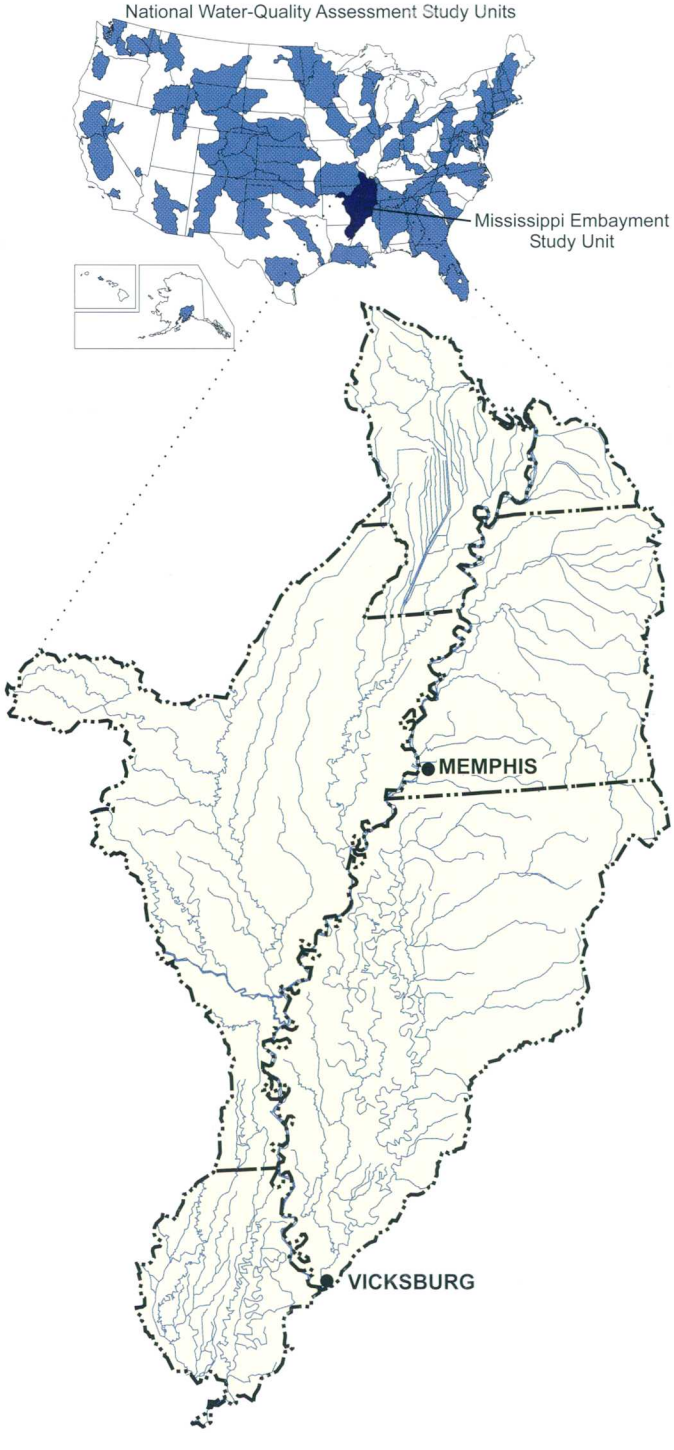
COMPARISON OF MULTI-HABITAT AQUATIC MACROINVERTEBRATE SAMPLING METHODS IN STREAMS OF THE MISSISSIPPI ALLUVIAL PLAIN ECOREGION

U.S. GEOLOGICAL SURVEY
Water-Resources Investigations Report 00–4216



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COMPARISON OF MULTI-HABITAT AQUATIC MACROINVERTEBRATE SAMPLING METHODS IN STREAMS OF THE MISSISSIPPI ALLUVIAL PLAIN ECOREGION

by B.G., Justus[1], D.G. Bray[2], A Dossett[2], M. Hicks[3], R. Sarver[4], and M. Rogers[5]

U.S. Geological Survey
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National Water-Quality Assessment Program

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Information regarding the National Water-Quality Assessment (NAWQA) Program is available via the Internet at: http://water.usgs.gov/nawqa/nawqa_home.html

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CONVERSION FACTORS AND ABBREVIATIONS

Multiply	By	To obtain
inch (in.)	2.54	centimeter
foot (ft)	0.3048	meter
mile (mi)	1.609	kilometer
square inch (in^2)	6.4516	square centimeter
square foot (ft^2)	0.09290	square meter
square mile (mi^2)	2.590	square kilometer
acres	0.4047	hectare
ounce (oz)	29.57	milliliter
quart (qt)	0.9464	liter

The following acronyms are used in this report:

MAP Mississippi Alluvial Plain
MISE Mississippi Embayment Study Unit
NAWQA National Water-Quality Assessment
USGS U.S. Geological Survey

FOREWORD

The U.S. Geological Survey (USGS) is committed to serve the Nation with accurate and timely scientific information that helps enhance and protect the overall quality of life, and facilitates effective management of water, biological, energy, and mineral resources.(http://www.usgs.gov/). Information on the quality of the Nation's water resources is of critical interest to the USGS because it is so integrally linked to the long-term availability of water that is clean and safe for drinking and recreation and that is suitable for industry, irrigation, and habitat for fish and wildlife. Escalating population growth and increasing demands for the multiple water uses make water availability, now measured in terms of quantity and quality, even more critical to the long-term sustainability of our communities and ecosystems.

The USGS implemented the National Water-Quality Assessment (NAWQA) Program to support national, regional, and local information needs and decisions related to water-quality management and policy. (http://water.usgs.gov/nawqa). Shaped by and coordinated with ongoing efforts of other Federal, State, and local agencies, the NAWQA Program is designed to answer: What is the condition of our Nation's streams and ground water? How are the conditions changing over time? How do natural features and human activities affect the quality of streams and ground water, and where are those effects most pronounced? By combining information on water chemistry, physical characteristics, stream habitat, and aquatic life, the NAWQA Program aims to provide science-based insights for current and emerging water issues and priorities. NAWQA results can contribute to informed decisions that result in practical and effective water-resource management and strategies that protect and restore water quality.

Since 1991, the NAWQA Program has implemented interdisciplinary assessments in more than 50 of the Nation's most important river basins and aquifers, referred to as Study Units. (http://water.usgs.gov/nawqa/nawqamap.html). Collectively, these Study Units account for more than 60 percent of the overall water use and population served by public water supply, and are representative of the Nation's major hydrologic landscapes, priority ecological resources, and agricultural, urban, and natural sources of contamination.

Each assessment is guided by a nationally consistent study design and methods of sampling and analysis. The assessments thereby build local knowledge about water-quality issues and trends in a particular stream or aquifer while providing an understanding of how and why water quality varies regionally and nationally. The consistent, multi-scale approach helps to determine if certain types of water-quality issues are isolated or pervasive, and allows direct comparisons of how human activities and natural processes affect water quality and ecological health in the Nation's diverse geographic and environmental settings. Comprehensive assessments on pesticides, nutrients, volatile organic compounds, trace metals, and aquatic ecology are developed at the national scale through comparative analysis of the Study-Unit findings. (http://water.usgs.gov/nawqa/natsyn.html).

The USGS places high value on the communication and dissemination of credible, timely, and relevant science so that the most recent and available knowledge about water resources can be applied in management and policy decisions. We hope this NAWQA publication will provide you the needed insights and information to meet your needs, and thereby foster increased awareness and involvement in the protection and restoration of our Nation's waters.

The NAWQA Program recognizes that a national assessment by a single program cannot address all water-resource issues of interest. External coordination at all levels is critical for a fully integrated understanding of watersheds and for cost-effective management, regulation, and conservation of our Nation's water resources. The Program, therefore, depends extensively on the advice, cooperation, and information from other Federal, State, interstate, Tribal, and local agencies, non-government organizations, industry, academia, and other stakeholder groups. The assistance and suggestions of all are greatly appreciated.

Robert M. Hirsch
Associate Director for Water

COMPARISON OF MULTI-HABITAT AQUATIC MACROINVERTEBRATE SAMPLING METHODS IN STREAMS OF THE MISSISSIPPI ALLUVIAL PLAIN ECOREGION

By B.G. Justus, D.G. Bray, A. Dossett, M. Hicks, R.J. Sarver, and M. Rogers

ABSTRACT

Most streams in the Mississippi Alluvial Plain ecoregion, the largest alluvial plain in the interior United States, have been hydrologically modified, and almost all receive nonpoint source agricultural runoff. Methods for conducting aquatic biological assessments in this ecoregion are not well established; few sites are sampled compared to other ecoregions, and no biological criteria (narrative or numeric indicators of stream condition) are in place. To compare methods, results, and efficiency of benthic macroinvertebrate sampling and processing techniques, three streams in the northern part of the Mississippi Alluvial Plain ecoregion were sampled by teams from four Federal and State agencies that share monitoring responsibilities of the ecoregion; duplicate samples were collected for a laboratory that participated only in laboratory processing.

All methods compared in this study are considered to be effective for use in the Mississippi Alluvial Plain ecoregion, and macroinvertebrate data collected by the teams can be added to existing databases and have value in establishing biological criteria. However, the following factors may influence monitoring accuracy and efficiency: (a) some intersite discriminating ability may be lost when a small sample size (of about 100 organisms) is used; (b) conversely, intersite discriminating ability may not increase for a large sample size (between 500 and 1,000 organisms), (c) processing a standard number of organisms at all sites may reduce some data variability by eliminating field picker subjectivity and (d) a large net-mesh size (about 800 micrometers) may be appropriate for sampling in this ecoregion.

Results from this study may be useful to others who monitor streams in the Mississippi Alluvial Plain ecoregion or monitor low-gradient streams elsewhere. Similarities between the methods used in this study and multi-habitat protocols established by the U.S. Environmental Protection Agency for wadeable, low-gradient streams in other ecoregions, suggest that the multi-habit protocols may also be applicable to nonwadeable streams and to other streams of the Mississippi Alluvial Plain ecoregion.

INTRODUCTION

Macroinvertebrate samples can be used to identify ecological factors that influence stream conditions. As a result, it is important to establish macroinvertebrate sampling protocols and the minimum resources necessary to collect macroinvertebrate samples for all ecoregions where sampling is planned. Sampling protocols have been established for most regions in the U.S. (Barbour and others, 1999; U.S. Environmental Protection Agency, 1997; Florida Department of Environmental Protection, 1997; Plafkin and others, 1989; Bode, 1988; Ohio Environmental Protection Agency, 1987), and there have been a number of interagency field and laboratory method comparisons and workshops to evaluate and perfect macroinvertebrate sampling protocols in problematic areas (Houston and

others, Alabama Department of Environmental Management, written commun., 1997; Lenz and Miller, 1996; Gurtz and Muir, 1994; Plafkin, 1989).

The Mississippi Alluvial Plain ecoregion (as defined by Omernik, 1987) is the largest alluvial plain in the interior United States. Macroinvertebrate sampling protocols have not been developed specifically for the Mississippi Alluvial Plain ecoregion, but have been developed for ecoregions that are physically similar (U.S. Environmental Protection Agency, 1997; Florida Department of Environmental Protection, 1997. Currently, it is not known if macroinvertebrate sampling protocols developed for other lowland ecoregions can be applied to streams in the Mississippi Alluvial Plain ecoregion. The U.S. Environmental Protection Agency (USEPA) recommends that protocols developed for other lowland ecoregions are applicable to streams that lack rocky substrates; however, USEPA also suggests these protocols not be used for nonwadeable streams (Barbour and others, 1999). Although streams in the Mississippi Alluvial Plain ecoregion lack rocky substrates, they are seldom wadeable and must routinely be sampled from a boat.

The following factors have deterred development of macroinvertebrate sampling protocols and other methods of aquatic biological assessment in the Mississippi Alluvial Plain ecoregion.

- There are few permitted discharges that require monitoring by State environmental agencies.
- Related physical and chemical characteristics (such as low stream velocities and low dissolved-oxygen concentrations) that can be stressful to aquatic life commonly influence biotic diversity of the least-impacted streams and can obscure anthropogenic impacts to diversity.
- In the past 100 years most streams in the Mississippi Alluvial Plain ecoregion have been modified for flood control and have been used for irrigation supply. Accordingly, the ecological integrity of the streams has not been a priority.
- Almost all streams receive nonpoint agriculture runoff, which is difficult to quantify.
- Monitoring responsibility for the ecoregion is divided among seven States (fig. 1).

One objective of the Clean Water Act (as amended in 1977) is to promote biological integrity or the ability of a stream to support and maintain a balanced, integrated, adaptive community of organisms having a composition, diversity, and functional organization comparable with those of natural habitats within the region (Frey, 1977). However, because biological integrity differs for all regions, establishing the range of aquatic conditions (or narrative or numeric indicators of stream condition as biological criteria), in an ecoregion is essential before an appropriate level of biological integrity can be identified. To identify this range of conditions, many sites need to be sampled, and for ecoregions shared by multiple states (such as the Mississippi Alluvial Plain ecoregion), sampling resources from different agencies need to be pooled.

Before sampling resources are pooled, participating agencies should know if biological data collected in an ecoregion yield comparable bioassessment results. Comparing results of various sampling methods used in an ecoregion will help determine if data can be pooled and can facilitate biocriteria development.

Macroinvertebrate sampling teams from each of the six States that share monitoring responsibilities for the Mississippi Alluvial Plain ecoregion were invited to participate with the U.S. Geological Survey (USGS) in a methods comparison effort. Teams representing three State agencies participated: the Arkansas Department of Environmental Quality (ADEQ), the Missouri Department of Natural Resources (MDNR), and the Mississippi Department of Environmental Quality (MDEQ). The USGS participated in two ways: (1) the Mississippi Embayment Study Unit (MISE) team of the National Water-Quality Assessment (NAWQA) Program collected and field-processed samples concurrently with the State teams; and (2) the MISE team collected additional benthic macroinvertebrate samples, which were processed at the USGS National Water Quality Laboratory (NWQL) in Denver, Colorado.

Purpose and Scope

This report describes macroinvertebrate sampling methods used by the sampling teams participating in the comparison, and addresses variability in the results by site and by team. Factors that could influence sampling efficiency and accuracy in the Mississippi Alluvial Plain ecoregion are described.

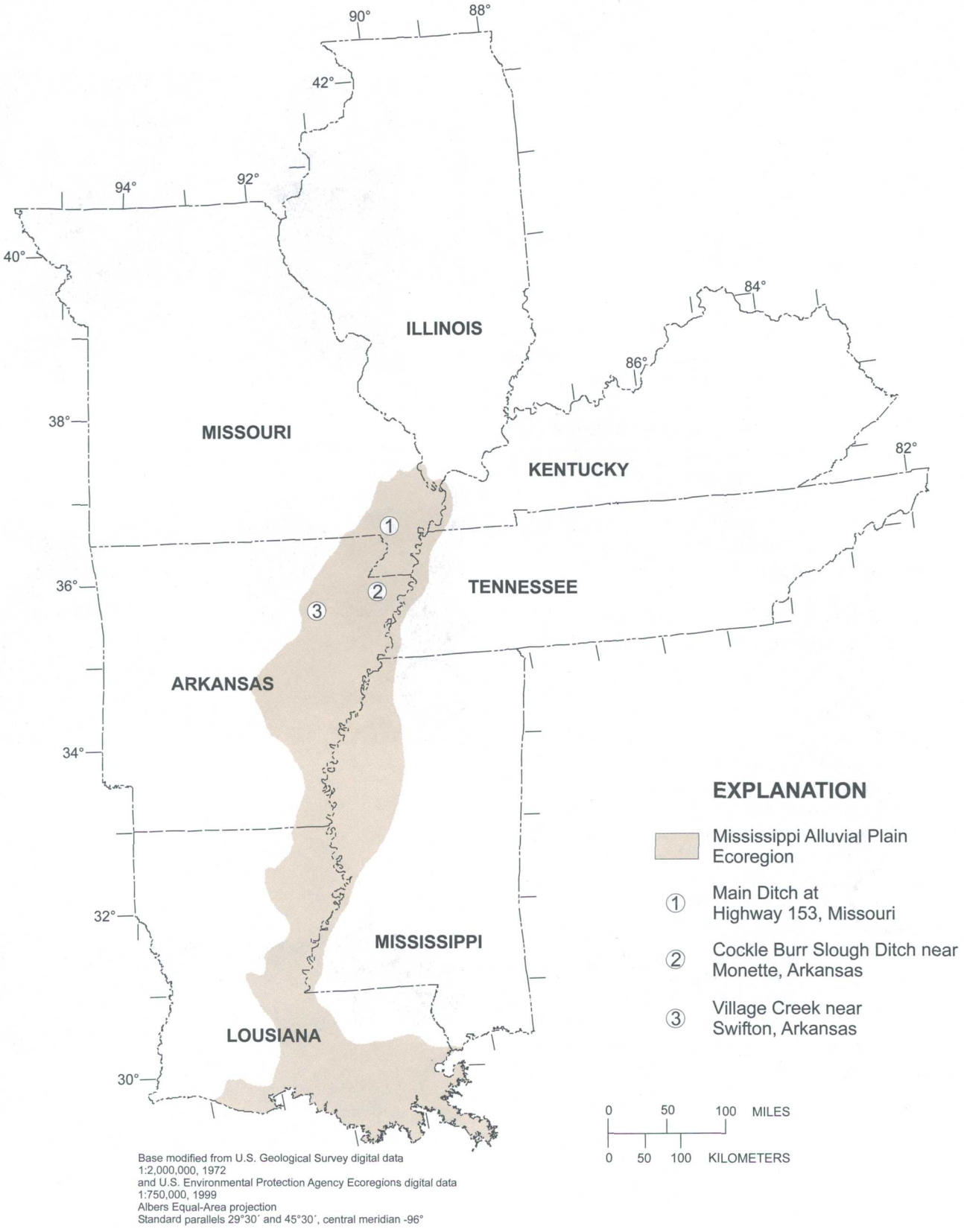


Figure 1. Location of sites sampled for macroinvertebrate methods comparison in the northern part of the *Mississippi Alluvial Plain ecoregion.*

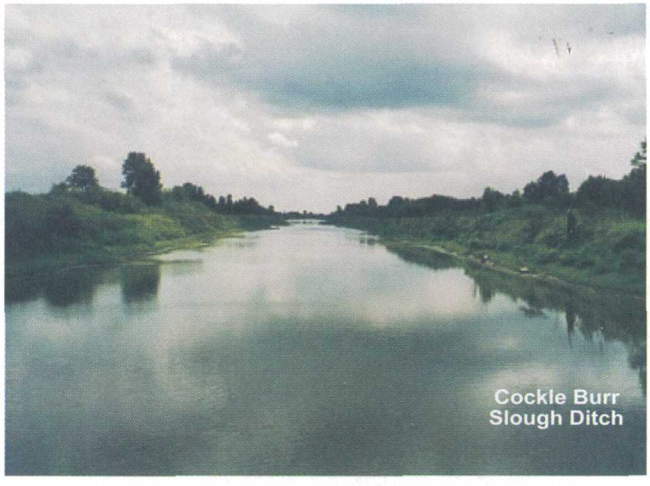
Description of the Study Area and Sampling Sites

The Mississippi Alluvial Plain ecoregion extends approximately 600 miles from Cairo, Illinois, to the Gulf of Mexico, and ranges in width from 25 to 125 mi (miles). The ecoregion lies east and west of the Mississippi River and encompasses more than 20 million acres (Brown and others, undated). Streams in the ecoregion have low gradients, and relief is sometimes less than 8.1 in/mi (inches per mile) (Arkansas Department of Pollution Control and Ecology, 1987).

Three streams in the northern part of the Mississippi Alluvial Plain ecoregion were selected for sampling: Main Ditch in Dunklin County, Missouri; Cockle Burr Slough Ditch in Craighead County, Arkansas; and Village Creek in Jackson County, Arkansas (fig. 1). Sites selected for sampling varied in channel sinuosity, flow velocity, and quality of instream and riparian habitat to ensure that factors that typically influence macroinvertebrate assemblages in the Mississippi Alluvial Plain ecoregion would be found. The sites selected also were in watersheds dominated by row crop agriculture (land use typical of the Mississippi Alluvial Plain ecoregion) and were centrally located to the participating sampling teams.

The drainage areas associated with Main Ditch and Cockle Burr Slough Ditch are similar in size, land use, and hydrologic alterations. The watershed areas upstream from the sampling sites at Main Ditch and Cockle Burr Slough Ditch are about 130 and 110 mi^2 (square miles), respectively. Cotton, soybeans, and corn are the dominant crops in both watersheds (Arkansas Agricultural Statistics Service, 1995; and Missouri Agricultural Statistics Service, 1997). Both streams are channelized extensively, and there is some evidence that these streams may be hydraulically connected to the underlying alluvial aquifer. If this is the case, then increased ground-water discharge may dilute surface runoff and improve water quality. Woody vegetation is well established on one bank of the sampling site at Main Ditch, but is totally lacking at the sampling site at Cockle Burr Slough Ditch. Average stream velocities were determined by dividing the total discharge by the cross-sectional area of the stream. Average stream velocities at Main Ditch and Cockle Burr Slough Ditch were 1.2 and 0.28 ft/s (foot per second), respectively, on the date of sampling.

In contrast, Village Creek is a braided lowland stream that flows through a cypress/tupelo gum swamp and has a watershed of approximately 157 mi^2. The dominant crops in the Village Creek drainage area are soybeans and rice (Arkansas Agricultural Statistics Service, 1995). The average stream velocity of Village Creek was 0.35 ft/s on the date of sampling.



Three sites sampled in the Mississippi Alluvial Plain ecoregion by four sampling teams comparing macroinvertebrate sampling methods.

(Photos by Billy G. Justus, U.S. Geological Survey)

METHODS

The following provides an overview of the methods used by the four sampling teams, a description of the methods used by each team, and a description of the metrics selected to evaluate and compare the data.

Methods Overview

Macroinvertebrate samples were collected at Main Ditch and Cockle Burr Slough Ditch on August 12, 1997, and at Village Creek on August 13, 1997. All teams used boats to find wadeable areas suitable for sampling at two or more of the sites, and to find habitats suitable for sampling in deep water. Care was taken to ensure that there was no overlap of sampling areas. All four teams used multi-habitat sampling methods similar to a method used by the Mid Atlantic Coastal Streams Workgroup (U.S. Environmental Protection Agency 1997) and recommended by the USEPA protocols (Barbour and others, 1999). The teams deviated somewhat from the USEPA protocols in regard to the equipment used for sampling, how the habitats were sampled, and the method of standardizing sampling (table 1). All teams used an aquatic dip net to sample most habitats. When stream flow was sufficient, the substrate was disturbed by kicking, and dislodged organisms were carried into the net by the water current. When flow was insufficient or where the substrate was too deep for kicking, the net was used to repeatedly sweep the habitat and collect dislodged organisms. Leaves and sticks were carefully removed from the water by hand or by using a net. Organisms were picked from all habitat samples using forceps.

Some teams used loppers to collect stick samples from streams in the Mississippi Alluvial Plain ecoregion. At many sites, deep water prevented wading, making it necessary to sample from a boat.

(Photo by Darrell T. Wilson, U.S. Geological Survey)

Table 1. Summary of methods used to sample three streams in the Mississippi Alluvial Plain ecoregion

[ADEQ, Arkansas Department of Environmental Quality; MDNR, Missouri Department of Natural Resources; MDEQ, Mississippi Department of Environmental Quality; MISE, Mississippi Embayment Study Unit; in., inches; µm, micrometers; x, multiplied by; --, not applicable]

	Sampling Teams			
Characteristics	ADEQ	MDNR	MDEQ	MISE
Net mesh dimensions (um)	800 x 900	500 x 500	800 x 900	425 x 425
Number of habitats sampled	3	4	3 – 5	3 – 5
Primary collection method	sweep/kick	sweep/kick	sweep	sweep
Primary tool	dip net	kick net	dip net	dip net
Quantification method	time	area	--	area
Reach length (yards)	--	20x stream width	--	550
Sample type	semiquantitative[1]	semiquantitative[1]	qualitative[2]	semiquantitative[1]
Secondary tool	--	nitex bag	--	young grab
Net mouth dimensions (in.)	10 x 13	9 x 18	10 x 13	10 x 13

[1] standardized sampling effort
[2] no standardization of sampling effort

A macroinvertebrate dip net was used to sample most macroinvertebrate habitats in streams of the Mississippi Alluvial Plain ecoregion.

(Photo by Carol P. Moss, U.S. Geological Survey)

The ADEQ, MDNR, and MISE teams sampled semiquantitatively (standardized sampling effort); the MDEQ team sampled qualitatively (did not standardize sampling efforts). The MISE and MDNR teams used area to standardize sampling, whereas the ADEQ team used time to standardize sampling (table 2).

Table 2. Habitats sampled and standardization measures used by three teams to sample three streams in the Mississippi Alluvial Plain ecoregion

[min., minutes; ft, linear feet; ft^2, square feet; ft^3, cubic feet; ADEQ, Arkansas Department of Environmental Quality; MDNR, Missouri Department of Natural Resources; MISE, Mississippi Embayment Study Unit; --, not sampled]

Habitats	ADEQ	MDNR	MISE
Depositional	--	65.0 ft^2	2.25 ft^2
Macrophytes	2 min	65.0 ft^2	2.25 ft^2
Sticks	--	6.5 ft^2	2.60 ft^2
Root mats	2 min	19.7 ft	2.25 ft^2
Leaf packs	2 min	--	0.35 ft^3

Samples were processed using one of two methods. Organisms were either separated from debris (organic material) while in the field (field picking) or samples were taken directly to the laboratory where they were subsampled (a predetermined number of organisms were randomly removed from the sample). The amount of effort (man hours) that each team spent collecting and preparing the samples for identification was tabulated and is listed in table 3.

All organisms were identified to the lowest practical taxonomic level. Larger macroinvertebrates were identified by using dissecting microscopes (6.7-80X magnification). Chironomids and oligochaetes were mounted on slides using mounting media, and then were identified using a compound microscope (40-1,000X magnification). All organisms were counted, the taxonomic determination was recorded on bench sheets, and specimens were preserved in vials or stored in slide boxes. Actual identification time was not compared because of the variability of taxonomic expertise and the preference of the participants for different taxonomic levels; however, an effort ratio was estimated (table 3). The effort ratio was calculated by dividing the number of taxa determined by each sampling team at each site by the lowest number of taxa (common denominator) for all samples collected at the site, and then averaging the three quotients from the three sites for each sampling team.

The MISE team collected duplicate samples at all three sites. One sample was field picked; the other sample was shipped to the NWQL to be subsampled.

The MDNR team collected two samples at Main Ditch for quality assurance purposes. Both samples were collected and processed in the same manner. Averages were calculated for each metric using results from both Main Ditch samples collected by the MDNR team.

Arkansas Department of Environmental Quality Methods

The ADEQ team collected samples in accordance with the USEPA rapid bioassessment protocols (Barbour and others, 1999), except that the sampling effort was standardized by time rather than by sampling area or the number of times the habitat was swept with the net. Each of three habitat types (sticks and leaf packs, root mats, and aquatic macrophytes) was sampled for 2 minutes. Material to be subsampled in the laboratory was composited, stored in 1-quart (qt) jars, and preserved in 70 percent ethanol. Sample volume was reduced in the field by inspecting large pieces of debris for organisms, removing the organisms, and discarding the debris; however, the majority of the organic material was returned to the laboratory for subsampling.

Table 3. Details of sampling and processing efforts at three streams in the Mississippi Alluvial Plain ecoregion

[ADEQ, Arkansas Department of Environmental Quality; MDNR, Missouri Department of Natural Resources; MDEQ, Mississippi Department of Environmental Quality; NWQL, U.S. Geological Survey, National Water Quality Laboratory; MISE, Mississippi Embayment Study Unit]

	Sampling Team				
Description A	DEQ	MDNR	MDEQ	NWQL	MISE
Average collection time (man hours)	1.3	2.0	4.0	5.3	5.3
Average processing time (man hours)	1.9	11.3	1.0	6.6	3.0
Mean number of individuals sampled	110	818	391	537	564
Ratio of effort for taxonomy[1]	1.0	3.0	2.5	2.8	2.7
Total collection and preparation time (man hours)	3.2	13.3	5.0	11.9	8.3
Type of processing	Subsample	Subsample	Field Pick	Subsample	Field Pick

[1]The effort ratio was determined by dividing the number of taxa determined by each sampling team at each site by the lowest number of taxa (common denominator) for all samples collected at the site, and then averaging the three quotients from the three sites for each team.

Subsamples were obtained in the laboratory by placing the composite sample in a 5-gallon (gal) bucket, adding approximately 3.5 gal of water, stirring the contents to suspend the material, and then immediately pouring off an 8-ounce (oz) aliquot. Large debris was hand picked from the aliquot, and the remaining material was placed in a 12 x 18-inch (in) sorting tray. Organisms were picked until at least 95 individuals were retrieved. The number of aliquots used depended on the number of organisms. No partial picking of aliquots was allowed; once the aliquot was removed from the composite sample, the entire 8-oz aliquot was picked free of organisms. This method results in variation of the number of organisms sampled, but reduces bias in the picking process. After subsampling was completed, the remaining composite sample was discarded and the subsampled aliquot was preserved in 70 percent ethanol.

The average collection time for the ADEQ samples was approximately 1.3 man hours. The average time to process, to pick organisms, and to make chironomid head slides was approximately 1.9 man hours. The average total collection and processing time was 3.2 man hours.

Missouri Department of Natural Resources Methods

The MDNR team collected samples from a stream reach selected at each site. The reach length was 20 times the width of the stream. Macroinvertebrates were collected from four habitats (if present) within each reach. These habitats included depositional habitat (sand, silt, clay, fine particulate organic material), macrophytes, large woody debris, and root mats.

Depositional habitat and macrophytes were sampled in the following manner. Six 10.8-ft^2 samples were collected from different depositional areas and macrophyte beds within each habitat type. A traveling kick method was used--organisms and debris were suspended in the water column using a foot-stirring action to disturb the substrate to a depth of 6 to 10 in., and the kick net was swept back and forth just above the substrate to collect dislodged organisms. The six samples were composited into a rectangular plastic container with a 9.5-gal capacity. Water was added to the plastic container, and all large pieces of debris were vigorously brushed (to remove organisms), inspected for organisms, and discarded. The remaining composite sample was concentrated by pouring off all excess water through a brine shrimp net (500-µm mesh); the concentrate was then scooped into a sample jar(s) using a 3-in-wide putty knife. Ambient water was used to flush any remaining debris from the container through the net. The net was then inverted and the contents were placed into a sample jar and covered with preservative.

Organisms associated with woody debris were collected by vigorously brushing 12 sticks or logs having a total area of approximately 6.5 ft^2 into a nitex bag

while the sticks remained in the stream (bag dimensions were 1.6 ft per side; and the bag was made by folding a 1.6 x 3.3 ft piece of 500-µm mesh nitex cloth in half on the long side and sewing the sides). When no natural current was present to carry dislodged organisms into the bag, an artificial current was created by sweeping the brush along the woody debris in the direction of the bag opening. After the 12 samples were composited into the nitex bag, the bag was emptied by concentrating all of the contents into a corner of the bag and emptying the contents into a sample jar. The contents of the sample jar were then covered with preservative.

Six root mat samples were collected, each from an approximate 3.3-ft length of shoreline. If stream velocity was sufficient, the net was placed downstream of the root mat and a kicking action was used to dislodge the organisms and sweep them into the net. If stream velocity was insufficient, the net was placed around the root mat; the net was then shaken to dislodge organisms into the net. The six samples were composited into a 9.4-gal plastic container. Water was added to the composite sample, and any large debris was vigorously washed, checked for organisms, and discarded. The remaining sample was then concentrated and covered with preservative.

A laboratory subsampling method was used to efficiently isolate 270 to 330 organisms per habitat. To remove the subsample, each habitat sample was transferred to a 14 x 20-in. stainless steel sieve with a 500-µm mesh screen [U.S. Standard Testing Sieve, American Society for Testing of Materials (ASTM) number 35 (Tyler Industrial Products, 1976)], and rinsed with water to dilute the preservative. Any large debris was scrubbed, rinsed, and removed. The sieve was then placed into a rectangular plastic container with a 9.5-gal capacity. Enough water was added to allow thorough mixing, and the sample was randomly distributed by stirring (on the sieve). Once the sample was distributed, the sieve was quickly lifted from the water and allowed to drain. A grating with seventy grids each with an area of 2.0 in^2 was placed onto the sieve to divide the sample, and a random number generator was used to select squares for subsampling. The dimensions of each grid were outlined into the sample using a spatula, the grid was then removed, and the contents were lifted out. Using a microscope, organisms from this subsample were separated and removed from the debris. Organisms were sorted into two vials filled with 80 percent ethanol; one vial contained slide-mountable organisms (chironomidae and oligochaeta) and a second vial contained all other organisms. When subsampling was completed, the remaining part of the habitat sample was inspected for any readily visible taxa not previously found; these organisms were preserved separately as large/rare specimens. Levels of identification and taxonomic references used are listed in Sarver and Humphrey (1998). Representatives of all new genera and species were placed in the MDNR macroinvertebrate reference collection. The average collection time for MDNR samples was 2 man hours. The average processing time was 11.3 man hours, and the average total collection and processing time for the samples was 13.3 man hours.

Mississippi Department of Environmental Quality Methods

The MDEQ team sampled root mats, macrophytes, depositional habitats, sticks, and leaf packs. Sampling sites were chosen to adequately reflect habitat variability in relation to stream velocity, water depth, and amount of sedimentation on the habitat. Three different areas were sampled in the same manner for root mats and macrophytes at each site. The root mat or macrophytes were positioned inside the net, and the net was shaken briefly to dislodge organisms and debris. Five seasoned sticks (sticks submerged for a period long enough to be colonized by an optimum number of aquatic macroinvertebrates) of approximately the same size were collected by hand at each site. Leaf packs were collected by placing the net just downstream from drifted leaves and then dislodging the material into the net by hand. This was done at several places until the net was approximately half full. Depositional substrates were sampled by scooping the top inch of substrate from the stream bottom. After sampling available habitats, the sampling site was inspected for any organisms that were not previously collected. These organisms were collected by sweeping or by any other means possible (for example, breaking clay apart by hand). Samples were composited by habitat into 5-gal plastic buckets.

Habitat samples were processed in the field according to habitat type. Specimens from each habitat type were picked from sample material by using forceps. Specimens were placed in vials that contained Dietrichs solution (Pennak, 1978), and then labeled with the appropriate habitat name. Root mat, aquatic

plant, and leaf pack samples were processed by placing a small aliquot of the sample in a white dissecting pan and then picking all visible macroinvertebrates. After this part of the sample was picked, the aliquot was discarded and another was added until all the collected material was processed. Aliquots picked last were inspected only for new taxa; no effort was made to collect all organisms of the dominant taxa.

For processing, depositional habitat samples were placed in a 5-gal plastic bucket containing a small amount of water. Organisms in the sampled material were suspended by stirring the mixture by hand. After a brief settling period of a few seconds, material that remained suspended was elutriated onto a sieve having a 600-µm-mesh screen [U.S. Standard Testing Sieve, ASTM number 30 (Tyler Industrial Products, 1976)]. After five elutriations, any material remaining on the sieve was rinsed with stream water, placed in a white dissecting pan, and processed as previously described.

Sticks were examined for macroinvertebrates attached to the outside surfaces and in crevices, and then washed and brushed over a white dissecting pan. Material in the pan was examined and then processed as previously described.

The average collection time for MDEQ samples was 4 man hours. The average processing time was 1 man hour, and average total collection and processing time for the samples was 5 man hours.

USGS Methods

The MISE collected two samples at each of the three sites, with one sample to be processed by the MISE at the District Office in Pearl, Mississippi, and the other sample to be shipped to the NWQL in Denver, Colorado. Both samples were collected from the same 550-yard (yd) reach of stream, but there was no overlap of sampling area. The same methods were used to collect both samples, but each sample was processed by different techniques as described below.

MISE Sampling and Processing Methods

Before sampling began, each site was evaluated for five habitat types --root mats, macrophytes, depositional habitat, leaf packs, sticks, and coarse woody drift. All five habitats were sampled at one or more of the three sites, and material collected from each habitat was stored separately in a 5-gal bucket immediately after collection.

Root mats and macrophytes were sampled by sweeping three different locations having an area equal to the mouth of the dip net (0.75 ft^2). Five seasoned sticks approximately 12 x 1 in. were composited for a stick sample. Sticks were cut with large loppers from submerged trees and limbs that had fallen into the stream. Three to four leaf packs that filled approximately one-third of the net were collected per site. The net was positioned downstream of drifted leaves, and the leaves were dislodged into the net by hand. Depositional habitat (sediment) was sampled using a "Young grab" sediment sampler. The top inch was collected from each of five grabs; this material was composited into one sample.

Processing involved field picking relatively large numbers of macroinvertebrates from sample debris to increase representation of all taxa. Sediment was washed from the sample by using a pressure sprayer to lightly spray the sample with water. Macroinvertebrates were preserved in 10 percent formalin, but were transferred to 70 percent ethanol after 72 hours.

Prior to identification, macroinvertebrates were sorted by taxa into separate vials. With the exception of the chironomids, all organisms were keyed to the lowest convenient taxon. The chironomids were separated from the remaining organisms and placed in gridded trays where 42 percent of the grids were picked free of organisms. Relative abundance was calculated for the chironomids and rounded to the nearest whole number prior to reporting. The average time for collecting and field picking the samples was 5.25 man hours. The average laboratory processing time was 3 man hours, and the average total collection and processing time for the samples was 8.25 man hours.

Field processing involved picking macroinvertebrates from sample debris.

(Photo by Brian J. Caskey, U.S. Geological Survey)

NWQL Processing Methods

The samples shipped to the NWQL were processed in the field according to NAWQA protocols (Cuffney and others, 1993). Two components were shipped to the laboratory from each site--a main body component and a large rare component. The main body component was obtained from the debris collected from all habitats sampled at a site. The volume of the main body component was reduced by discarding large objects, and by washing the sample through a sieve [425 μm, U.S. Standard Testing Sieve, ASTM number 40 (Tyler Industrial Products, 1976)] with ambient water until the total volume was reduced to 0.2 gal. The large rare component consisted of large, rare organisms collected by scanning the entire sample and removing organisms that were not uniformly distributed throughout the sample. Samples sent to the NWQL were subsampled in the laboratory by placing the sample in a tray with 0.8-in^2 grids, and picking all macroinvertebrates from randomly selected grids until approximately 500 organisms (±20 percent) were removed.

Laboratory processing of the NWQL samples, exclusive of identification, was approximately 6.6 manhours. The average collection time for NWQL samples was 5.25 manhours (the same as for MISE samples). The average total collection and processing time for the samples was 11.85 manhours.

Metrics Selected for Data Analysis

Data collected at each site by each sampling team were evaluated using the following nine metrics to determine if variability existed among the various sample collection and processing techniques: total taxa, percent of all taxa collected at the site (the number of taxa collected at the site divided by the number of taxa collected at all three sites), the number of organisms, biotic index values, mean tolerance of all taxa, number of ephemeropteran and trichopteran (ET) taxa, percent of the taxa that were not insect, number of chironomid taxa, and diversity. Biotic and mean tolerance values were taken from Lenat (1993), Bode and others (1991), and Huggins and Moffett (1988). A measure of diversity was calculated by dividing Shannon Diversity (d of log base 2) by the maximum diversity (dMAX) possible (Zar, 1984). This was done to eliminate variability associated with sample size; diversity is reported as d/dMax.

RESULTS AND DISCUSSION

Comparison of Metrics Results

Results for each sampling team (table 4) and the average metric values for all teams (table 5) were comparable. Results of five of the nine metrics were similar for all samples collected at each site: biotic index, ET taxa, percent of taxa that were not insect, mean tolerance, and diversity (figs. 2-3). Variability was observed for four metrics, which are more dependent on sample size; these included the number of taxa, number of individuals, the percent of the total number of taxa collected, and number of chironomid taxa (fig. 4). Most of the variability was associated with the ADEQ method, which sampled the fewest organisms.

The number of organisms sampled in an ecoregion is an important consideration in developing an efficient and accurate sampling plan. In an ecoregion where biological diversity is naturally limited, small differences between metrics of reference sites and test sites may be significant simply because the range for some metrics may be more constricted. This was a concern in the Mississippi Alluvial Plain ecoregion where the number of organisms sampled had to be large enough to maximize the range of the metrics. Although the 100-organism subsampling approach used by the ADEQ team has less overall intersite discriminating ability than other processing methods, data collected by ADEQ can be used by others if the metrics selected are the least dependent on sample size. An obvious benefit of the subsampling method used by ADEQ is that the method required only 24 to 64 percent of the effort required by the other sampling teams to collect and process samples.

Processing Differences Between Subsampling and Field Picking

With the exception of the subsampling effort by ADEQ, samples that were subsampled in the laboratory by MDNR and NWQL took 2.2 - 11.3 times as much effort to process as samples that were field picked (table 3). However, metric values for samples that were subsampled in the laboratory were similar to metric values from field picked samples, and no additional accuracy was observed for the additional laboratory effort. These results indicate that the MDNR and NWQL methods could reduce sample size in the Mis-

Table 4. Macroinvertebrate metric results for five sampling and processing teams and three streams in the Mississippi Alluvial Plain ecoregion

[ADEQ, Arkansas Department of Environmental Quality; MDNR, Missouri Department of Natural Resources; MDEQ, Mississippi Department of Environmental Quality; MISE, Mississippi Embayment Study Unit; NWQL, U.S. Geological Survey, National Water Quality Laboratory; ET, ephemeroptera and trichoptera; d/dMAX, Shannon Diversity/Maximum Diversity]

Sampling Team	Metric								
	Total Taxa	Percent of all taxa collected at the site	Number of individuals	Biotic index	Mean tolerance	ET taxa	Percent noninsect	Chironomid taxa	Diversity (d/dMAX)
Main Ditch									
ADEQ	19	16	118	5.8	6.9	7	2	4	0.49
MDEQ	59	51	443	5.7	6.7	7	1	20	0.49
MDNR	66	57	893	6.4	6.8	13	7	25	0.49
MISE	57	49	793	6.0	6.6	9	5	22	0.36
NWQL	59	51	545	6.3	6.6	10	7	20	0.57
Cockle Burr Slough Ditch									
ADEQ	22	24	96	8.1	7.6	2	51	5	0.48
MDEQ	40	34	247	7.2	7.5	5	23	18	0.57
MDNR	47	41	543	7.4	7.3	7	15	22	0.45
MISE	43	37	342	7.8	7.3	4	27	16	0.50
NWQL	50	43	506	7.0	7.3	4	13	17	0.54
Village Creek									
ADEQ	19	16	115	7.8	7.0	2	35	4	0.43
MDEQ	64	54	484	7.8	7.2	4	15	20	0.53
MDNR	60	51	943	7.0	7.8	4	29	22	0.45
MISE	58	49	559	7.7	7.5	4	39	14	0.48
NWQL	59	50	561	7.5	7.7	4	42	16	0.53

Table 5. Average macroinvertebrate metric results for five sampling and processing teams and three streams in the Mississippi Alluvial Plain ecoregion

[ET, ephemeroptera and trichoptera; d/dMAX, Shannon Diversity/Maximum Diversity]

Site	Total Taxa	Percent of all taxa	Number of individuals	Biotic index	Mean tolerance	ET taxa	Percent noninsect	Chironomid taxa	Diversity (d/dMAX)
Main Ditch	52	45	558	6.0	6.7	9	4	18	0.48
Cockle Burr Slough Ditch	40	36	347	7.5	7.4	4	26	16	0.51
Village Creek	52	44	532	7.6	7.4	4	32	15	0.48

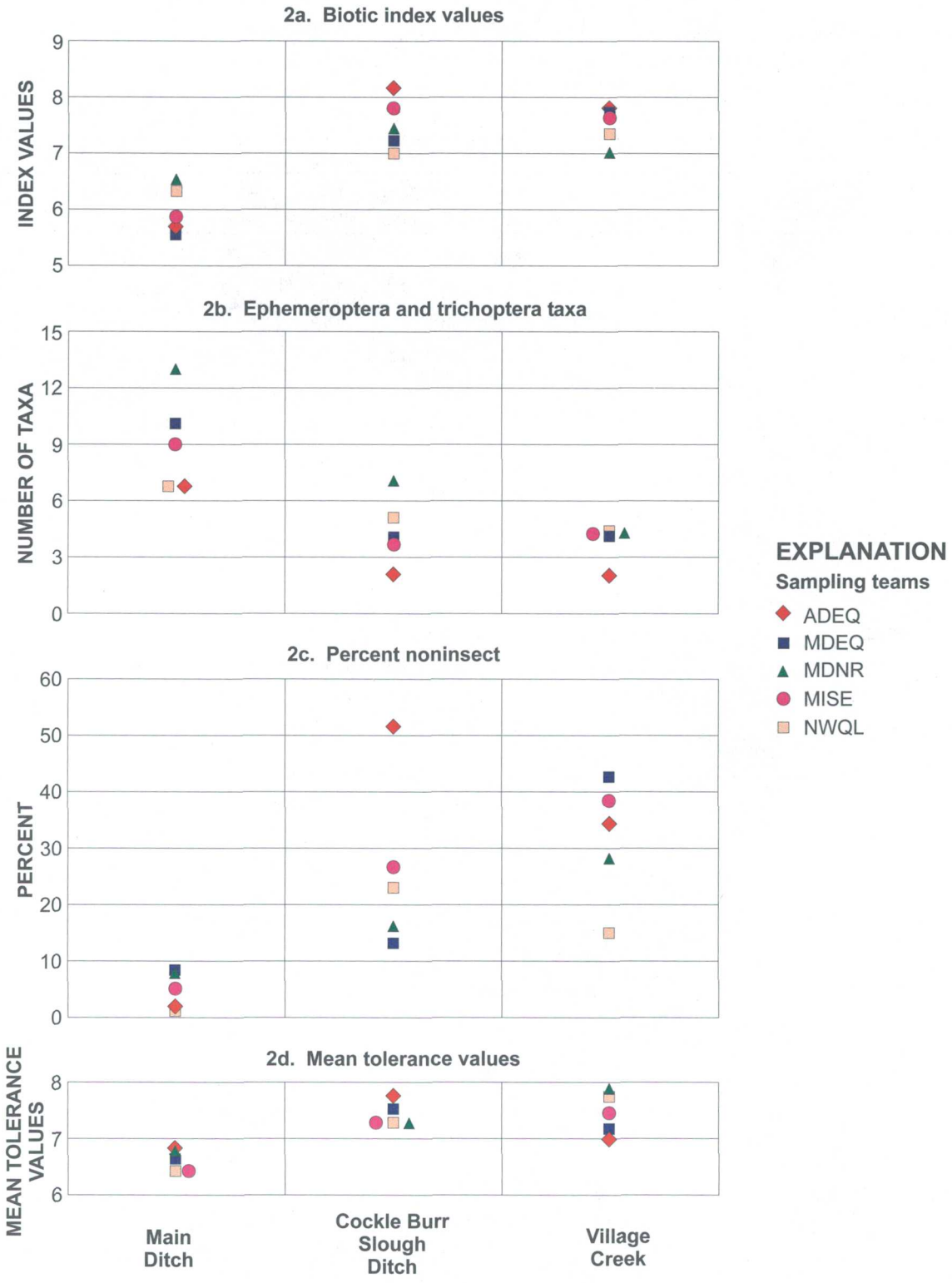


Figure 2. Metrics least affected by sample size for macroinvertebrate samples collected from three streams in the Mississippi Alluvial Plain ecoregion.

Figure 3. Diversity values (d/dMax) for macroinvertebrate samplescollected from three streams in the Mississippi Alluvial Plain ecoregion.

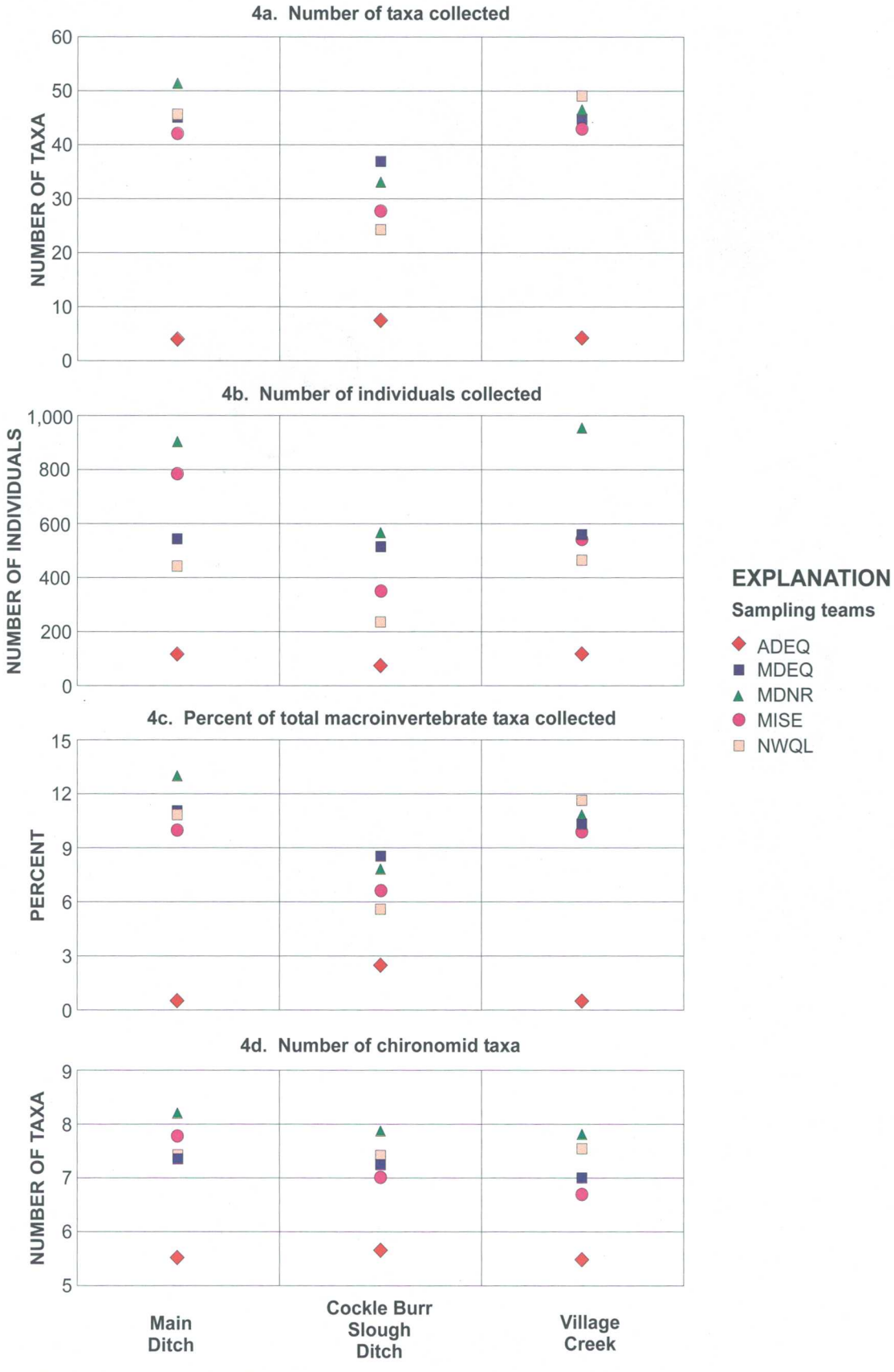


Figure 4. Metrics most affected by sample size for macroinvertebrate samples collected from three streams in the Mississippi Alluvial Plain ecoregion.

sissippi Alluvial Plain ecoregion without sacrificing any discriminating ability. In contrast, the ADEQ method could lose some intersite discriminating ability for some metrics that are related to sample size (the number of taxa, and the number of individuals) because the team sampled only about 100 organisms and spent less effort.

Differences in diversity values indicate that some variability exists between samples subsampled in the laboratory and samples that were field picked. Diversity values were comparable within each processing method (laboratory subsampling and field picking), but were slightly different between the two processing methods--for all sites subsampled, the highest diversity values were at Main Ditch, and for all sites field-picked, the highest diversity values were at Cockle Burr Slough Ditch and the lowest diversity values were at Main Ditch (fig. 3). Some of this variability may be attributed to subjectivity of field pickers as they determined when an appropriate number of organisms had been picked. Processing a standard number of organisms at all sites, as was the case for samples that were subsampled, may serve to reduce variability for some diversity indices because subjectivity (concerning the appropriate number of organisms to be sampled) is eliminated.

No differences in the data could be associated with different net-mesh sizes. Because typically less effort is expended the larger the net-mesh size (fewer small organisms are collected), a large mesh size (about 800 μm) may be the most appropriate for use in the Mississippi Alluvial Plain ecoregion.

Focus of Future Work

Biological metrics and biocriteria need to be developed specifically for streams of the Mississippi Alluvial Plain ecoregion. Compared to upland ecoregions, few biological metrics have been developed specifically for lowland ecoregions, and the application of metrics developed specifically for upland ecoregions to lowland ecoregions is not always appropriate. Metrics developed for upland ecoregions can produce false-negative results for biotic integrity in lowland streams, even under reference conditions, because upland metrics favorably weight organisms that require high dissolved oxygen concentrations. Streams in the Mississippi Alluvial Plain ecoregion that have the most intact riparian zones typically have low stream velocities and high organic content. As a result, dissolved oxygen concentrations commonly exhibit dramatic diurnal shifts placing natural limitations on some biota. For example, the biotic integrity of Village Creek is probably nearer that of a reference site for a least-impacted Mississippi Alluvial Plain ecoregion stream than the biotic integrity of Main Ditch. However, organisms collected at Main Ditch are more intolerant to organic pollution than organisms collected at Village Creek. Differences in pollution tolerance at the two sites may reflect the positive influence of ground water on surface-water quality at Main Ditch (as a result of the hydraulic connection to the underlying alluvial aquifer).

SUMMARY

Results for each sampling team and the average metric values for all teams were comparable, which implies that site assessments by individual teams are in good agreement. Because the team assessments were comparable, the collection and processing methods used are considered effective in the Mississippi Alluvial Plain ecoregion. Macroinvertebrate data collected by any of the sampling teams should have value for increasing existing macroinvertebrate databases and establishing biological criteria for the ecoregion.

Some factors that were observed, which could influence monitoring efficiency and accuracy in the Mississippi Alluvial Plain ecoregion include:

- some intersite discriminating ability may be lost when a small sample size (of about 100 organisms) is used;
- conversely, there may be no gain of intersite discriminating ability associated with a large sample size (between 500 and 1,000 organisms);
- processing a standard number of individuals at all sites may reduce variability because subjectivity of the field picker is reduced; and
- a large net-mesh size (about 800 μm) may be the most practical size for use in the Mississippi Alluvial Plain ecoregion.

Results from this study may be useful to those who monitor low-gradient streams in the Mississippi Alluvial Plain ecoregion and elsewhere. The similarities between the methods used in this study and the USEPA multi-habitat protocols established for wadeable, low-gradient streams in other ecoregions, implies that the USEPA protocols may also be applicable to nonwadeable streams and to streams of the Mississippi Alluvial Plain ecoregion.

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