United States
Environmental Protection
Agency

Pilot Study for Montgomery County and Maryland DNR Data Integration: Comparison of Benthic Macroinvertebrate Sampling Protocols for Freshwater Streams

EPA903/R-03/005
May 2003

Pilot Study for Montgomery County and Maryland DNR Data Integration: Comparison of Benthic Macroinvertebrate Sampling Protocols for Freshwater Streams

Prepared for:
U.S. Environmental Protection Agency
Office of Environmental Information and
Mid-Atlantic Integrated Assessment Program
701 Mapes Road
Fort Meade, MD 20755-5350
Wayne Davis, Project Officer

Under subcontract from:
Technology Planning & Management Corporation
Mill Wharf Plaza, Suite 208
Scituate, MA 02066

Prepared by:
J.H. Vølstad, N.E. Roth,
M.T. Southerland, G. Mercurio

Versar, Inc.
ESM Operations
9200 Rumsey Road
Columbia, MD 21045

Printed on chlorine free 100% recycled paper with
100% post-consumer fiber using vegetable-based ink.

NOTICE

This document has been reviewed and approved in accordance with U.S. Environmental Protection Agency policy. Mention of trade names, products, or services does not convey and should not be interpreted as conveying official EPA approval, endorsement, or recommendation for use.

Funding was provided by the U.S. Environmental Protection Agency under U.S. Department of Commerce, Commerce Information Technical Solutions Contract No. 50-CMAA-900065 with Technology Planning and Management Corporation.

The appropriate citation for this report is:

Vølstad, J.H, N.E. Roth, M.T. Southerland and G. Mercurio. 2003. Pilot Study for Montgomery County and Maryland DNR Data Integration: Comparison of Benthic Macroinvertebrate Sampling Protocols for Freshwater Streams. Prepared by Versar, Inc., Columbia, Maryland for U.S. Environmental Protection Agency, Office of Environmental Information and Mid-Atlantic Integrated Assessment Program, Region 3, Ft. Meade, MD.

This document can be downloaded from EPA's website for Biological Indicators of Watershed Health:

http://www.epa.gov/bioindicators

ACKNOWLEDGEMENTS

EPA funded this study through contract no. 50CMAA900065 to TPMC, Project No. 6073-012 to Versar subcontract. We are grateful to Wayne Davis (EPA, Office of Environmental Information), Keith Van Ness (Montgomery County), and Ron Klauda and Paul Kazyak (Maryland Department of Natural Resources) for supporting this project. Montgomery County staff collected all field data, sorted organisms from debris, and subsampled organisms in the laboratory. The Versar benthic laboratory performed the taxonomic identification of organisms in all samples.

ABSTRACT

At both state and local levels, bioassessment programs supply valuable information to guide stream resource management. For example, a regulatory decision-making framework is currently being developed by the Maryland Department of the Environment (MDE) for listing watersheds (Maryland 8-digit and 12-digit watersheds) as impaired based on indices of biotic integrity (IBIs), initially for freshwater, non-tidal streams. The primary source of data for developing and implementing the biocriteria framework is the Maryland Biological Stream Survey (MBSS) conducted by the Maryland Department of Natural Resources (DNR). Several counties in Maryland conduct biological sampling of streams and produce more spatially intensive results that can be of use for biocriteria and other stream management activities.

To successfully integrate IBI data collected by both county and state monitoring in the same watersheds, differences in sampling protocols must be evaluated. This report presents the results of a quantitative comparison of benthic sampling protocols used by MBSS and Montgomery County to assess freshwater, non-tidal streams. Montgomery County Department of Environmental Protection (DEP) has monitored streams since 1994 and is currently exploring adopting MBSS protocols for benthic macroinvertebrates. This comparison study involved paired sampling at a random subset of sites. The experimental sites were allocated in a balanced manner into catchments with both high and low percentage urban land use and small and large stream size, ensuring that paired sampling was conducted across a range of stream condition.

This study supports the contention that Montgomery County and Maryland DNR stream monitoring of benthic macroinvertebrate communities can be effectively integrated. In the case of sampling protocol differences, integration options include (1) continuing to use different protocols when the mean results are comparable but of differing precision; (2) adjusting the result from one protocol to match the other, usually with a loss of precision; and (3) agreeing to adopt the same protocol.

The study demonstrates that D-Net sampling protocol can provide more reliable benthic indices of biotic integrity (B-IBI) indices than the Kick Seine protocol because sampling from more plots is more representative of the stream segment. This study also indicates that Montgomery County could improve the precision of their B-IBIs by increasing the level of chironomid and oligochaete identification to genus level. For the same overall survey cost, however, we conclude that the identification of chironomids to tribe, in conjunction with an appropriate increase in the number of sampling sites, could yield a similar level of precision in mean B-IBI scores. For some monitoring programs, the moderate improvements in IBI precision obtained by identifying chironomids to genus may not warrant the needed investments in equipment and training. One option for such programs is to identify these taxa to tribe as part of a B-IBI for watershed screening and to identify these taxa to genus only at impaired stations to support stressor identification.

CONTENTS

Page

NOTICE ..... **ii**
ACKNOWLEDGEMENTS ..... **iii**
ABSTRACT ..... **iv**
FIGURES ..... **vi**
TABLES ..... **vii**
1.0 INTRODUCTION **1-1**
2.0 METHODS **2-1**
2.1 Experimental Design 2-1
2.2 Replicate Sampling 2-3
2.3 Compilation of Field and Laboratory Data 2-3
2.3.1 Field Sampling 2-3
2.3.2 Laboratory Subsampling and Taxonomic Identification 2-4
2.4 Analytical Methods 2-6
3.0 RESULTS **3-1**
3.1 Comparisons by Stream Order and Human Disturbance Class 3-1
3.2 Comparisons of MBSS and Montgomery County Field and Laboratory Methods 3-2
3.2.1 MBSS B-IBI and Individual Metrics 3-2
3.3 Comparisons of 100- versus 200-organism Subsampling 3-5
3.3.1 MBSS B-IBI and Individual Metrics 3-5
3.4 Effects of Sampling Method and Laboratory Protocol on the Precision of Mean B-IBI Scores 3-9
3.5 Comparisons by Taxonomic Level for Chironomids and Oligochaetes 3-9
3.6 Cost-Benefit Analysis of Taxonomic Identification Level 3-14
4.0 DISCUSSION **4-1**
5.0 CONCLUSIONS **5-1**
6.0 REFERENCES **6-1**

APPENDIX
Frequency Distribution (%) of Individual Taxa Across All 24 Sites by Sampling Method and Laboratory Protocol **A-1**

FIGURES

Figure No. **Page**

2-1 Locations of 24 stream sites in Montgomery County, Maryland, used for paired comparison of field sampling methods during spring 2001 2-2

3-1 Mean B-IBI (MBSS method) for D-Net "100-organism" samples by urban land use class and stream order (SO) 3-3
3-2 Mean B-IBI (MBSS method) for Kick Seine "200-organism" samples by urban land use class and SO 3-3
3-3 Mean B-IBI scores (MBSS method) by urban land use class and SO for (1) *D-Net* samples with "100-organism" subsample, and (2) *Kick Seine* samples with "200-organism" subsample 3-4
3-4 Paired comparison of B-IBI scores (MBSS method) for D-Net samples with "100-organism" subsample, and Kick Seine samples with "200-organism" subsample, using linear regression 3-4
3-5 Mean B-IBI scores (MBSS method) by urban land use class and SO for (1) D-Net samples with "100-organism" subsample, and (2) D-Net with "200-organism" subsample 3-6
3-6 Mean B-IBI scores (MBSS method) by urban land use class and stream order for Kick Seine samples with "100-organism" and "200-organism" subsamples 3-6
3-7 Paired comparison of B-IBI scores (MBSS method) for D-Net samples with "100-organism" versus "200-organism" subsamples using linear regression 3-7
3-8 Paired comparison of B-IBI scores (MBSS method) for Kick Seine samples with "100-organism" versus "200-organism" subsamples using linear regression 3-7
3-9 Mean B-IBI (MBSS method) across all sites (n=24) by field method and laboratory subsampling procedure 3-10
3-10 Relative SE (RSE= SE/$\bar{x}$) of mean B-IBI (MBSS method) scores across all sites (n=24) by field method and laboratory subsampling procedure 3-10
3-11 Relationship between standard B-IBI scores for D-Net "100 organism" samples with chironomids and oligochaetes identified to genus, and B-IBI scores with chironomids and oligochaetes lumped to tribe. 3-11
3-12 Relationship between standard B-IBI scores for D-Net "100 organism" samples with chironomids and oligochaetes identified to genus, and B-IBI scores with oligochaetes lumped to family 3-11
3-13 Relationship between standard B-IBI scores for D-Net "100 organism" samples with chironomids and oligochaetes identified to genus, and B-IBI scores from Kick Seine "200 organism" samples with chironomids and oligochaetes lumped to tribe 3-12
3-14 Relationship between standard B-IBI scores for D-Net "100 organism" samples with chironomids and oligochaetes identified to genus, and B-IBI scores from Kick Seine "200 organism" samples with chironomids and oligochaetes lumped to family 3-12

TABLES

Table No. **Page**

2-1 Summary of experimental design.. 2-1

2-2 Description of different taxonomic identification methods compared, and the associated laboratory processing time for chironomids and oligochaetes. 2-6

3-1 Number of organisms in the laboratory subsamples for “100 organism” and “200 organism” target sample size. 3-1

3-2 Comparison of MBSS B-IBI and individual metrics from paired samples using MBSS and Montgomery County methods (D-Net “100-organism” versus Kick Seine “200-organism”) with test statistic for paired t-test 3-5

3-3 Mean difference between MBSS B-IBI scores and individual metrics for D-Net “100-organism” and “200-organism” subsamples with associated SEs and p-values for paired t-test. 3-8

3-4 Mean difference between MBSS B-IBI scores and individual B-IBI metrics for Kick Seine “100-organism” and “200-organism” subsamples with associated SEs and p-values for paired t-test 3-8

3-5 B-IBI scores for MBSS 8-digit watersheds for 2000 for three levels of taxonomic identification for chironomids and oligochaetes 3-13

3-6 Mean predicted B-IBI scores for 8-digit watersheds (at genus level) for MBSS 2000 from samples where chironomids and oligochaetes were lumped to tribe or family 3-13

1.0 INTRODUCTION

To meet the requirements of the Clean Water Act (CWA), the MDE, with the assistance of the Biological Criteria Advisory Committee, has developed an interim regulatory framework for the application of biocriteria to Maryland's water quality inventory (305(b) report) and list of impaired waters (303(d) list). The proposed biocriteria apply to wadeable, non-tidal (first- to fourth-order) streams, and rely on two biological indicators from the Maryland Biological Stream Survey (MBSS or the Survey; Klauda et al. 1998), the fish Index of Biotic Integrity (IBI) (Roth et al. 1998) and the benthic macroinvertebrate IBI (Stribling et al. 1998). The MDE applies the results of MBSS biological sampling for management and regulatory purposes (e.g., CWA §303(d) listing) at the same spatial resolution (Maryland 8-digit watershed) currently used in the state's Water Quality Inventory (305(b) report). Maryland defines 8-digit watersheds and 12-digit subwatersheds at a scale finer than the USGS 8-digit Hydrologic Unit Codes (HUCs). In some but not all cases, these state-defined units are true topographic watersheds (Omernik and Bailey 1997). Maryland 8-digit watersheds (average area 194 km^2) are subunits of USGS 8-digit HUCs (average area in Maryland 1295 km^2). The first round of MBSS (1995-1997) focused on the basin level. When sample sizes are sufficient, the results from the first round can also be applied to the 8-digit watershed level because the inclusion probabilities of all samples are known. In this case, each 8-digit watershed is considered to be a sub-population (domain) of the basins (see Cochran, 1977; Vølstad et al. 2003a).

The primary source of data for developing and implementing the biocriteria framework is the MBSS conducted by the Maryland DNR. However, other bioassessment programs conducted at both state and local levels supply valuable information to guide stream resource management. When local programs are probability-based, and provide IBI data that are compatible with data from the MBSS, it is desirable to include these data in the state's biocriteria framework to increase sample sizes and enhance the reliability of the water quality assessments. The interim biocriteria framework for Maryland requires that fish or benthic IBI data from ten or more MBSS sites be used to evaluate impairment of a Maryland 8-digit watershed. To apply biocriteria where sample size is insufficient to characterize the 8-digit watershed, the smaller 12-digit subwatersheds (statewide average area 21 km^2) contained within the 8-digit watershed are evaluated to determine impairment. A 12-digit subwatershed is determined to be impaired if either the fish or the benthic IBI fails to meet a predetermined threshold (as defined in the biocriteria framework) at any site. In the future, fish and benthic IBI data from biological surveys conducted by the counties could increase the sampling coverage. In addition, the potential exists for integrating volunteer monitoring data that have a probability-based study design with state and local program data.

In Maryland, several counties conduct biological sampling of streams, and IBI data are currently available from more than one survey for some watersheds. When the surveys are probability-based, as is the case for one component of the Montgomery County stream survey, mean IBIs that are more precise than the separate estimates can be achieved by using composite estimators

(Korn and Graubard 1999; Vølstad et al. 2003a). Both state and local program managers recognize the advantages of integrating stream monitoring. Potential advantages to monitoring program integration include (1) consistent statements to the public about stream condition, (2) increased accuracy in estimates of stream condition, and (3) reduced cost of sampling programs. Specifically, integrated data analysis has the potential to increase the precision of stream condition estimates (e.g., mean IBIs) for each program. In addition, the potential exists for integrating volunteer monitoring data with state and local program data. An evaluation of the effects of field and laboratory protocols on estimates of stream condition is one critical component of an effective integration of stream monitoring programs.

The objective of this project was to provide a quantitative comparison of how differences in benthic sampling protocols used by MBSS and Montgomery County affect the assessments of freshwater, non-tidal streams based on IBIs. Montgomery County Department of Environmental Protection (DEP) has monitored streams since 1994 and is currently exploring adoption of MBSS protocols for benthic macroinvertebrates. This methods comparability study was conducted to (1) evaluate how the change in methods could affect Montgomery County assessment results and (2) facilitate use of County data by MBSS in developing consistent assessments of stream condition. This comparison study, involving two established programs, also affords an opportunity to develop a statistical design and methods comparison approach that has general applicability for the integration of county and state monitoring programs.

Historically, Montgomery County has sampled benthic organisms with field and laboratory methods that differed somewhat from those used by MBSS, as detailed in Section 2, with two Kick Seine samples (2.00 m^2 total) in riffle habitat only. The organisms collected from these two samples were composited, and a target of 200 organisms were subsampled from this composite sample in the lab and identified. Most taxa were identified to genus, but chironomids and oligochaetes were only identified to family level. Starting in 2001, Montgomery County began using D-Nets as the standard gear for sampling benthic macroinvertebrates following MBSS protocol. In the MBSS, a composite sample of benthic organisms is collected at each station from 20 jabs (1.85 m^2 total) with a D-Net in a variety of habitats (primarily riffles). A 100-organism subsample is identified in the laboratory. Chironomids and oligochaetes are slide mounted and identified to genus or lowest possible taxonomic level.

A previous study to compare Montgomery County Kick Seine and MBSS D-Net methods was conducted jointly by the two programs in 1997, with paired sampling at 12 sites selected ad hoc. Although scores from the two programs generally were in the same (or neighboring) assessment categories (Roth et al. 2001), the results were inconclusive because of (1) low sample sizes and (2) a study design that resulted in little spread in IBI scores among the experimental sites. Therefore, a more extensive study that covers a wide range of stream conditions was recommended. In this study, we applied a stringent experimental design that was implemented by Montgomery County to effectively compare the effects of differences in the Montgomery County and MBSS benthic sampling and laboratory processing protocols on IBI scores. Sampling for this comparison study was conducted in spring 2001.

2.0 METHODS

2.1 EXPERIMENTAL DESIGN

A stringent experimental design was implemented for the 2001 comparison study to increase the power for (1) detecting differences between field sampling methods and (2) determining the effects of different laboratory protocols for subsampling organisms prior to identification. A randomized paired comparison design (Box et al. 1978) was employed to study the effects of benthic sampling protocols and gear (D-Net versus Kick Seine) on IBI scores and individual metrics under a variety of stream conditions. The experiment involved paired sampling at a random selection of sites within four blocks defined by stream order and urban land use (Table 2-1). These blocks were introduced to eliminate unwanted sources of variability; the randomization within blocks supports valid inferences in the face of the remaining variability which could not be controlled. The percentage of impervious surface in the catchments was used as a proxy variable for poor and good stream condition. Streams were also classified into size according to their stream order: The sites were grouped into two land use classes: high urban (catchments > 15% impervious) and low urban (catchments < 15% impervious). Stream order 1-2 (small streams) versus 3-4 (large streams), with the stream order being based on the Strahler convention (Strahler 1957), using the Montgomery County 1:24,000 scale map. The impervious area percentages were based on the County-wide Stream Protection Strategy (CSPS), 1998. These impervious area percentages are based on actual ground cover from aerial photos performed in 1998. Any development that may have occurred between 1998 through 2002 was minimal in the rural station areas.

Table 2-1. Summary of experimental design. Paired D-Net and Kick Seine sampling was conducted at each site.

Percentage of Impervious Surface	Stream Order (1:24K map)	Number of Sites
Catchments with > 15% impervious surface	1,2	6
	3+	6
Catchments with ≤ 15% impervious surface	1,2	6
	3+	6
Both Classes of Land Use	1-4	24

Sites for paired sampling methods comparisons (Kick Seine and D-Nets) were randomly selected from a list of Montgomery County sites within each block. This experimental design ensured that paired sampling was conducted across a wide range of stream conditions (Figure 2-1). Paired comparisons were conducted at six sites per block, for a total number of 24 sites. Because of the relatively low total sample size, it was critical that the design be balanced (i.e., that paired sampling is conducted at an equal number of sites within each of the four blocks) to maintain adequate statistical power.

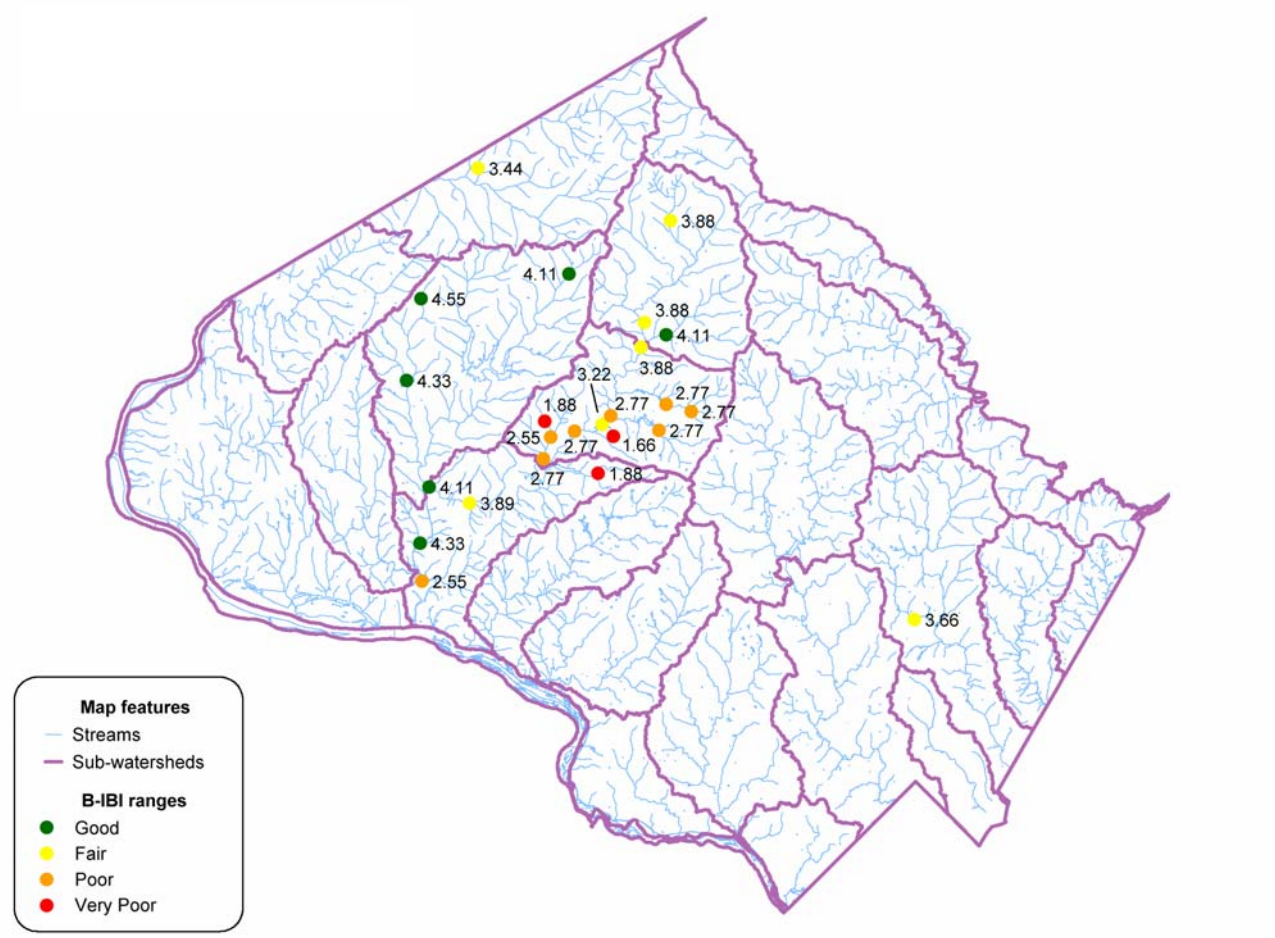

Figure 2-1. Locations of 24 stream sites in Montgomery County, Maryland, used for paired comparison of field sampling methods during spring 2001. The benthic indices of B-IBI are based on the MBSS method applied to the D-Net 100-organism subsamples.

2.2 REPLICATE SAMPLING

In addition to obtaining data for the comparison study, Montgomery County DEP is interested in quantifying variability in its B-IBI scores resulting from repeated sampling at a single site using the MBSS protocols. Similar replicate sampling (collection of two MBSS samples at a site) is conducted at approximately 5% of sites per year in the MBSS; these data have provided Maryland DNR with an estimate of small-scale variance, which is useful in data interpretation. Montgomery County DEP requested guidance on how many replicate samples it should take to estimate the within-site variance. We recommended that Montgomery County follow the MBSS protocol and collect two samples during each site visit (replicates) with D-Nets within a random subset of 5-10% of the total number of sites sampled in each year. Over time, this will provide sufficient data for evaluating measurement error in IBI scores. Such information is particularly useful when evaluating temporal trends in IBI scores at individual sites. The County reported that it was able to sample this minimum number of sites as replicates during spring 2001. When a sufficient number of samples becomes available, analysis of these replicate sample data can be performed at a future time, but this is outside the scope of this project.

2.3 COMPILATION OF FIELD AND LABORATORY DATA

2.3.1 Field Sampling

At each experimental site, paired sampling was conducted in a 75-m stream segment using the two field sampling methods:

(1) D-Net method: 20 jabs in multiple habitats with 600-micron-mesh D-frame dipnet (D-Net) used in MBSS (Kazyak 2001), and

(2) Kick Seine method: two Kick Seine collections in riffles with a mesh size of 530 microns used by the Montgomery County (Van Ness et al. 1997) from 1994 to 2001.

The Montgomery County Water Quality Monitoring Program staff conducted D-Net sampling within each stream segment (mainly from riffles) to collect organisms from habitats likely to support the greatest taxonomic diversity; the benthic macroinvertebrates collected from the 20 jabs were pooled into one composite sample. The Montgomery County staff received training from Maryland DNR in the standard 20 jabs D-Net method used in the MBSS before the experimental field data were collected. Within the same 75-m stream segments, the Montgomery County staff collected a paired sample using a Kick Seine, following standard Montgomery County protocol. On each sampling event, two Kick Seine samples were collected from the same stream segment – one from an area of fast current velocity and one from an area of slower current velocity. These two Kick Seine samples were then combined to provide one composite sample for each site.

2.3.2 Laboratory Subsampling and Taxonomic Identification

For this comparison, the B-IBI scores for all samples were calculated using the standard MBSS B-IBI method (Stribling et al. 1998). The standard Montgomery County and MBSS laboratory protocols were modified to improve the sensitivity of the study to detect differences in B-IBI scores and B-IBI metrics attributable specifically to a “100-organism” versus “200-organism” subsampling protocol in the laboratory. The standard laboratory subsampling of benthos conducted by MBSS and Montgomery County involves the distribution of organisms in the composite sample across a gridded tray; organisms are then picked from randomly selected grids. When the cumulative number of organisms from random grids reaches the target sample size of 100 or 200 organisms, the remaining organisms in the last grid are also included in what is called the “100-organism” or “200-organism” subsample. Thus, the actual number of organisms in a sample may exceed the target sample size. For this methods comparison study, the subsamples were selected in two stages: First a "100-organism" subsample was collected from the required number of random grids; and second, additional random grids were picked until a "200-organism" subsample was achieved. The organisms collected in each stage were placed in two separate containers for identification. An example of this three-stage procedure is as follows:

> The laboratory technician picks grids up to and including the grid containing the 100th organism. This first subsample contains 134 organisms. All go into the first container (subsample 1).
>
> The 135th organism goes into a second container. The technician continues picking organisms from random grids up to and including the grid containing the 200th organism. These organisms go into the second container (subsample 2).
>
> After the laboratory identification, data from the two containers are combined to make up the 200-organism subsample.

By identifying the two groups of organisms separately for each sample, we calculated separate IBI scores for the first group (the “100-organism” subsample) and for the combined groups (“200-organism” subsample). This information was used to assess how subsample size affects B-IBI scores and individual B-IBI metrics. Note that if the first “100-organism” subsample contains a large number of individuals (i.e., approaching or exceeding 200), then few, if any, additional organisms are needed to achieve the combined “200-organism” subsample, and the two subsamples will be similar or identical.

In addition, the remaining “sortate” (debris containing the remainder of individuals in the composite sample) was preserved and retained. These data could be analyzed at a later stage to evaluate the effects of larger subsample sizes, different grid sampling techniques, or other questions of interest.

For this study, the taxonomic identifications for all samples were conducted to genus level so that the effects of taxonomic identification level on IBI scores could be evaluated. Montgomery County protocol involves identifying chironomids and oligochaetes to family, and all other specimens to genus. For this comparison, all chironomids and oligochaetes were slide mounted and identified to genus; these data can be aggregated to family during data analysis for the comparison of IBI scores. Identification procedures employed here differed slightly from the MBSS standard protocol, which employs some subsampling of chironomids. As outlined in Boward and Friedman (2000), MBSS standard laboratory protocols are to identify most organisms to genus, if possible. Exceptions, and their corresponding target taxonomic level, include chironomids and oligochaetes (family), Nematoda (phylum), Nematomorpha (family). Those taxa not identifiable to genus (due to small size or damage) may be identified to family level or higher. The MBSS process for identifying chironomid larvae (Boward and Friedman 2000) is as follows:

Divide chironomid larvae into subfamily (i.e., Chironominae, Orthocladiinae, Tanypodinae, Diamesinae) or tribe (i.e., Tanytarsini, Chironomini) and count the total number in each group.

Identify using slide mounts for a subsample of approximately 20% of the individual larvae within each subfamily or tribe. Once these subsamples are identified, multiply the counts of all genera by five and record the total extrapolated number of genera for the entire chironomid group.

If either the total number of chironomids or the total number of individuals within a subfamily or tribe is ten or less, all larvae are identified (no subsampling is performed).

The four levels of taxonomic identification that were compared in this study and the estimated laboratory processing time are summarized in Table 2-2. We assume that collection of each benthic field sample takes 2 hours, with a crew of two.

Table 2-2. Description of different taxonomic identification methods compared, and the associated laboratory processing time for chironomids and oligochaetes. The additional laboratory processing time for other macroinvertebrates is estimated as 1 - ¼ hour per 100-organism subsample.

Method Name as Used in Text and Tables	Taxonomic Level of Identification	Relative Level of Laboratory Effort	Estimated Number of Hours for Laboratory Processing (100-organism subsample)
Genus	Chironomidae - Genus Oligocheata - Genus	Most Effort	1
Genus - Chironomidae only	Chironomidae - Genus Oligocheata - Family		¾
Tribe	Chironomidae - Tribe Oligocheata - Family		½
Family	Chironomidae - Family Oligocheata - Family	Least Effort	¼

2.4 ANALYTICAL METHODS

The randomization and blocking of sites by stream order and land use in conjunction with the paired comparison of methods support the use of paired *t* tests for testing differences in B-IBI scores and the suite of individual B-IBI metrics (see Box et al. 1978, p. 101). Analysis of variance (ANOVA) for a two-factor experiment was also used to further evaluate differences in mean B-IBI scores between stream orders and the two classes of urban land use using the model:

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + \varepsilon_{k(ij)} \qquad (1.1)$$

where Y_{ijk} is the B-IBI score for sample *k* in stream order *i* and urban land use class *j*, *A* represents the stream order factor (*i*=1,2); *B* represents the urban land use factor (*j*=1,2), and *k*=1,2,...,6 signify the observations collected within each cell. This model was applied separately to D-Net "100-organism" samples and Kick Seine "200-organism" samples.

Model 1.1 was also expanded to include the effects of field and laboratory methods on differences in
B-IBI scores, using the following model:

$$Y_{ijkl} = \mu + T_l + A_i + B_j + AB_{ij} + T_l(AB_{ij}) + \varepsilon_{k(ij)} \qquad (1.2)$$

where T_l represents the combination of field method and laboratory subsampling configurations (l = 1,2,..,4), and $T_l(AB_{ij})$ is the effect of method within each cell defined by stream order and

land use. The factor of interest here is T_l (alone, or within stream order and land use); the other factors were introduced to remove or lessen the effects of stream order and land use on B-IBI scores and thereby increase the sensitivity of the analysis for detecting significant differences in B-IBI scores caused by the choice of sampling method. We used model 1.2 to examine the variation in B-IBI scores between D-Net with "100-organism" subsamples and Kick Seine with "200-organism" subsamples, as well as variation caused by subsampling sizes within each field method (e.g., D-Net with "100-organism" versus "200-organism" subsamples).

We also conducted a linear regression analysis of B-IBI scores from replicated paired samples of the same stream segments using the model,

$$Y = \alpha + \beta X \qquad (1.3)$$

where Y is the IBI score for the second sample, and X is the IBI score for the first sample. When equation 1.3 is used to predict mean IBI scores, the standard error will be inflated because of uncertainty in the regression parameters. In the prediction of mean genus level B-IBI from mean tribe or family B-IBIs, the intercept in equation 1.3 was not significant, and was, therefore, not included in the regression. An approximate estimator for the variance (S_y^2) of a predicted mean IBI when the intercept $\alpha = 0$ is

$$S_y^2 = (\hat{\beta}\hat{\sigma}_{\bar{x}})^2 + (\bar{X}\hat{\sigma}_{\beta})^2 - \hat{\sigma}_{\bar{x}}^2 \times \hat{\sigma}_{\beta}^2 \qquad (1.4)$$

where $\sigma_{\bar{x}}$ is the standard deviation of the mean variable $\bar{X}$, σ_{β} is the standard deviation of the estimated slope ($\hat{\beta}$). This equation is based on the variance estimator for a product of two independent random variables (Goodman 1960; Kendall et al. 1987, p. 342). The square root of model 1.4 is an estimator of the standard error (SE) of the mean predicted score.

Model 1.3 was used to compare paired benthic IBI scores for (1) D-Net "100-organism" versus "200-organism"; (2) Kick Seine "100-organism" versus "200-organism"; (3) D-Net "100-organism" versus Kick Seine "200-organism", and (4) IBI scores at different taxonomic identification levels for chironomids and oligochaetes. The regressions can be used to calibrate B-IBI results from Montgomery County and MBSS and to evaluate effects of taxonomic levels on the rating of stream condition. The similarity of the IBI scores was assessed by the slopes and the R^2. The regression plots also offered a simple visual means of determining whether the variability in IBI scores within stream segments tended to be greater for high or low mean scores.

3.0 RESULTS

3.1 COMPARISONS BY STREAM ORDER AND HUMAN DISTURBANCE CLASS

The 24 experimental sites represent a wide range of stream sizes and degree of anthropogenic disturbance as intended by the experimental design (Table 3-1). On average, the “100 organism” subsamples for each method significantly exceeded the target number of 100 specimens (at 5% alpha level). In three cases the target “100 organism” subsample exceeded 200 organisms, and thus the “100 organism” and the “200 organism” samples were identical. Two sites (GSGN 104 and GSLD 110) did not have sufficient number of specimens to achieve the target sample sizes.

Table 3-1. Number of organisms in the laboratory subsamples for “100 organism” and “200 organism” target sample size. The percent impervious area in the catchments is indicated for the urban class. The mean number of organisms across sites and the associated SEs by sampling method and laboratory protocol are shown in the last row.

Station	Stream Order	Urban	D-Net		Kick Seine	
			"100 Organism"	"200 Organism"	"100 Organism"	"200 Organism"
GSCB 111	1	High, 23%	123	240	179	282
GSGN 104	1	High, 23%	32	32	32	32
GSLD 110	1	High, 28%	101	101	97	198
GSLS 101	1	Low, 4%	99	208	136	228
GSLS 102	1	Low, 5%	121	248	111	261
GSMS 112	1	High, 30%	95	168	105	245
LSBL 110	1	Low, 4%	101	219	111	204
GSCB 207	2	High, 23%	108	210	108	227
GSGN 205	2	High, 23%	96	235	122	206
LSBL 203	2	Low, 5%	130	244	106	219
LSLS 202	2	Low, 5%	109	228	194	314
LSLS 203	2	Low, 5%	184	368	237	237
GSGB 303	3	Low, 5%	292	292	252	406
GSGN 302	3	High, 23%	118	241	96	203
GSGS 303	3	Low, 5%	145	248	184	235
GSWR 302	3	High, 33%	157	266	156	289
GSWR 305	3	High, 33%	108	251	175	319
GSGS 402	4	Low, 5%	216	216	198	263
GSLS 430	4	Low, 5%	209	209	142	284
GSLS 438	4	Low, 4%	84	195	97	208
GSMS 404	4	Low, < 15%	177	266	104	202
GSMS 406	4	High, 21%	152	285	407	677
GSMS 413	4	High, 21%	133	212	135	223
GSMS 415	4	High, 21%	164	276	109	258
Mean count across all sites (SE in brackets)			132 (10)	227 (13)	150 (15)	259 (22)

For this analysis, MBSS B-IBI scores were computed following the protocols described in Stribling et al. (1998). Sites with high urban land use (catchments >15% impervious surface) generally had significantly lower mean B-IBI scores than sites with low urban land use (catchments ≤15% impervious surface), while only small differences in mean scores by stream order were observed (Figures 3-1 and 3-2).

The model 1.1 ANOVA applied to B-IBI values based on D-Net "100-organism" samples was highly significant (F=22.72; p<0.0001) with R^2 =0.77. The ANOVA showed highly significant differences in mean B-IBI scores between the two urban land use classes (F = 56.59; p <0.001) and for the interaction between land use and stream order factors (F = 10.67; p = 0.0032), while stream order alone had no significant effect on IBI scores (F = 0.39; p = 0.54) from the D-Net samples. The same ANOVA model applied to the Kick Seine "200-organism" samples also was highly significant (F=18.12; p<0.0001), with R^2 =0.73. As for the D-Net, a highly significant difference for urban land use (F=49.84; p<0.001) was observed, but neither stream order (F=2.52; p=0.13) nor the interaction between stream order and urban land use class (F=1.99; p=0.17) had a significant effect.

3.2 COMPARISONS OF MBSS AND MONTGOMERY COUNTY FIELD AND LABORATORY METHODS

3.2.1 MBSS B-IBI and Individual Metrics

The standard MBSS D-Net and the Montgomery County Kick Seine sampling protocols resulted in similar mean B-IBI scores by stream order and urban land use (Figure 3-3). The paired t-test showed no significant difference in B-IBI scores (MBSS method, Stribling et al. 1998) between the D-Net with "100-organism" and the Kick Seine "200-organism" samples (Table 3-2). However, the Kick Seine "200-organism" samples had significantly larger values on average for many of the individual B-IBI metrics (e.g., over 13 more taxa per site were collected on average by the Kick Seine) as compared to the D-Net "100-organism" samples. The linear relationship and a coefficient of determination (R^2) of 0.77 suggest that the scores from the two methods are comparable on average. However, the fairly large spread of scores around the regression line suggest that the prediction of stream condition at individual sites could vary substantially depending on the sampling protocol with increasing uncertainty for streams that had B-IBI scores above 3.0 (Figure 3-4).

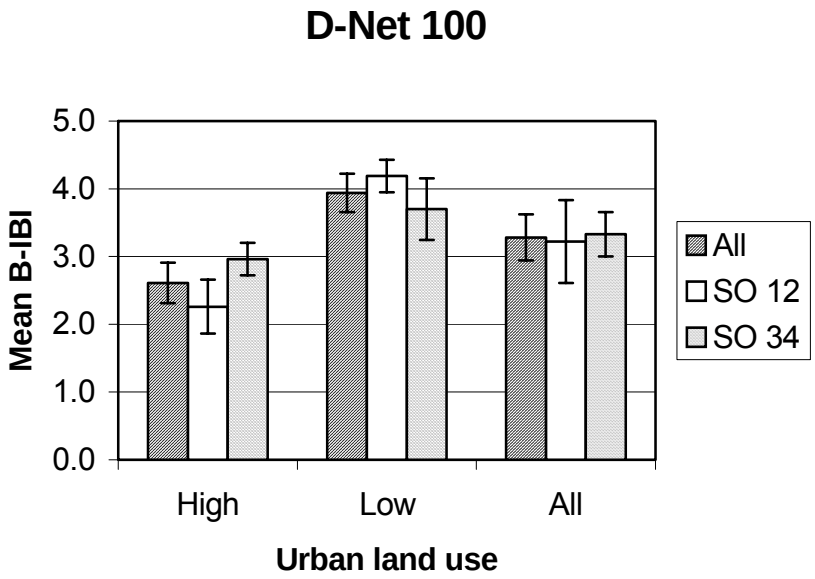

Figure 3-1. Mean B-IBI (MBSS method) for D-Net "100-organism" samples by urban land use class and stream order (SO). ***High*** and ***Low*** urban land use defines catchments with >15% and ≤15% impervious surface, and ***All*** represents both classes. *SO 12* represents orders 1 and 2 combined, *SO 34* represents orders 3 and 4 combined, and *All* represents stream orders 1–4 combined.

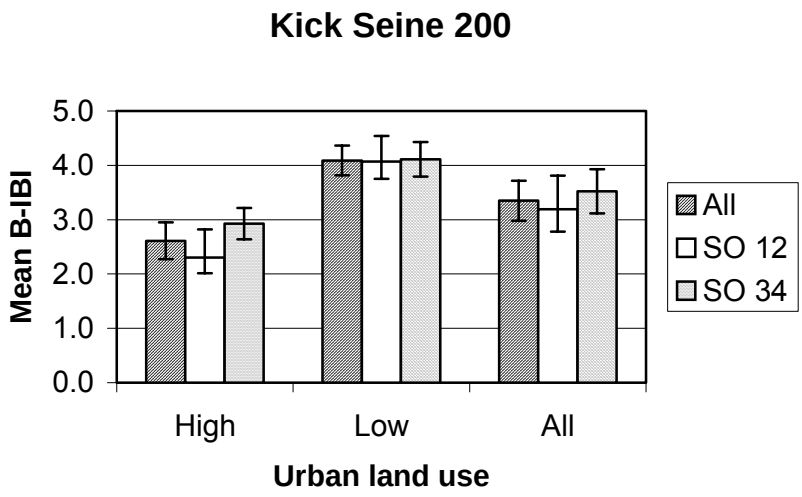

Figure 3-2. Mean B-IBI (MBSS method) for Kick Seine "200-organism" samples by urban land use class and SO. ***High*** and ***Low*** urban land use defines catchments with >15% and ≤15% impervious surface, and ***All*** represents both classes. *SO 12* represents orders 1 and 2 combined, *SO 34* represents orders 3 and 4 combined, and *All* represents orders 1–4 combined.

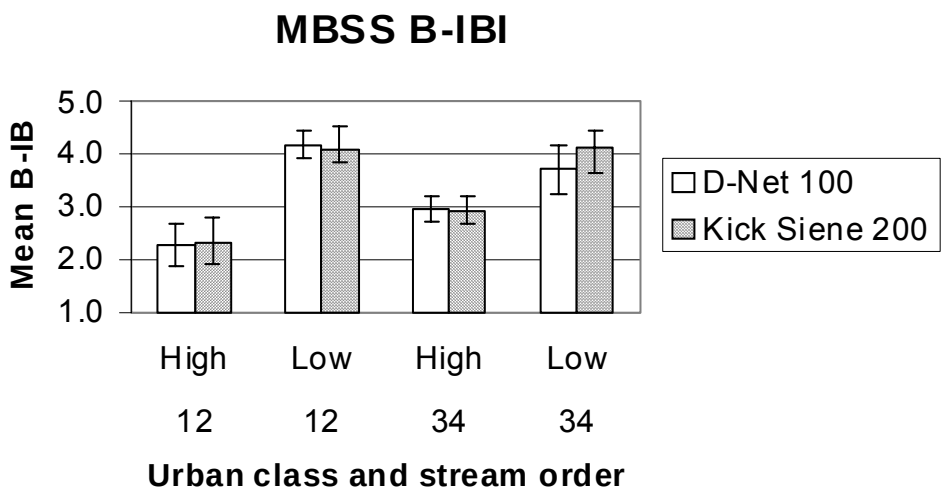

Figure 3-3. Mean B-IBI scores (MBSS method) by urban land use class and SO for (1) *D-Net* samples with "100-organism" subsample, and (2) *Kick Seine* samples with "200-organism" subsample. ***High*** and ***Low*** urban land use define catchments with >15% and ≤15% impervious surface, respectively. *SO 12* represents order 1 and 2 combined, while *SO 34* represents order 3 and 4 combined.

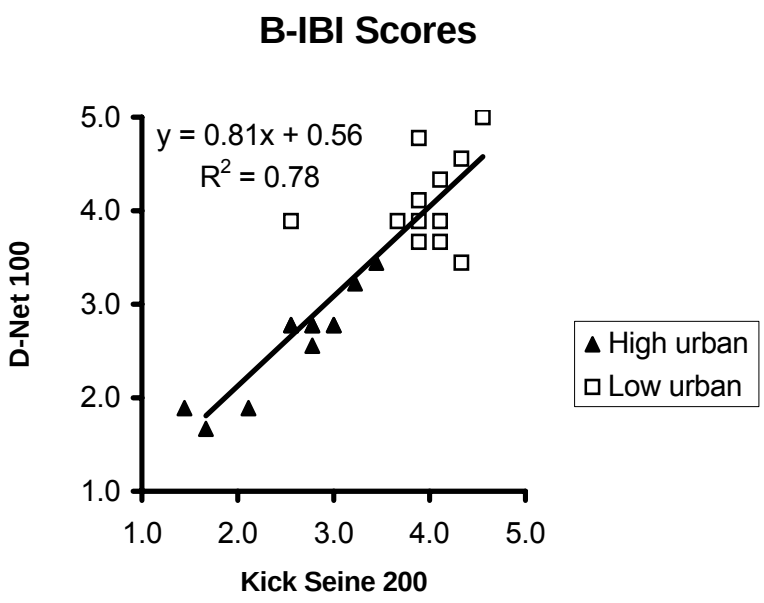

Figure 3-4. Paired comparison of B-IBI scores (MBSS method) for D-Net samples with "100-organism" subsample, and Kick Seine samples with "200-organism" subsample, using linear regression. High and low urban land uses are defined by catchments with >15% and ≤15% impervious surface, respectively. The regression coefficients were estimated using SAS (SAS Institute 1999) and may not correspond exactly with the regression line fitted Microsoft® Excel.

Table 3-2. Comparison of MBSS B-IBI and individual metrics from paired samples using MBSS and Montgomery County methods (D-Net "100-organism" versus Kick Seine "200-organism") with test statistic for paired t-test. Total number of paired samples across stream order and land use is n =24.

Parameter	Δ = Difference in D-Net and Kick Seine paired B-IBI values		Pr > $\lvert t \rvert$
	Mean Δ	SE	
B-IBI	-0.07	0.09	0.42
Number of Taxa	-13.58	2.27	<0.0001
Number of EPT Taxa	-3.42	0.67	<0.0001
Number of Diptera Taxa	-9.33	1.80	<0.0001
Percentage Ephemeroptera Individuals of Total Number of Individuals	0.60	1.15	0.61
Percent Tanytarsini of Total Number of Individuals	3.35	1.19	0.0097
Number of Intolerant Taxa	-1.88	0.58	0.0038
Percent Tolerant Individuals	-0.26	5.00	0.96
Percent Collectors	4.59	1.80	0.018

The ANOVA using model 1.2 was highly significant (F=17.22; p<0.0001) with R^2 =0.75, showing no significant effect of method (T_l) alone (F=0.29; p=0.59), nor of method within stream order and land use cells (F=0.70; p=0.56) on B-IBI scores. This indicates that the four combinations of field method and subsampling procedures produce comparable B-IBI scores. Regression results (model 1.3) suggest that B-IBI scores from the two sampling protocols could be used interchangeably but at a cost of increased standard errors. The estimated intercept and slope in the linear regression of D-Net 100 IBI values against Kick Seine 200 values (model 1.3) were 0.56 (SE=0.32) and 0.81 (SE=0.92), respectively. The mean predicted scores had a standard error of 0.47 (model 1.4), as compared to 0.17 and 0.19 for the mean IBI scores from D-Net 100 and Kick Seine 200 samples, respectively.

3.3 COMPARISONS OF 100- VERSUS 200-ORGANISM SUBSAMPLING

3.3.1 MBSS B-IBI and Individual Metrics

The regression analysis of scores from "100-organism" versus "200-organism" subsamples by method show that the B-IBI score for a "200-organism" subsample was 8% higher for D-Net and 12% higher for Kick Seine. The mean B-IBI scores for the "200-organism" samples were significantly higher than for the "100-organism" (Tables 3-3 and 3-4) as expected, and were consistently larger in both small and large streams, and for both high and low urban land use (Figures 3-5 and 3-6). The high coefficient of

determination (R^2 >0.91) for the regressions also suggests that the scores for a "200-organism" sample can be predicted quite accurately from the first sample of "100-organism" (Figures 3-7 and 3-8).

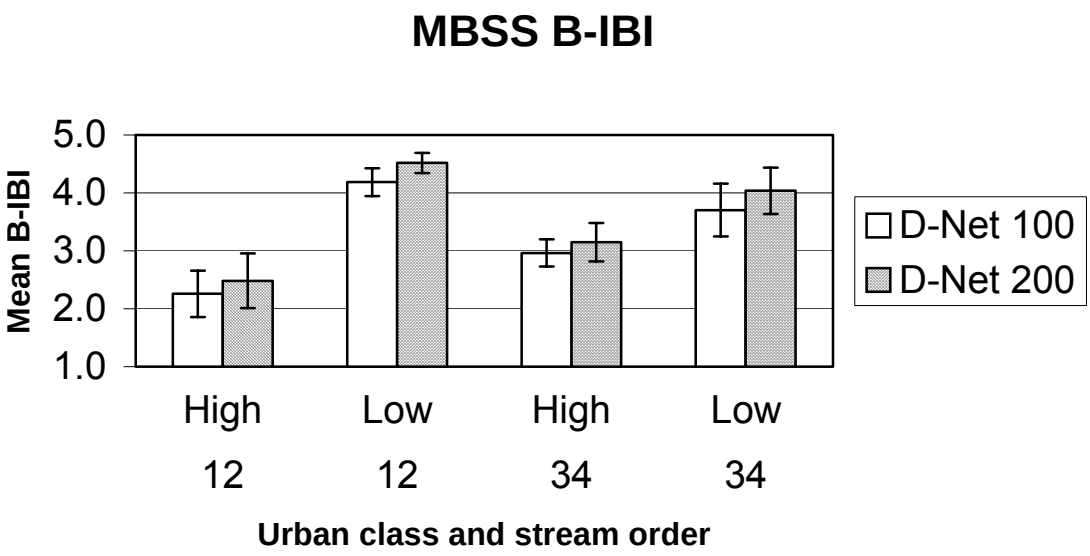

Figure 3-5. Mean B-IBI scores (MBSS method) by urban land use class and SO for (1) D-Net samples with "100-organism" subsample, and (2) D-Net with "200-organism" subsample. ***High*** and ***Low*** urban land use define catchments with >15% and ≤15% impervious surface, respectively. *SO 12* represents order 1 and 2 combined, while *SO 34* represents order 3 and 4 combined.

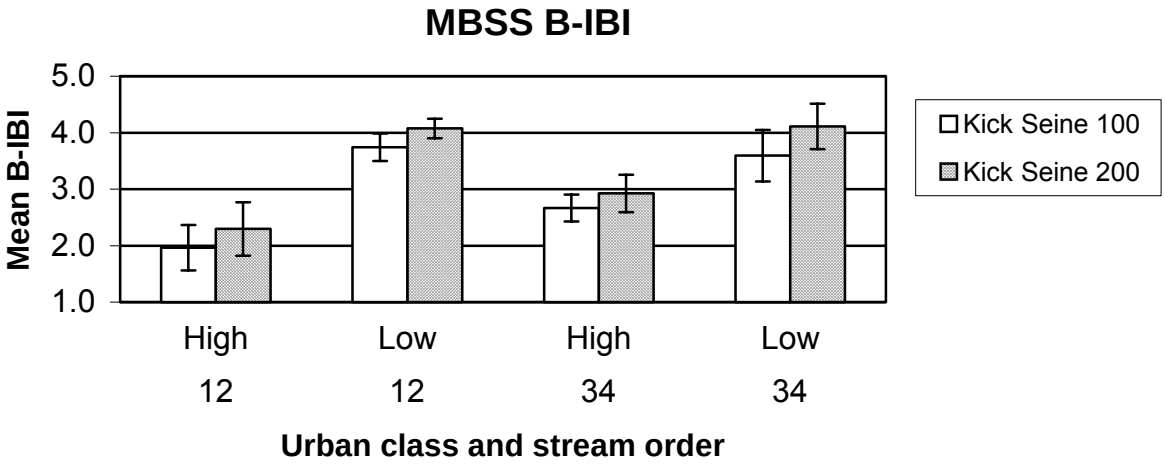

Figure 3-6. Mean B-IBI scores (MBSS method) by urban land use class and stream order for Kick Seine samples with "100-organism" and "200-organism" subsamples. ***High*** and ***Low*** urban land use define catchments with >15% and ≤15% impervious surface, respectively. *SO 12* represents order 1 and 2 combined, while *SO 34* represents order 3 and 4 combined.

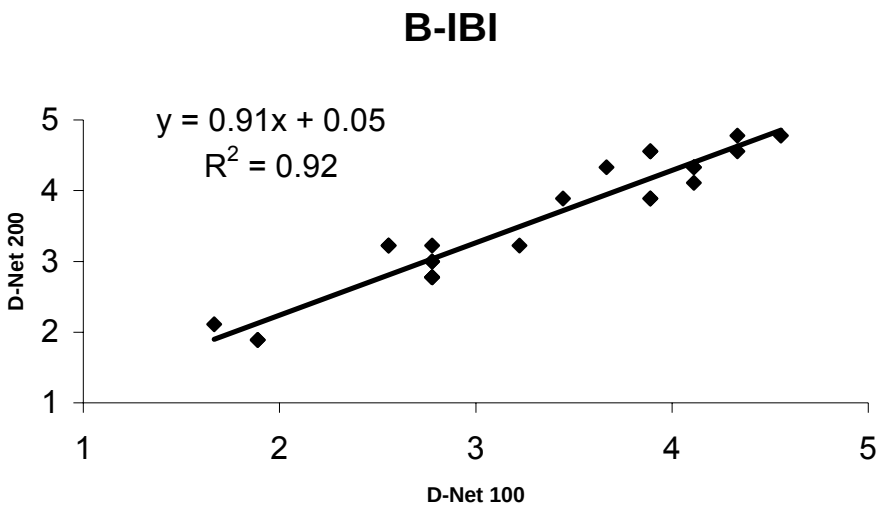

Figure 3-7. Paired comparison of B-IBI scores (MBSS method) for D-Net samples with "100-organism" versus "200-organism" subsamples using linear regression. The regression coefficients were estimated using SAS (SAS Institute 1999) and may not correspond exactly with the regression line fitted in Microsoft® Excel.

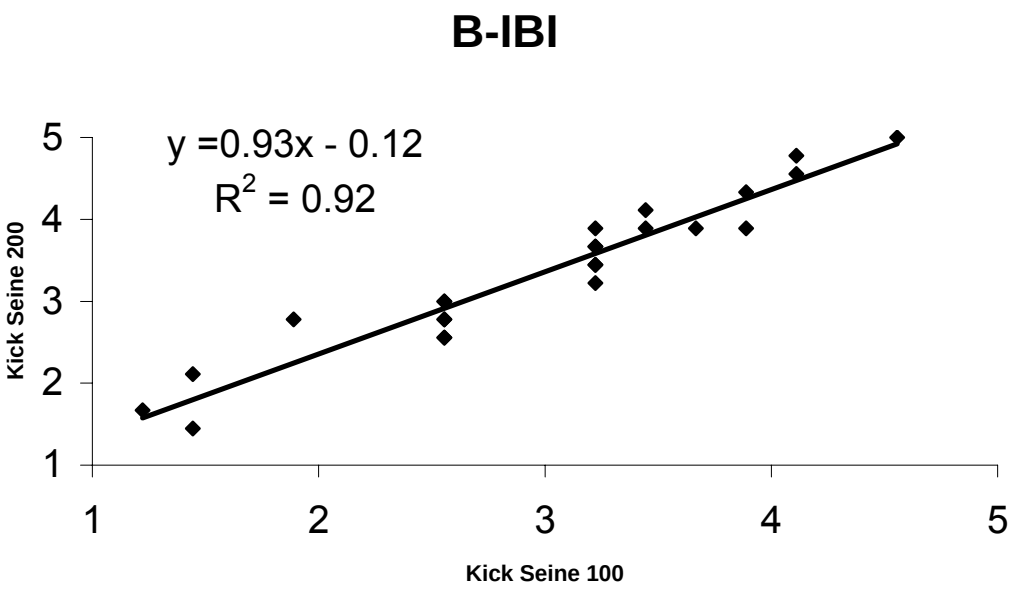

Figure 3-8. Paired comparison of B-IBI scores (MBSS method) for Kick Seine samples with "100-organism" versus "200-organism" subsamples using linear regression. The regression coefficients were estimated using SAS (SAS Institute 1999) and may not correspond exactly with the regression line fitted in Microsoft® Excel.

Table 3-3. Mean difference between MBSS B-IBI scores and individual metrics for D-Net "100-organism" and "200-organism" subsamples with associated SEs and p-values for paired t-test. Total number of paired samples across SO and urban land use is n =24.

Parameter	Δ = Difference between 100- and 200-organism subsamples		Pr > $\lvert t \rvert$
	Mean Δ	SE	
B-IBI	-0.27	0.05	<0.0001
Number of Taxa	-22.21	2.59	<0.0001
Number of EPT Taxa	-5.04	0.98	<0.0001
Number of Diptera Taxa	-9.33	1.80	<0.0001
Percentage Ephemeroptera Individuals of Total Number of Individuals	-0.31	0.33	0.36
Proportion Tanytarsini of Total Number of Individuals	0.22	0.37	0.56
Number of Intolerant Taxa	-2.38	0.62	0.0008
Percent Tolerant Individuals	-0.26	5.00	0.96
Percent Collectors	-0.08	0.59	0.88

Table 3-4. Mean difference between MBSS B-IBI scores and individual B-IBI metrics for Kick Seine "100-organism" and "200-organism" subsamples with associated SEs and p-values for paired t-test. Total number of paired samples across SO and urban land use is n =24.

Parameter	Δ = Difference between 100- and 200-organism subsamples		Pr > $\lvert t \rvert$
	Mean Δ	SE	
B-IBI	-0.36	0.05	<0.0001
Number of Taxa	-18.83	1.71	<0.0001
Number of EPT Taxa	-4.37	0.68	<0.0001
Number of Diptera Taxa	-11.75	1.14	<0.0001
Percentage Ephemeroptera Individuals of Total Number of Individuals	0.60	1.15	0.61
Percent Tanytarsini of Total Number of Individuals	-0.12	0.20	0.0097
Number of Intolerant Taxa	-2.04	0.42	<0.0001
Percent Tolerant Individuals	0.79	0.54	0.15
Percent Collectors	0.46	0.57	0.43

3.4 EFFECTS OF SAMPLING METHOD AND LABORATORY PROTOCOL ON THE PRECISION OF MEAN B-IBI SCORES

The mean B-IBI scores and their associated precision varied across field sampling methods and laboratory sub-sampling protocols. The D-Net produced mean B-IBIs with a slightly lower relative SE than for the Kick Seine samples (Figure 3-10). D-Net samples are taken from 20 small locations (1.89 m^2 total) within the stream segment as opposed to 2 large locations (2.00 m^2 total) within the segment for the Kick Seine. Because of the patchy distribution of benthic organisms, the larger number of small samples appeared to produce a composite sample that is more representative of the overall composition in the 75-m stream segment than a composite sample based on few samples of larger size. The frequency distribution of individual taxa by sampling method and laboratory protocol is provided in the Appendix.

This study indicates that the precision of mean B-IBI scores only marginally improves when the target subsample sizes increase from 100 to 200 organisms for either D-Net or Kick Seine samples (Figures 3-9 and 3-10).

3.5 COMPARISONS BY TAXONOMIC LEVEL FOR CHIRONOMIDS AND OLIGOCHAETES

The regression of B-IBI scores for D-Net "100 organism" samples where chironomids are identified to genus on scores based on their identification to tribe or family suggests that the standard MBSS B-IBI scores can be fairly accurately predicted from the samples lumped to tribe (Figures 3-11), with R^2 of 0.89. The prediction of standard MBSS B-IBI scores from samples where the chironomids are identified to family is less reliable (Figure 3-12) with R^2 of 0.53. The same tendency holds when predicting standard MBSS B-IBI scores from Kick Seine samples, with $R^2 = 0.67$ when the chironomids are identified to tribe, and $R^2 = 0.54$ when they are identified to family (Figures 3-13, 3-14). The consequences of these different levels of taxonomic identification is that in the experimental study (which provides the widest range of B-IBI conditions), the percentage of sites designated as degraded (B-IBI < 3) using tribe is not significantly different than the percentage designated as degraded using genus (9% fewer using tribe) (Figure 3-11). In contrast, the percentage of sites designated as degraded using family was significantly different ($p = .05$) than the percentage designated as degraded using genus (36% more using family) (Figure 3-12). We conducted further investigations to evaluate the implications of these different taxonomic approaches on watershed estimates, a spatial level at which bioassessment results are often employed (e.g., in the Maryland biocriteria framework).

As an example, we calculated the mean B-IBI scores for eleven 8-digit watersheds sampled by MBSS during 2001. The mean scores based on family and tribe were biased downwards compared to standard MBSS B-IBI scores based on genus, as expected, because the number of different taxa are reduced (Table 3-5). The predicted B-IBI scores from samples lumped to tribe using the fitted regression reduced the bias, but the predicted scores were generally lower than the observed (Table 3-6). The standard errors (model 1.4) for the predicted standard mean IBI by

watershed from samples where chironomids were lumped to family was slightly larger, on average, than the SEs for the IBI based on genus.

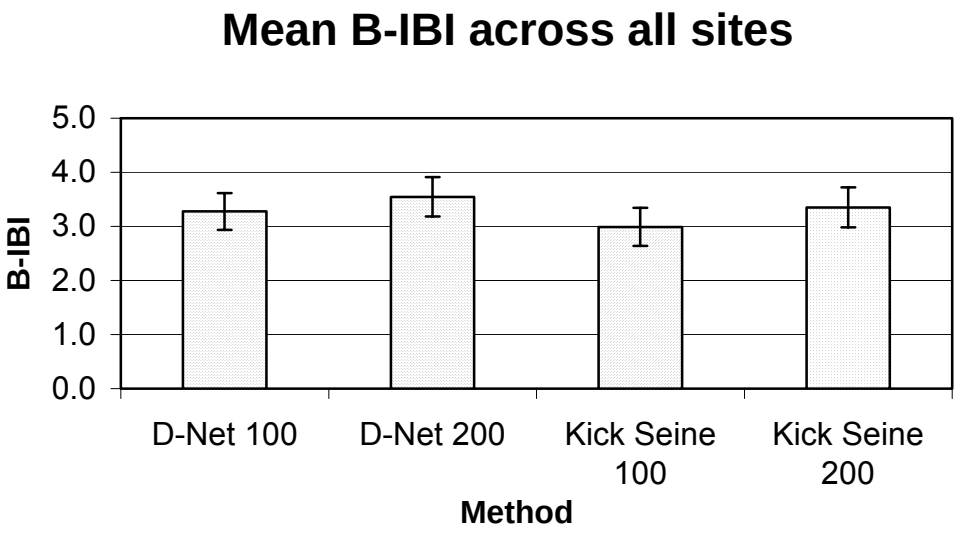

Figure 3-9. Mean B-IBI (MBSS method) across all sites (n=24) by field method and laboratory subsampling procedure. Error bars represent 95% confidence intervals.

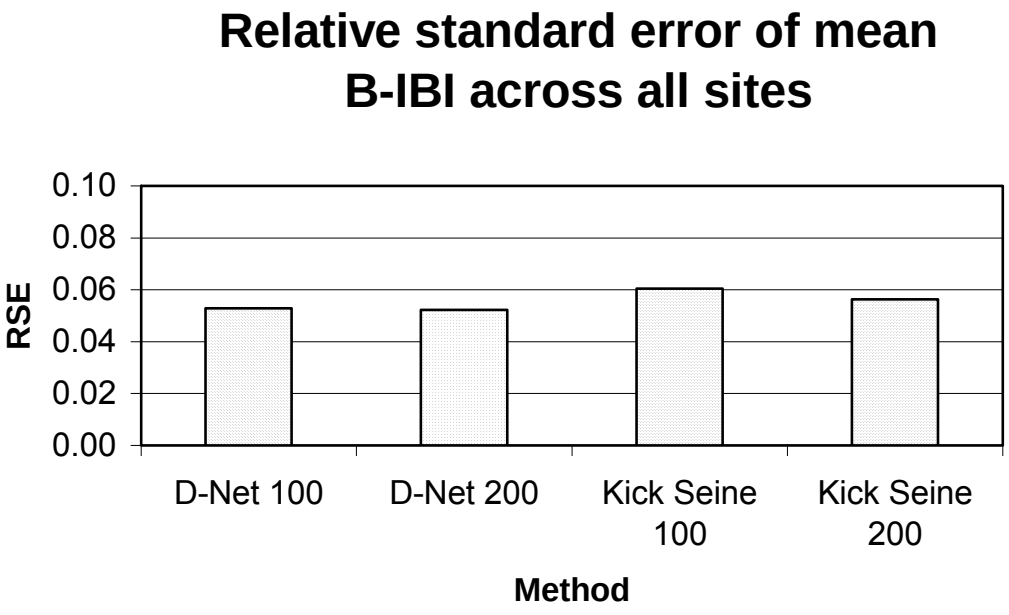

Figure 3-10. Relative SE (RSE= SE/ $\bar{x}$) of mean B-IBI (MBSS method) scores across all sites (n=24) by field method and laboratory subsampling procedure.

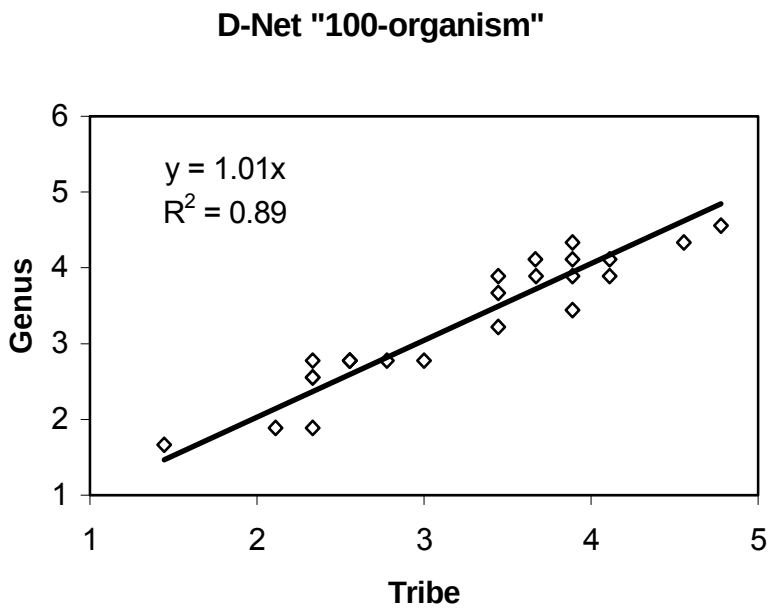

Figure 3-11. Relationship between standard B-IBI scores for D-Net “100 organism” samples with chironomids and oligochaetes identified to genus, and B-IBI scores with chironomids and oligochaetes lumped to tribe. The SE of the regression coefficient is 0.02, estimated using SAS (SAS Institute 1999).

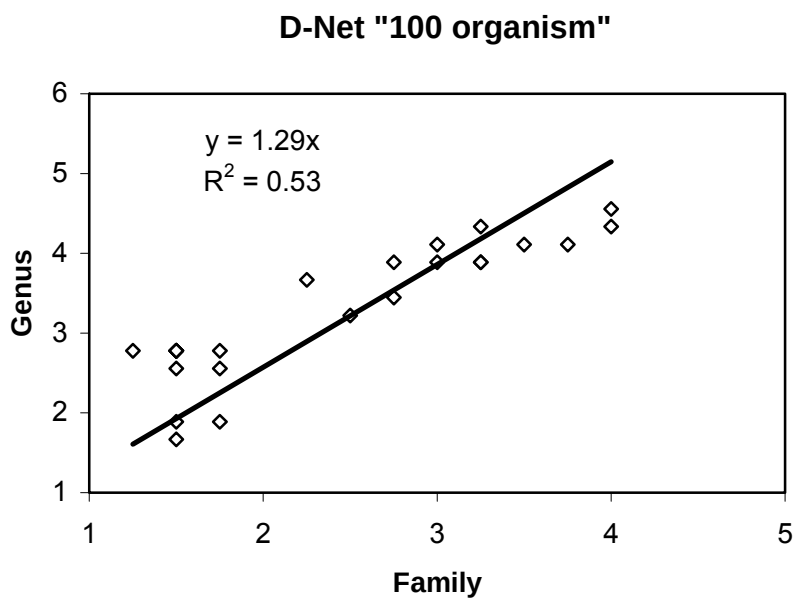

Figure 3-12. Relationship between standard B-IBI scores for D-Net “100 organism” samples with chironomids and oligochaetes identified to genus, and B-IBI scores with oligochaetes lumped to family. The SE of the regression coefficient is 0.05, estimated using SAS (SAS Institute 1999).

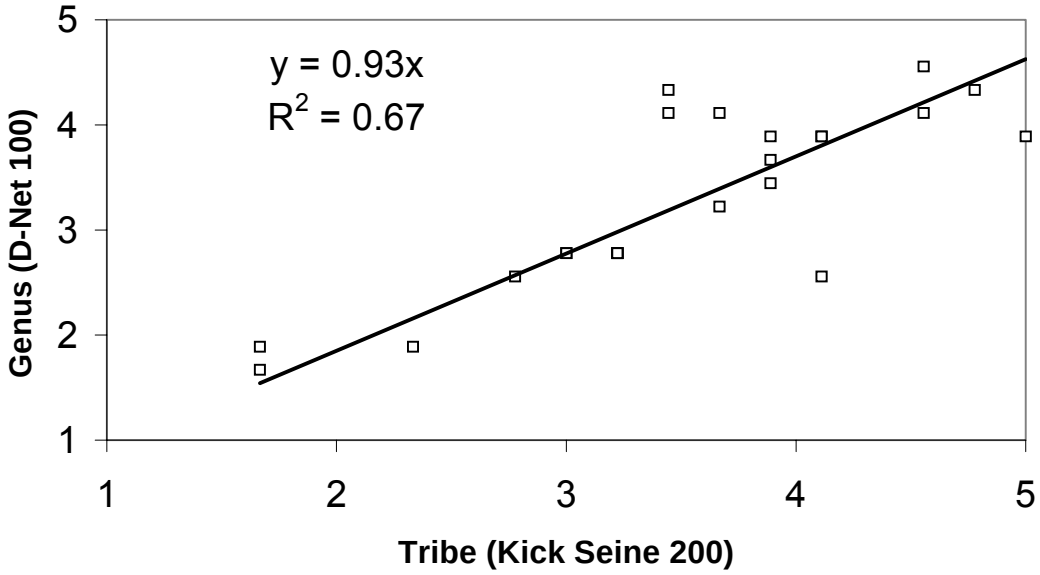

Figure 3-13. Relationship between standard B-IBI scores for D-Net "100 organism" samples with chironomids and oligochaetes identified to genus, and B-IBI scores from Kick Seine "200 organism" samples with chironomids and oligochaetes lumped to tribe. The SE of the regression coefficient is 0.03, estimated using SAS (SAS Institute 1999).

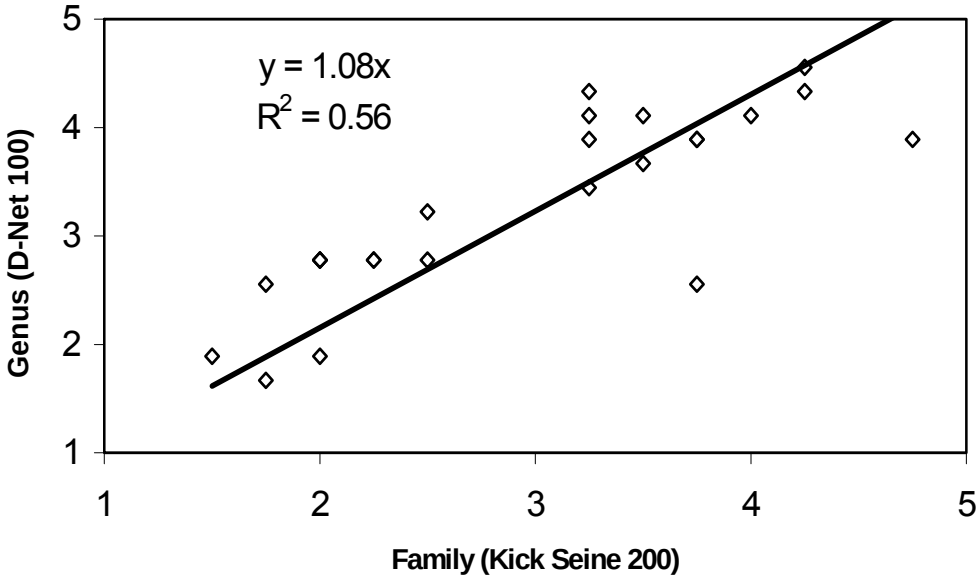

Figure 3-14. Relationship between standard B-IBI scores for D-Net "100 organism" samples with chironomids and oligochaetes identified to genus, and B-IBI scores from Kick Seine "200 organism" samples with chironomids and oligochaetes lumped to family. The SE of the regression coefficient is 0.04, estimated using SAS (SAS Institute 1999).

Table 3-5. B-IBI scores for MBSS 8-digit watersheds for 2000 for three levels of taxonomic identification for chironomids and oligochaetes.

Watershed	N	Genus		Tribe		Family		Ratio	
		Mean	SE	Mean	SE	Mean	SE	Genus/ Tribe	Genus/ Fam
Casselman River	10	3.38	0.4	3.04	0.41	2.58	0.36	1.11	1.31
Liberty Reservoir	16	3.6	0.14	3.17	0.17	2.47	0.16	1.14	1.46
Little Patuxent River	13	2.79	0.29	2.68	0.19	2.21	0.17	1.04	1.26
Lower Monocacy River	21	3.32	0.21	2.92	0.19	2.41	0.18	1.14	1.38
Mattawoman CR	10	3.34	0.3	2.82	0.26	2.38	0.27	1.18	1.40
Patapsco River Lower North BR	14	2.87	0.16	2.35	0.19	2.00	0.15	1.22	1.44
Potomac R WA County/ Marsh Run/Tonoloway/Little Ton	12	2.81	0.15	2.52	0.16	2.07	0.17	1.12	1.36
Prettyboy Reservoir	10	3.96	0.18	3.71	0.20	2.89	0.20	1.07	1.37
Town CR	10	3.82	0.21	3.44	0.23	2.91	0.22	1.11	1.31
Upper Choptank	11	2.38	0.27	2.29	0.17	1.61	0.13	1.04	1.48
Upper Monocacy River	17	3.2	0.19	2.88	0.20	2.57	0.16	1.11	1.25
Average		3.22	0.23	2.89	0.22	2.37	0.20	1.12	1.36

Table 3-6. Mean predicted B-IBI scores for 8-digit watersheds (at genus level) for MBSS 2000 from samples where chironomids and oligochaetes were lumped to tribe or family. The SEs for the predicted IBI means were estimated using model 1.4.

Watershed	N	From Tribe		From Family	
		Mean	SE	Mean	SE
Casselman River	10	3.07	0.42	3.33	0.48
Liberty Reservoir	16	3.20	0.18	3.19	0.24
Little Patuxent River	13	2.71	0.20	2.85	0.25
Lower Monocacy River	21	2.95	0.20	3.11	0.26
Mattawoman CR	10	2.85	0.27	3.07	0.37
Patapsco River Lower North BR	14	2.37	0.20	2.58	0.22
Potomac R WA County/Marsh Run/ Tonoloway/Little Ton	12	2.55	0.17	2.67	0.24
Prettyboy Reservoir	10	3.75	0.22	3.73	0.30
Town CR	10	3.47	0.24	3.75	0.32
Upper Choptank	11	2.31	0.18	2.08	0.19
Upper Monocacy River	17	2.91	0.21	3.32	0.24
Average		2.92	0.23	3.06	0.28

3.6 COST-BENEFIT ANALYSIS OF TAXONOMIC IDENTIFICATION LEVEL

The number of samples that can be collected and processed for a fixed survey cost depends on the taxonomic identification level for chironomids and oligochaetes. Specifically, the identification of these organisms below the family level (i.e., to tribe or genus) requires more time and taxonomic expertise. Most programs follow EPA recommended sampling methods that utilize an "index period" within which all benthic sampling is done to minimize seasonal variability between sampling years. There is a maximum number of samples that can be collected during this index period dependent on staff, hours allocated to the sampling effort, costs of processing, and weather. The identification of chironomids is more pertinent since very few oligochaetes were collected in our samples. To address this issue, we surveyed several taxonomic experts from Maryland, Ohio, and New York and chose the most often reported time estimates for laboratory processing, a four-fold increase in time associated with identification to genus as compared to family. It should be noted that some of the surveyed taxonomists estimated a two-fold increase in time; running the analysis below with this estimate did not significantly change the conclusions, so the more conservative estimate was used.

For simplicity, we have expressed these laboratory costs in terms of personnel hours, and have assumed that the field and laboratory personnel hours are equivalent in terms of cost. As a reference, the total cost of collecting and processing 100 samples to genus level (taxonomic level 1), with a laboratory sub-sample size of 100, is 625 personnel hours. We have assumed that collection of each field sample takes two hours with a staff of two biologists (i.e., 4 personnel hours). Monitoring programs should strive to collect and process the number of samples that are required to achieve adequate precision in the IBIs for the sampling design employed, but often they are constrained by a shortage of staff and resources. For a given monitoring cost, the sample size depends on the field effort, as well as the cost of processing the samples. It costs more to identify chironomids to genus than to tribe or family, and this additional cost would only be justified if it resulted in increased accuracy and precision in the resulting assessment. In some cases, the same accuracy and precision may be achieved at a lower taxonomic level if it allows for more samples to be processed for the same cost. To a certain extent, the increased sample size compensates for the higher variability in genus IBI scores that are predicted from lower taxonomic levels. Using the laboratory processing times for four different taxonomic levels (Table 2-2), we estimated that for a fixed cost of 625 personnel hours, it would be possible to collect and process:

100 samples for taxonomic level 1 (genus);
104 samples for taxonomic level 2 (genus for chironomids only);
109 samples for taxonomic level 3 (tribe); and
114 samples for taxonomic level 4 (family).

In evaluating expected precision for a given sample size, we used the mean coefficient of variation (*cv*) for the eleven 8-digit watersheds sampled in MBSS 2000 as a measure of the natural variability between samples in a Maryland 8-digit watershed. Precision in estimated mean B-IBI from a sample of size *n* is measured by the RSE. The mean coefficient of variation

for the taxonomic level 1 B-IBI (genus level) was 26%, as compared to 28% and 33% when the B-IBI was predicted from samples processed to level 3 (tribe) or level 4 (family), respectively. The variability between B-IBI values that are predicted from level 3 or level 4 taxonomic levels is higher then the variability in genus level B-IBI values as a result of prediction errors. We used the respective *cv*s to estimate expected RSE for mean B-IBI as a function of taxonomic level and sample size, using the relationship $RSE = cv / \sqrt{n}$ (Cochran 1977). On average, ten samples identified to genus level would be expected to produce a mean B-IBI with RSE of 8.2%. For the same cost as collecting and processing 10 samples to genus level, the sample size could be increased by 9% if processed only to tribe (level 3) and 14% if processed to family (level 4). The resulting precision (including the additional samples that could be collected and processed for the fixed cost) in predicted B-IBI (compared to taxonomic level 1) would only marginally decrease (RSE = 8.5%) for taxonomic level 3, and decrease further (RSE = 9.8%) for taxonomic level 4. Thus, the 14% increase in sample size that could be achieved if chironomids and oligochaetes were identified to family level, as compared to genus, would not be sufficient to offset the added variability from predicting B-IBI (i.e., reduced precision in estimating stream condition).

4.0 DISCUSSION

The balanced allocation of the 24 experimental sites into catchments with both high and low percentage of urban land use and a small and large stream size ensured that this study could compare the field sampling and laboratory protocols across a wide range of stream condition, as intended. The similarity in mean B-IBI scores for large and small streams suggest that the calibration for stream order in the scoring method is effective. As expected, the sites in catchments with high urban land use (>15% impervious surface) had lower B-IBI scores than sites with low urban land use (≤15% impervious surface), on average. This is consistent with results in Vølstad et al. (2003b), which indicated that the likelihood of stream sites failing biocriteria in Maryland doubles for every 10% increase in the extent of urban land use in their catchments. Many other studies have linked stream degradation to increases in impervious surface (Center for Watershed Protection 1998; Schueler 1994; Smart et al. 1981; Wang et al. 1997). Steedman (1988) found that more than 25-50% urban land use led to severe impacts on stream quality in southern Ontario. We chose the 15% impervious surface cut-off in the study design, because it sufficiently separated the scores in each group while covering a range of stream degradation. We did not target sites in catchments with very high impervious surface for this comparison study, because B-IBI scores at these sites would have low expected values, regardless of the field sampling and laboratory protocols we tested. Vølstad et al. (2003b) found that sites in catchments with more than 40-50% urban land use had greater than 80% probability of failing the Maryland interim biocriteria, on average.

Using this design, our study produced robust answers to the following questions.

Are D-Net or Kick Seine sampling protocols comparable?
Are 100- and 200-organism subsampling protocols comparable?
Are different levels of taxonomic identification for chironomids and oligochaetes comparable?

D-Net versus Kick Seine Sampling. The MBSS and Montgomery County sampling methods produced similar mean B-IBI scores across a wide range of stream conditions, suggesting that results from the two field sampling programs could be integrated. However, the MBSS D-Net sampling appears to produce slightly more precise area-wide estimates of mean B-IBI than the Kick Seine sampling. This is likely a result of the D-Net collecting benthic macroinvertebrates from more areas within each stream segment than does the Kick Seine. In principle, both programs employ two-stage sampling (Cochran 1977; Gilbert 1987). In the first stage, a representative sample of streams segments is selected from each watershed, and in the second stage, macroinvertebrates are collected from representative areas (plots) within each stream segment. Macroinvertebrates generally have a patchy distribution, both at the local spatial scale (e.g., 75-m stream segment) and at the larger scales, such as a Maryland 8-digit watershed. Hence, the sampling from many small plots may better characterize the benthic community within a stream segment than sampling from a few larger plots. The subsampling of benthos

within stream segments adds to the variance of estimated mean B-IBI scores for streams, but the exact amount of added uncertainty cannot be assessed from the standard MBSS and Montgomery County data, because the within-segment samples are composited. The increased plot-size in Kick Seine sampling by Montgomery County does not appear to compensate for the uncertainty associated with sampling fewer plots within each stream segment when compared to D-Net sampling from more plots. The use of a small plot sizes is also supported by Karr and Chu (1999).

100-organism Versus 200-organism Subsampling. For the Maryland DNR and Montgomery County data evaluated, it appears that the precision in area-wide estimates of mean B-IBI scores only marginally improves if the target subsample size in the laboratory is increased from 100 to 200 organisms, regardless of the field sampling method. For a fixed survey cost, the optimum subsample size depends on the cost of collecting field samples and the cost of the taxonomic identification. A net loss in precision of mean B-IBI scores could result if an increased subsample size reduces the number of stream segments that can be collected in the field.

The choice of number of individual organisms to be counted and identified from each field sample is controversial (Karr and Chu 1999). Recommendations range from 100 organisms to a complete count of all organisms. A number of other studies have shown that species richness as a function of sample size reaches an asymptotic level for a count of between 100 and 900 organisms depending on overall richness in the sample (May 1975, Barbour and Gerritsen 1996). Somers et al. (1998) conclude that counts of 100 animals are sufficient to distinguish the littoral benthic communities of small inland lakes in south-central Ontario. Karr and Chu (1999) advocate subsample sizes of more than 100 organisms and point out that a fixed subsample size is a potential source of bias. The subsampling protocol employed by Montgomery County and the MBSS does not involve a fixed number of organisms (i.e., can exceed the 100- or 200-count target because all the organisms in the last grid are included) and thus do not introduce this type of bias. The actual number of organisms selected is a random variable, although the target sample size is 100 or 200 organisms. Although the target sample size of 100 organisms appears to be sufficient for characterizing Maryland streams well, a greater number of organisms would likely encounter more rare taxa and potentially produce a more sensitive index. However, a larger count would also add significant laboratory costs for a fixed number of field samples.

Underestimation of rare species can reduce the sensitivity of community-based assessment methods to detect ecological changes and thus reduce the effectiveness of bioassessment (Cao et al. 1998; Karr and Chu 1999). Karr and Chu (1999) and Cao et al. (1998) advocate a larger sample size than the standard of 100 to 300 individuals used in EPA rapid bioassessment protocols (RBPs) to reliably differentiate between reference and impacted sites. However, the actual count of organisms required to achieve adequate power for distinguishing between reference and impaired sites depends on characteristics of the biota and on how each site is sampled in the field and thus may differ among studies. Somers et al. (1998), for example, compared biological indices for assessing health of lakes based on counts of 100, 200, and 300 organisms and found that doubled or tripled effort resulted in little improvement in the ability to distinguish between lakes.

Our study indicates that the number of plots sampled in each stream segment may have a larger affect on the precision in estimates of species richness and in particular the likelihood of detecting rare species than the subsample size of individuals from each composite sample. Because virtually all species have a patchy distribution, the sampling of only a few plots from a stream segment may not provide accurate data on the benthic community in that segment even if all specimens from the composite samples are identified. Therefore, this effect should be taken into account when evaluating the effects of subsample sizes on mean IBI scores.

Taxonomic Identification Level for Chironomids and Oligochaetes. Identification of chironomids and oligochaetes to genus improved the precision of B-IBI scores. When the genus data were aggregated to tribe or family, the resulting B-IBI scores were biased downwards, as expected. Although this bias could be adjusted for somewhat by predicting genus B-IBI values from regressions, the use of such predictions reduces the precision of mean B-IBI scores. The reason for lowered precision is that the estimated regression parameters also have associated variances that influence the predictions.

The cost-benefit analysis conducted indicates that finer levels of taxonomic identification of chironomids and oligochaetes improve the precision in mean B-IBI for the same survey cost. An approximately constant level of precision can be obtained by only identifying these taxa to tribe, if the laboratory cost saving is converted into additional samples. In contrast, this same level of precision cannot be achieved by converting cost savings from identification to the family level. It should be noted that considerable investments in equipment and training are needed to identify chironomids and oligochaetes to genus, and that the moderate improvements in IBI precision may not warrant these investments for some programs. One option would be identification of chironomids to tribe for use in a B-IBI for watershed screening purposes. Those stations that are identified as impaired could then be reevaluated by having the chironomids in these fewer stations identified to genus to provide information on potential stressors.

5.0 CONCLUSIONS

This study supports the contention that Montgomery County and Maryland DNR stream monitoring of benthic macroinvertebrate communities can be effectively integrated. In the case of sampling protocol differences, integration options include (1) continuing to use different protocols when the mean results are comparable but of differing precision; (2) adjusting the result from one protocol to match the other, usually with a loss of precision; and (3) agreeing to adopt the same protocol.

The study demonstrates that D-Net sampling protocol can provide more reliable B-IBI indices than the Kick Seine protocol, possibly because sampling from 20 smaller plots is more representative than sampling from 2 larger plots of the stream segment. This study also indicates that Montgomery County could improve the precision of their B-IBIs by increasing the level of chironomid and oligochaete identification to genus level. For the same overall survey cost, however, we conclude that the identification of chironomids to tribe, in conjunction with an appropriate increase in the number of sampling sites, could yield a similar level of precision in mean B-IBI scores. For some monitoring programs, the moderate improvements in IBI precision obtained by identifying chironomids to genus may not warrant the needed investments in equipment and training. One option for such programs is to identify these taxa to tribe as part of a B-IBI for watershed screening and to identify these taxa to genus only at impaired stations to support stressor identification.

The determination of an optimum subsample size is complex and would require detailed information about the cost of both the taxonomic identification step and field sampling step. For the stream networks sampled in this study (and using the B-IBI developed based on 100 organisms), it appears that Montgomery County could reduce its subsampling to 100 organisms to save costs with only a marginal loss in precision of mean B-IBI scores. Before such a decision is made, further study should be undertaken to determine if a B-IBI developed using 200 organisms would produce cost-effective benefits in precision.

6.0 REFERENCES

Barbour, M.T. and J. Gerritsen. 1996. Subsampling of benthic samples: A defense of the fixed-count method. *J. N. Am. Benthol. Soc.* **15**: 386-391.

Box, G.E.P., W.G. Hunter, and J.S. Hunter. 1978. *Statistics for Experimenters*. John Wiley & Sons. New York. 653pp.

Boward, D. and E. Friedman. 2000. Maryland Biological Stream Survey: Laboratory Methods for Benthic Macroinvertebrate Processing and Taxonomy. Maryland Department of Natural Resources, Monitoring and Non-tidal Assessment Division. CBWP-MANTA-EA-00-6

Cao, Y., D.D. Williams, and N.E. Williams. 1998. How important are rare species in aquatic community ecology and bioassessment? Limnol. Oceanogr. 43(7): 1403-1409.

Center for Watershed Protection.1998. Rapid Watershed Planning Handbook: A Comprehensive Guide for Managing Urbanizing Watersheds. Center for Watershed Protection, Ellicott City, MD.

Cochran, W.G. 1977. Sampling Techniques. 3rd Ed. John Wiley & Sons. New York. 428pp.

Gilbert, R.O. 1987. Statistical methods for Environmental Pollution Monitoring. Van Nostrand, New York. 320pp.

Goodman, L.A. 1960. On the exact variance of products. J. Amer. Statist. Ass. 55: 708.

Karr, J.R. and E.W. Chu. 1999. Restoring Life in Running Waters. Island Press, Washington DC. 206pp.

Kazyak, P.F. 2001. Maryland Biological Stream Survey Sampling Manual. Maryland Department of Natural Resources, Monitoring and Non-tidal Assessment Division.

Kendall, M.A. Stuart, and J.K. Ord. 1987. Kendall's Advanced Theory of Statistics – 5th Ed. Volume 1: Distribution Theory. Charles Griffin & Co. London.

Klauda, R., P. Kazyak, S. Stranko, M. Southerland, N. Roth, and J. Chaillou. 1998. Maryland Biological Stream Survey: A state agency program to assess the impact of anthropogenic stresses on stream habitat quality and biota. Environmental Management and Assessment 51:299-316

Korn, E.L. and B.I. Graubard. 1999. Analysis of Health Surveys. John Wiley & Sons. New York. 382 pp.

May, R.M. 1975. Patterns of species abundance and diversity. In: M.L. Cody and J.M. Diamond (editors). Ecology and evolution of communities. Harvard University Press, Cambridge, Massachusetts. pp. 81-120.

Omernik, J.M. and R.G. Bailey. 1997. Distinguishing between watersheds and ecoregions. Journal of the American Water Resources Association 33: 935-949

Roth, N., M. Southerland, J. Chaillou, R. Klauda, P. Kazyak, S. Stranko, S. Weisberg, L. Hall, Jr., and R. Morgan II. 1998a. Maryland Biological Stream Survey: Development of a Fish Index of Biotic Integrity. Environmental Management and Assessment 51:89-106.

SAS Institute, Inc. 1999. SAS/STAT User's Guide, Version 8.0, SAS Institute Inc., Cary, North Carolina.

Schueler, T. 1994. The importance of imperviousness. Watershed Protection Techniques 1:100-111.

Smart, .M., T. Barney, and J.R. Jones. 1981. Watershed impact on stream quality: a technique for regional assessment. Journal of Soil and Water Conservation 36:297-300.

Somers, K.M., R.A. Reid, and S.M. David. 1998. Rapid biological assessments: how many animals is enough? J. N. Am. Benthol. Soc. 17: 348-358.

Steedman, R.J. 1988. Modification and assessment of an index of biotic integrity in southern Ontario. Can. J. Fish. Aquat. Sci. 45: 492-501.

Strahler, A.N. 1957. Quantitative analysis of watershed geomorphology. Transactions of the American Geophysical Union 38(6): 913-920.

Stribling, J.B., B.K. Jessup, J.S. White, D. Boward, and M. Hurd. 1998. Development of a Benthic Index of Biotic Integrity for Maryland Streams. Prepared by Tetra Tech, Inc., Owings. Mills, MD and Maryland Department of Natural Resources, Monitoring and Non-Tidal Assessment Division. CBWP-MANTA-EA-98-3.

Van Ness, K., K. Brown, M.S. Haddaway, D. Marshall, and D. Jordahl. 1997. Montgomery County Water Quality Monitoring Program Stream Monitoring Protocols. Montgomery County Department of Environmental Protection, Watershed Management Division, Rockville, MD. February 20, 1997.

Vølstad, J.H., N.K. Neerchal, N.E. Roth and M.T. Southerland. 2003a. Combining biological indicators of watershed condition from multiple sampling programs─a case study from Maryland. Ecological Indicators 3:13-25.

Vølstad, J.H., N. Roth, G. Mercurio, M. Southerland, and Don Strebel. 2003b. Incorporating environmental factors for predictive screening of stream condition based on biological indicators. Environmental Monitoring and Assessment (In press).

Wang, L., J. Lyons, P. Kanehl, and R. Gatti. 1997. Influences of watershed land use on habitat quality and biotic integrity in Wisconsin streams. Fisheries 22(6): 6-12.

APPENDIX

FREQUENCY DISTRIBUTION (%) OF INDIVIDUAL TAXA ACROSS ALL 24 SITES BY SAMPLING METHOD AND LABORATORY PROTOCOL

Table A-1. Frequency distribution of individual taxa across all 24 sites by sampling method and laboratory protocol.

Name	D-Net 100	D-Net 200	Kick Seine 100	Kick Seine 200
Cheumatopsyche	3.26	3.31	3.98	4.17
Cricotopus	3.11	2.91	3.29	3.69
Orthocladius	2.81	2.75	3.63	3.98
Rheotanytarsus	2.81	2.67	3.11	3.11
Parametriocnemus	2.37	2.67	2.77	3.01
Rheocricotopus	2.37	2.34	2.25	2.52
Tanytarsus	2.37	2.34	2.94	2.91
Hydrobaenus	2.22	2.10	1.90	2.14
Polypedilum	2.22	2.58	2.08	2.43
Conchapelopia	2.07	2.26	2.08	1.94
Meropelopia	2.07	2.10	2.42	2.23
Microtendipes	2.07	2.02	1.90	2.23
Simulium	2.07	1.94	1.90	1.94
Antocha	1.63	1.53	2.25	2.33
Clinocera	1.63	1.21	1.73	2.04
Paratanytarsus	1.63	1.53	0.52	0.39
Tvetenia	1.63	1.53	2.08	2.14
Ephemerella	1.48	1.45	1.90	2.04
Eurylophella	1.48	1.78	1.21	1.07
Stenelmis	1.48	1.62	2.60	2.43
Amphinemura	1.33	1.29	1.56	1.46
Brillia	1.33	1.53	1.04	1.07
Chelifera	1.33	1.29	1.04	0.97
Hemerodromia	1.33	1.45	2.25	1.94
Hydropsyche	1.33	1.53	1.90	2.04
Micropsectra	1.33	1.29	1.21	1.17
Phaenopsectra	1.33	1.53	0.87	0.68
Nais	1.19	1.21	2.77	2.72
Stenonema	1.19	1.29	1.73	1.75
Ablabesmyia	1.04	0.89	0.35	0.19
Ceratopsyche	1.04	1.05	1.38	1.26
Dubiraphia	1.04	0.97	0.35	0.19
Limnodrilus	1.04	0.73		0.10
Neophylax	1.04	1.05	1.38	1.17
Tipula	1.04	1.37	1.56	1.26
Zavrelimyia	1.04	0.89	0.35	0.19
Cricotopus/Orthocladius	0.89	0.97	0.87	1.17
Imm. Tubificid w/o Cap. Chaete	0.89	0.65	0.52	0.58
Parakiefferiella	0.89	0.73	1.21	1.17

Table A-1 (cont'd). Frequency distribution of individual taxa across all 24 sites by sampling method and laboratory protocol.

Name	D-Net 100	D-Net 200	Kick Seine 100	Kick Seine 200
Prosimulium	0.89	0.81	1.04	0.97
Thienemannimyia grp.	0.89	0.73	0.17	0.10
Argia	0.74	0.81		
Corynoneura	0.74	0.65	0.52	0.29
Helopelopia	0.74	0.65	0.69	0.58
Hydropsychidae	0.74	0.48	0.52	0.58
Potthastia	0.74	0.73	1.38	1.36
Rhyacophila	0.74	0.57	0.52	0.68
Thienemanniella	0.74	0.89	0.35	0.39
Ameletus	0.59	1.05	0.17	0.19
Calopteryx	0.59	0.81		
Chaetocladius	0.59	0.57	0.52	0.39
Crangonyx	0.59	0.73	0.17	0.29
Diamesa	0.59	0.57	1.04	0.97
Enchytraeidae	0.59	0.57	0.35	0.39
Leptophlebiidae	0.59	0.65	0.17	0.19
Lumbricidae	0.59	0.81	0.87	0.78
Macronychus	0.59	0.65	0.35	0.49
Optioservus	0.59	0.48	0.69	0.68
Orthocladiinae	0.59	0.40	0.17	0.19
Oulimnius	0.59	0.65	1.56	1.36
Physella	0.59	0.57		
Stempellinella	0.59	0.81	1.04	0.87
Acerpenna	0.44	0.32	0.69	0.58
Caenis	0.44	0.57	0.52	0.58
Dicrotendipes	0.44	0.32	0.17	0.19
Helichus	0.44	0.32	0.17	0.19
Nigronia	0.44	0.57	0.35	0.39
Pycnopsyche	0.44	0.32		
Rheopelopia	0.44	0.40		
Strophopteryx	0.44	0.40	0.35	0.39
Tanytarsini	0.44	0.24	0.52	0.39
Trissopelopia	0.44	0.73	0.52	0.49
Bezzia/Palpomyia grp.	0.30	0.40	0.69	0.49
Boyeria	0.30	0.32		
Caecidotea	0.30	0.16		
Coenagrionidae	0.30	0.24		
Collembola	0.30	0.32		0.19
Corbicula fluminea	0.30	0.32		
Corixidae	0.30	0.16		

Table A-1 (cont'd). Frequency distribution of individual taxa across all 24 sites by sampling method and laboratory protocol.

Name	D-Net 100	D-Net 200	Kick Seine 100	Kick Seine 200
Dasyhelea	0.30	0.16	0.17	0.19
Diphetor	0.30	0.24	0.17	0.19
Diplectrona	0.30	0.24	0.17	0.29
Empididae	0.30	0.24	0.35	0.19
Ephemerellidae	0.30	0.32	0.17	0.19
Gomphidae	0.30	0.16	0.17	0.39
Heptageniidae	0.30	0.32	0.17	0.29
Hexatoma	0.30	0.32	0.17	0.19
Hydroptila	0.30	0.16	0.17	0.19
Isonychia	0.30	0.16	0.52	0.58
Limnophyes	0.30	0.16		
Nanocladius	0.30	0.57		0.10
Natarsia	0.30	0.40	0.69	0.58
Nematoda	0.30	0.16	0.52	0.58
Ostrocerca	0.30	0.40	0.17	0.10
Sparganophilus	0.30	0.16	0.35	0.29
Stegopterna	0.30	0.16	0.52	0.49
Stenacron	0.30	0.16		0.10
Stenochironomus	0.30	0.16	0.17	0.10
Sublettea	0.30	0.24	0.69	0.87
Tanypodinae	0.30	0.16	0.17	0.10
Acentrella	0.15	0.24		0.10
Agabus	0.15	0.16		
Allocapnia	0.15	0.16		
Anchytarsus	0.15	0.16		0.10
Ancyronyx	0.15	0.08	0.35	0.19
Aulodrilus	0.15	0.08		
Chaetogaster	0.15	0.08		
Chimarra	0.15	0.16	0.35	0.39
Crangonyctidae	0.15	0.08		
Cryptochironomus	0.15	0.24	0.17	0.10
Cura	0.15	0.16		
Dixella	0.15	0.08		
Dugesia	0.15	0.08	0.17	0.10
Eccoptura	0.15	0.08	0.17	0.10
Ephemera	0.15	0.08		
Eukiefferiella	0.15	0.08	0.69	0.97
Glossosoma	0.15	0.32	0.35	0.19
Glyptotendipes	0.15	0.08		
Habrophlebia	0.15	0.08	0.17	0.19

Table A-1 (cont'd). Frequency distribution of individual taxa across all 24 sites by sampling method and laboratory protocol.

Name	D-Net 100	D-Net 200	Kick Seine 100	Kick Seine 200
Haploperla	0.15	0.08	0.17	0.10
Heterotrissocladius	0.15	0.08	0.35	0.19
Imm. Tubificid w/ Cap. Chaete	0.15	0.16		
Ironoquia	0.15	0.08		
Ischnura	0.15	0.24		
Leptophlebia	0.15	0.08		
Limnephilus	0.15	0.08		
Microcylloepus	0.15	0.08		
Neoporus	0.15	0.08		
Paraleptophlebia	0.15	0.08	0.17	0.10
Paraphaenocladius	0.15	0.16		
Pedicia	0.15	0.08		
Perlesta	0.15	0.16	0.17	0.10
Pilaria	0.15	0.16	0.17	0.10
Pisidium	0.15	0.16		
Procloeon	0.15	0.08		
Prodiamesa	0.15	0.08		
Prostoia	0.15	0.16		
Prostoma	0.15	0.08	0.35	0.29
Pseudochironomus	0.15	0.08		
Serratella	0.15	0.16	0.17	0.10
Sialis	0.15	0.08	0.17	0.29
Sigara	0.15	0.08		
Slavina	0.15	0.16		
Spirosperma	0.15	0.24	0.17	0.10
Stygonectes	0.15	0.16		
Sympotthastia	0.15	0.24	0.17	0.19
Syrphidae	0.15	0.08		
Taeniopteryx	0.15	0.08		
Triaenodes	0.15	0.16		
Tribelos	0.15	0.08		
Trichocorixa	0.15	0.08		
Acariformes			0.17	0.10
Acroneuria				0.10
Apsectrotanypus			0.17	0.10
Baetidae		0.08		
Branchiura				0.10
Capniidae			0.17	0.10
Cardiocladius		0.16		
Chaoborus		0.08		

Table A-1 (cont'd). Frequency distribution of individual taxa across all 24 sites by sampling method and laboratory protocol.

Name	D-Net 100	D-Net 200	Kick Seine 100	Kick Seine 200
Cladotanytarsus				0.19
Corydalus				0.10
Cultus			0.17	0.19
Dicranota		0.16		
Diplocladius			0.17	0.19
Diura			0.17	0.10
Dolophilodes		0.08		
Dytiscidae		0.08		
Eclipidrilus		0.08	0.52	0.29
Elmidae		0.08	0.17	0.19
Enallagma		0.08		
Endochironomus				0.10
Ferrissia		0.08	0.17	0.10
Helochares		0.08		
Lanthus			0.17	0.10
Leptoceridae		0.08		
Limnophila		0.08	0.17	0.10
Limonia			0.17	0.10
Lype		0.08		0.10
Microvelia		0.08	0.17	0.10
Molophilus		0.16		
Nemouridae		0.08	0.35	0.19
Orconectes				0.10
Paracladopelma		0.08		
Paratendipes			0.17	0.10
Paratrichocladius		0.16	0.17	0.19
Perlodidae		0.16	0.17	0.19
Planariidae			0.17	0.10
Polycentropus		0.08		0.19
Rhithrogena			0.17	0.10
Rhyacodrilus				0.10
Saetheria			0.17	0.10
Sperchon			0.17	0.10
Sphaerium			0.17	0.19
Stempellina			0.17	0.10
Stictochironomus		0.08		0.10
Stilocladius spp.		0.24	0.17	0.10
Synorthocladius			0.17	0.10
Taeniopterygidae				0.10

United States
Environmental Protection
Agency

Please make all necessary changes on the below label, detach or copy, and return to the address in the upper left-hand corner.

If you do not wish to receive these reports CHECK HERE ☐; detach, or copy the cover, and return to the address in the upper left-hand corner.

PRESORTED STANDARD
POSTAGE & FEES PAID
EPA
PERMIT No. G-35

Office of Environmental Information
and Mid-Atlantic Integrated
Assessment Program
Environmental Science Center
701 Mapes Road
Fort Meade, MD 20755-5350

Official Business
Penalty for Private Use
$300

EPA903/R-03/005
May 2003