Ecol. 10:199-207.

Waltz, R.D., W.P. McCafferty, and J.H. Kennedy. 1985. Barbaetis: a new genus of eastern nearctic Mayflies (Ephemeroptera: Baetidae). The Great Lakes Entomologist:161-165.

Watanabe, T., K. Asai, and A. Houki. 1988. Numerical water quality monitoring of organic pollution using diatom assemblages. In Round, F.E. (ed). Proc. 9th Inter. Diatom Symp.; Bristol (1986): 123-141.

Weber, C.I., (ed.). 1973. Biological field and laboratory methods for measuring the quality of surface waters and effluents. Nat. Environ. Res. Cent., Off. Res. Devel., U.S. Environmental Protection Agency, Cincinnati, OH. EPA-670/4-73-001.

Weber, C.I., and R. Raschke. 1970. Use of a floating periphyton sampler for water pollution surveillance. U.S. Environmental Protection Agency; Water Qual. Res. Lab.; Cincinnati, OH.

Wehr, J.D., and R.G. Sheath (eds). 2003. Freshwater algae of North America: ecology and classification. Academic Press, San Diego, CA.

Weitzel, R.L. 1979. Periphyton measurements and applications. In Weitzel, R.L.ed. Methods and measurements of periphyton communities. Amer. Soc. Test. And Mat. ASTM STP 690:3-33.

Wentworth, C.K. 1922. A scale of grade and class terms for classic sediments. J. Geology, 30: 377-392.

Westfall, M.J., and M.L. May. 1996. Damselflies of North America. Scientific Publishers. Gainesville, FL.

White, D S. 1978. A revision of the nearctic Optioservus (Coleoptera: Elmidae), with descriptions of new species. Systematic Entomology 3:59-74.

Whitford, L.A., and G.J. Schumacher. 1963. Communities of algae in North Carolina streams and their seasonal relations. Hydrobiol. 22:133-196.

Whitford, L.A., and G.J. Schumacher. 1973. A manual of fresh-water algae. Sparks Press, Raleigh, NC.

Whittaker, R. H. 1952. A study of foliage insect communities in the Great Smoky Mountains. Ecol. Monogr. 22:1-44.

Whittaker, R. H., and C. W. Fairbanks. 1958. A study of planktonic copepod communities in the Columbia Basin, Southeastern Washington. Ecology 39: 46-65.

Wiederholm, T. (editor). 1983. Chironomidae of the holarctic region. Keys and diagnoses, Part 1, Larvae. Entomologica Scandinavica Supplement 19, 1-457.

Wiederholm, T. (editor). 1986. Chironomidae of the holartic region. Keys and diagnoses. Part 2, Pupae. Entomologica Scandinavica Supplement 28: 1-482.

Wiggins, G.B. 1995. Larvae of the North American caddisfly genera (Trichoptera), 2nd ed. University of Toronto Press, Toronto, Canada.

Wilhm, J.L., and T.C. Dorris. 1966. Species diversity of benthic macroinvertebrates in a stream receiving domestic and oil refinery effluents. Amer. Midl. Nat. 76:427-449.

Williams, J.D., M.L. Warren, Jr., K.S. Cummings, J.L. Harris, and R. J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries, Vol.18, No. 9: 6-22.

Winger, P.V. 1981. Physical and chemical characteristics of warmwater streams: A review. Am. Fish. Soc. Warmwater Streams Symp., 1981:32-44.

Wolman, Gordan M. 1954. A Method of Sampling Coarse River-Bed Material, Trans., American Geophysical Union, 35(6), pp. 951-956.

Woods, A. J., J. M. Omernik, W. H. Martin, G. J. Pond, W.M Andrews, S. M. Call, J.A Comstock, and D. D. Taylor. 2002. Ecoregions of Kentucky (2-sided color poster with map, descriptive text, summary tables, and photographs): Reston, VA, U.S. Geological Survey (map scale 1:1,000,000).

Wujek, D.E., and R.F. Rupp. 1980. Diatoms of the Tittabawassee River, MI. Biblio. Phyc. 50:1-100.

Yoder, C.O., and M.A. Smith. 1999. Using fish assemblages in a state biological assessment and criteria program: Essential concepts and considerations. Pages 17-56 in T.P Simon (ed). Assessing the sustainability and biological integrity of water resources using fish communities. CRC Press, Boca Raton, FL