

**Analysis of variability in New York State benthic macroinvertebrate samples**

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## ABSTRACT

*Variability of single-habitat kick sampling and 100-organism subsampling for benthic invertebrates was measured across a wide range of stream water quality. These methods have been used for stream biomonitoring in New York State since 1984. Streams representing non, slight, moderate, and severe water quality impacts were sampled, taking three kick samples from each, and three 100-organism subsamples from each sample, using species level identification. Although not all individual metrics could discriminate between all levels of impact, the multimetric index provided balance between these metrics, and was able to classify water quality according to predicted level of impact for all subsamples. It is concluded that these methods are accurate and consistent, and appropriate for performing water quality assessments.*

## INTRODUCTION

Integral to most rapid biological water quality assessment methods for wadeable streams are four elements: kick sampling, subsampling, reduced taxonomic level of identification, and multimetric data analysis (Resh and Jackson 1993). Monitoring programs use these elements to achieve time-saving cost-effectiveness, while attempting to preserve assessment accuracy, reliability, and low variability. Various combinations of these elements are found in many state agency bioassessment programs, and are recommended by the U.S. Environmental Protection Agency in their Rapid Bioassessment Protocols (Barbour et al. 1999; Plafkin et al. 1989).

The New York State Department of Environmental Conservation (NYSDEC) Stream Biomonitoring Unit protocol utilizes three of the four rapid assessment elements for stream monitoring proposed by Resh and Jackson (1993) in its water quality assessment program (Bode et al., 2002). A 100-organism subsample is analyzed from a traveling kick sample of benthic invertebrates. Organism identification is taken to the species level when practicable. Information from the subsampling is used to calculate the multimetric Biological Assessment Profile score (BAP). The BAP score integrates four individual metrics, Species richness (Plafkin et al. 1989), Ephemeroptera-Plecoptera-Trichoptera richness (Lenat 1988), Hilsenhoff's Biotic Index (Hilsenhoff 1987), and Percent Model Affinity (Novak and Bode 1992) into a common scale of water quality (Bode et al. 2002; Riva-Murray et al. 2002). Maxted et al. (2000) developed an index similar in structure to New York's BAP, for use by mid-Atlantic coastal plain states, concluding that the most accurate measures were a tolerance metric, a richness metric, and a composition metric similar to Percent Model Affinity. New York's Biological Assessment Profile score is used to place the

combined water quality information from the individual metrics into one of four assessment categories, ranging from non-impacted to severely impacted (Table 1). The goal is to produce results that can be readily used by water quality managers, and be easily understood by the public.

The majority of U.S. regulatory agencies use a multimetric approach to biological monitoring (Reynoldson et al. 1997). Arguments for and against such an approach have been well documented in recent literature. Karr and Chu (1999) reported difficulty in discerning complex biological patterns from data when using multivariate approaches. They concluded that a major loss of biological information occurred due to reliance on lists of taxa and their abundances without consideration of aspects of life history. Conversely, Reynoldson et al. (1997) stated that multivariate approaches are attractive in that they do not require prior assumptions in creating groups from reference sites or in the comparison of test sites with reference sites. Reynoldson et al. (1997) did, however, state that multimetrics are beneficial in that they provide a single score comparable to a target value with the inclusion of ecological information, except that errors may be compounded and some metrics can be redundant. They advised making water quality assessments based on a combination of information from both techniques.

The primary purpose of this study was to examine variability in the results of the single habitat kick sample and the 100-organism fixed-count sub-sample. Plafkin et al. (1989) found single habitat riffle samples and multiple-habitat samples to result in similar responses, and suggested the use of only riffle habitats in order to reduce variability. The effects of taxonomic resolution and multimetric data analysis on the ability to detect water quality impairment were also investigated, since they are integral to the assessment process. Several previous papers have addressed the scientific validity of sampling and subsampling from different standpoints. Hornig and Pollard (1978) concluded that the traveling kick method provided more highly reproducible data than Surber samples. Similar conclusions were reached by Storey et al. (1991) and Mackey et al. (1984), although Mackey et al. (1984) and Furse et al. (1981) noted significant operator differences with the kick method. With regard to fixed count subsampling methods, some studies have supported its valid use (Barbour and Gerritsen 1996; Somers et al. 1998; Vinson and Hawkins 1996) while others have urged greater caution in the use of small subsample sizes (Courtemanch 1996; Karr and Chu 1999). In their comparison of selective 50, 100 and 150 organism subsampling methods, Gowns et al. (1997) concluded significant differences resulted between reference and polluted sites, using

HBI, EPT richness, and Family richness as well as an analysis of similarity.

Differing opinions have also been expressed regarding the preferred level of taxonomic resolution, from the standpoint of cost-effectiveness versus ability of water quality impairment assessment. Resh and Unzicker (1975) stated that the degree of variation in tolerances to environmental stressors at the species level is cause for identification to the “lowest possible taxon” to adequately assess water quality. However, Bailey et al. (2001) found that when calculating some metrics such as Hilsenhoff’s biotic index (Hilsenhoff 1987), greater taxonomic resolution does not seem to contribute more information. Reduced taxonomic resolution is also currently a concern pertaining to volunteer biological monitoring and the use of volunteer data. A large number of volunteer groups utilize family level or even ordinal level identification. Due to financial limitations many state agencies have begun to incorporate such data into their programs. By 1995 greater than half of state regulatory agencies were using some form of volunteer data in their management decisions (Penrose and Call 1995). Recent literature reflects investigations into the accuracy of volunteer data and how they compare to data collected by state regulatory professionals (Engel and Voshell 2002; Nerbonne and Vondracek 2003; Penrose and Call 1995).

Beyond its use in the assessment of degree of water quality impact, increased taxonomic resolution may be more pertinent to the diagnosis of causes of water quality impairment. Riva-Murray et al. (2002) demonstrated that the range of environmental preferences within some genera made species level identifications crucial to the determination of type of impact.

The suitability of any technique must be evaluated from the perspective of the ultimate use of the data. In New York State the objective of the stream biomonitoring program is to “evaluate the relative biological health of the State’s streams and rivers through the collection and analysis of macroinvertebrate communities” (Bode et al. 2002). This is achieved within the framework of a 4-tiered impact assessment regime. The objective of this study was to determine whether or not these methods of sampling and subsampling can produce accurate and consistent assessments of water quality within the 4-tiered framework. This question was examined across a full range of impacts within the regime, from non-impacted to severely impacted.

## **SITE DESCRIPTION**

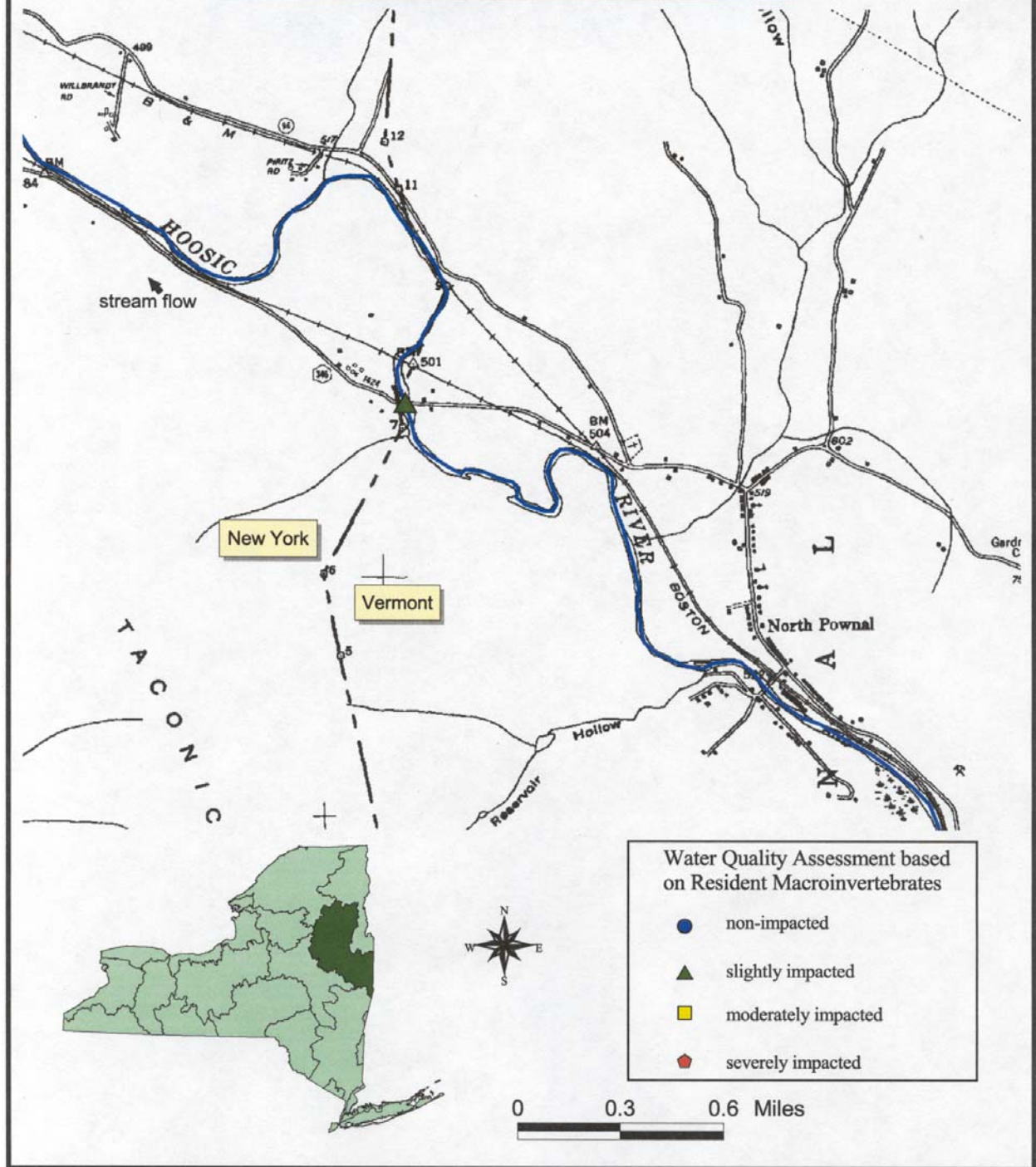
The streams sampled were chosen because they were predicted to represent the four different levels of water quality impact (non, slight, moderate and severe) used in the New York State hierarchy of biological water quality assessments. The four streams- Bloody Brook, Onondaga Creek, Pine Creek, and the Hoosic River - are cold-water streams ranging from 2<sup>nd</sup> order to 4<sup>th</sup> order, representing three watersheds: Black River, Hudson River, and Oswego River. Maps of stream locations are provided in figures 1 through 4. All samples were taken in wadeable riffles. Bloody Brook and Onondaga Creek were both collected during September of 2001, while Pine Creek was surveyed in August of 2002, and the Hoosic River in October of 2002. The following non-biological factors collected prior to, but on the same day as biological sampling were used to predict water quality: land use, habitat, and chemical parameters, primarily conductivity, which has been shown to be a good indicator of human activity (Yuan and Norton 2003). A Hydrolab Surveyor and Sonde™ recorded stream chemical attributes consisting of temperature, conductivity, pH, dissolved oxygen and percent saturation. Results of these measurements are summarized in Table 2. An on-site examination of the kick samples confirmed that the selected sites accurately represented the four impact categories.

The non-impacted stream selected, Pine Creek, is in a forested watershed, and had a conductivity of 30  $\mu\text{mhos/cm}$ , on the day of sampling. The macroinvertebrate fauna was diverse, and was dominated by Ephemeroptera and Trichoptera. The slightly impacted stream, the Hoosic River, was sampled downstream of an agricultural area; the watershed has heavy streambank erosion (New York State Department of Environmental Conservation 1996a), some upstream wastewater effluent discharges, and is partially forested; conductivity was 244  $\mu\text{mhos/cm}$ . The macroinvertebrate fauna was less diverse, and was dominated by Hydropsychidae (Trichoptera) and Elmidae (Coleoptera). The moderately-impacted stream, Bloody Brook, lies entirely in an urban/industrial setting, has previously documented findings of sewage inputs and elevated body burdens of several metals in invertebrates (New York State Department of Environmental Conservation 1996b), and had a conductivity of 912  $\mu\text{mhos/cm}$ . The macroinvertebrate fauna was greatly reduced, and was dominated by Elmidae (Coleoptera), Gammaridae (Amphipoda), and Chironomidae (Diptera). The severely impacted stream, Onondaga Creek, receives 78 combined sewer overflow discharges, had a conductivity of 5640  $\mu\text{mhos/cm}$ , primarily from upstream mud boils, salt springs, and brine well discharges (New York State Department of Environmental Conservation 1996b). The macroinvertebrate fauna had greatly reduced diversity, and was

composed almost entirely of Oligochaeta and tolerant Chironomidae (Diptera).



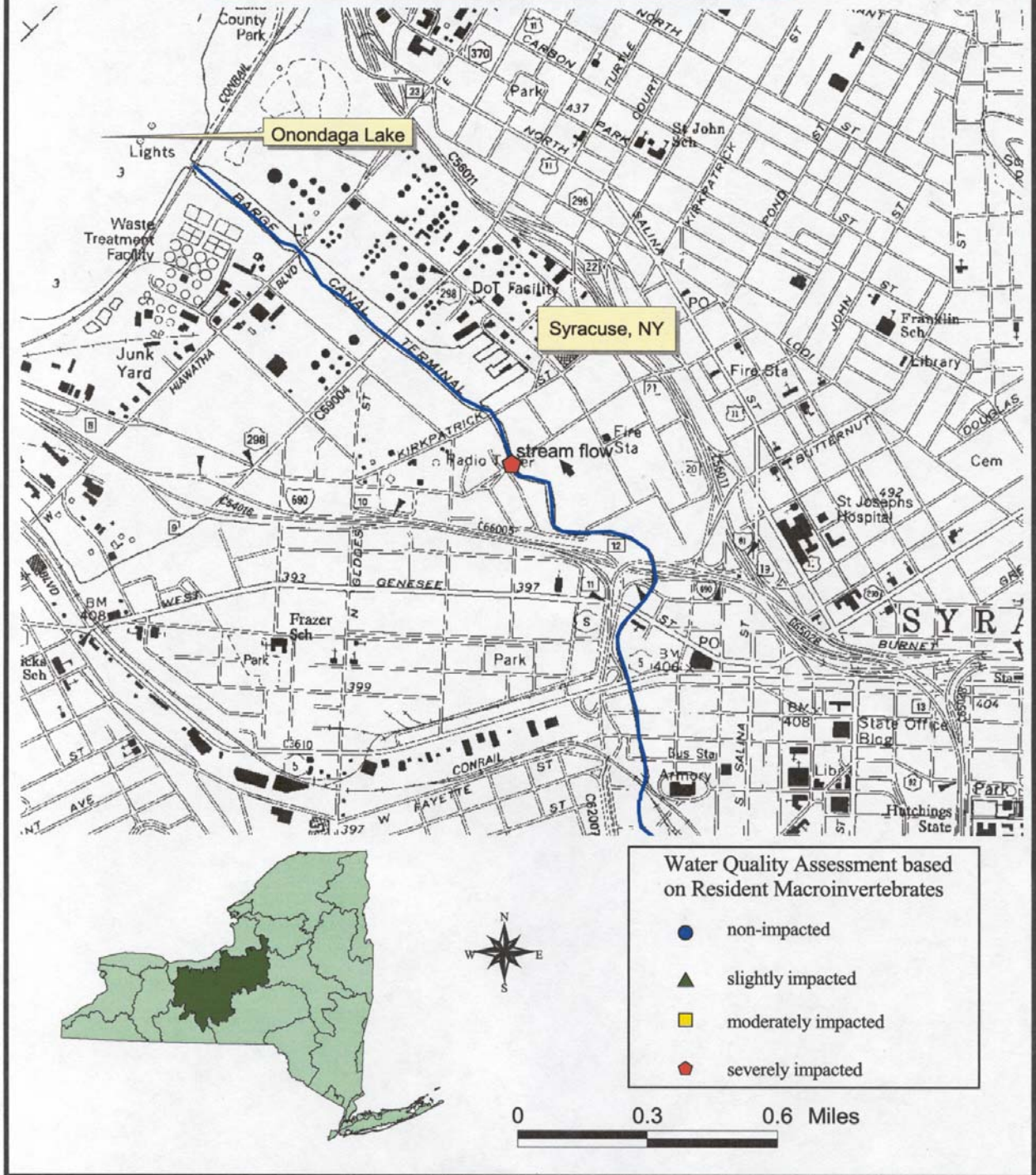
Figure 2. Hoosic River







**Figure 4. Onondaga Creek**



## METHODS

Three macroinvertebrate samples were collected from each stream using the traveling kick method over a five-meter transect of a riffle for a five minute period as described in the Quality Assurance Work Plan for Biological Stream Monitoring in New York State (Bode et al. 2002) and summarized in Appendix I - VIII. At each stream the samples were collected from the same section of riffle. Kick sampling was begun at the head of the riffle using a 9-inch by 18-inch kick net with a mesh opening of 0.8 mm by 0.9 mm. Transects proceeded diagonally through the section. Each kick sample was separately preserved in 95% ethyl alcohol. In the laboratory, three separate 100-organism sub-samples were removed from each kick sample. The sample detritus was mixed and spread thoroughly over a 25 x 20cm white enamel pan. A single tablespoon of detritus was removed and placed in a 9cm diameter petri dish and sorted under a dissecting microscope. All invertebrates larger than 1.5 mm were randomly removed by removing each organism encountered as small amounts of detritus were drawn towards the investigator. This technique continued until 100 organisms had been obtained. If the single tablespoon of sample was exhausted of its organisms another was examined. Macroinvertebrates were identified to the lowest possible taxonomic level (Bode et al. 2002). All samples were collected and processed by the same individual to ensure procedural consistency.

Water quality assessments were generated using the four community metrics utilized by the NYSDEC: species richness (Plafkin et al. 1989), EPT richness (Lenat 1988), Hilsenhoff's Biotic Index (HBI)(Hilsenhoff 1987), and Percent Model Affinity (PMA) (Novak and Bode 1992). Overall water quality assessments were made for each sub-sample by converting each community metric value to a ten-scale scoring regime and averaging these to calculate the Biological Assessment Profile score (BAP) (Bode et al. 2002; Riva-Murray et al. 2002).

Wilcoxon rank analysis was performed on the data to determine if significant differences were observed between streams, using results from each 100-organism subsample for the four community metrics and the Bioassessment Profile, and to identify any significant difference between kick samples within sites. Tukey's test on ranked scores was performed (level of significance 0.05) to evaluate differences identified by the Wilcoxon rank analysis.

To investigate the implications of reduced taxonomic resolution on detecting water quality

impairment, data were subjected to a reduction from species to family level identification. The four community metrics and the Bioassessment Profile Score for each subsample were then recalculated using this new information, and compared to provisional family-level impact criteria.

## RESULTS

Raw data from sample identification are provided in Appendix IX. Water quality in all four streams was assessed as predicted using the multimetric BAP score calculation. None of the four metrics had the ability to discriminate between the different classes of impairment for all samples and subsamples (Tables 1 and 3). However, results from the Wilcoxon Rank Test suggest significant differences between the four sites with regard to metric values ( $P < .05$ ) (Table 4). When using the same test to investigate differences between replicated kick samples within the four sites, no significant differences were observed (Table 5). Once the individual scores were converted to the common scale values and combined in a multimetric BAP score, the results from each subsample correctly identified the predicted level of water quality impact (Figure 5). Results of Tukey's test showed species richness and HBI from each site are significantly different ( $P < .05$ ) from each other (Table 6). Differences in EPT richness values are not significant between moderately and severely impacted sites ( $P < .05$ ), and PMA values are not significantly different between slightly and moderately impacted sites (Table 6). Despite the inability of EPT richness to discriminate between all moderately and severely impacted samples, and the inability of PMA to discriminate between all slightly and moderately impacted samples, the multimetric BAP score achieved the necessary balance to classify sites according to predicted level of impact for all subsamples in all impact categories (Table 1, 4, 6, and Figure 5).

Results of family level identification caused initial difficulty in distinguishing between certain water quality impact levels. Censoring the data to family-level identification and using New York State's provisional scales of water quality for family richness, EPT richness, HBI, and PMA resulted in the inability to distinguish between moderate and severe impact at the family level (See Appendix IX. for family level macroinvertebrate indices). Results of richness measurements at the family level do not separate more severe levels of impact to the extent that HBI and PMA do. However the data suggest that PMA has difficulty in distinguishing between slight and moderate impacts. A revision of the scales and calculations used to generate the family level Biological Assessment Profile score was made by revising the ranges at which impact classes were defined, to

increase the ability of the data to properly assess water quality. Upon revision this censored data placed 92% of subsamples in the predicted water quality impact assessment category (Figure 6.), compared to 100% correct placement with genus/species level identification.

Table 1. Individual metric criteria and the multimetric BAP score range for water quality assessment of wadeable waters in New York State. SPP = Species Richness, HBI = Hilsenhoff's Biotic Index, EPT = Ephemeroptera, Plecoptera, Trichoptera Richness, PMA = Percent Model Affinity, and BAP = Biological Assessment Profile Score.

	SPP	HBI	EPT	PMA	BAP
Non-Impacted	>26	0.00-4.50	>10	>64	7.51-10.0
Slightly Impacted	19-26	4.51-6.50	6-10	50-64	5.01-7.5
Moderately Impacted	11-18	6.51-8.50	2-5	35-49	2.51-5.0
Severely Impacted	0-10	8.51-10.00	0-1	<35	0.0-2.5

Table 2. Summary of physical and chemical data collected from all four streams. Stream names have been abbreviated; PINC = Pine Creek, HOOS = Hoosic River, BLDY = Bloody Brook, and ONON = Onondaga Creek. Parameters not recorded in the field are represented by a dash.

	PINC	HOOS	BLDY	ONON
Order	2 <sup>nd</sup>	4 <sup>th</sup>	2 <sup>nd</sup>	4 <sup>th</sup>
Depth (meters)	0.2	0.2	0.2	0.3
Width (meters)	5	30	5	20
Current (cm/sec)	100	110	-	-
Canopy (%)	25	10	10	10
Embeddedness (%)	50	40	25	25
Temperature (°C)	22.3	13.9	20	16.6
Conductivity (umhos)	30	244	912	5640
pH	6.8	8.4	8.5	7.8
D.O. (mg/l;ppm)	7.5	12.9	10.5	8.6
% Saturation	91	124	-	-

Table 3. Metric results. Sub. = Subsample, SPP = Species Richness, EPT = Ephemeroptera, Plecoptera, Trichoptera richness, HBI = Hilsenhoff's Biotic Index, PMA = Percent Model Affinity, BAP = Biological Assessment Profile Score.

Site	Kick	Sub	SPP	EPT	HBI	PMA	BAP
Pine Crk.	1	1	27	12	3.99	69	8.1
		2	27	13	3.56	73	8.4
		3	27	16	3.5	71	8.6
	2	1	28	15	3.15	78	8.9
		2	29	17	3.75	74	8.75
		3	31	15	3.57	59	8.60
	3	1	29	18	3.3	80	9.00
		2	24	13	3.4	80	8.33
		3	27	14	3.1	78	8.7
Hoosic Riv.	1	1	24	10	4.91	54	6.72
		2	20	9	4.95	45	5.9
		3	18	8	4.58	44	5.65
	2	1	22	8	4.8	53	6.3
		2	20	10	4.47	45	6.12
		3	20	10	4.98	48	6.14
	3	1	19	10	4.78	51	6.72
		2	22	10	4.71	55	5.90
		3	20	9	5.11	55	5.65
Bloody Brk.	1	1	14	0	6.05	45	3.32
		2	11	1	6.05	41	3.28
		3	13	0	6.09	45	3.28
	2	1	16	1	6.08	49	3.95
		2	16	3	6.3	53	4.68
		3	15	2	6	46	4.28
	3	1	12	0	6.21	45	3.18
		2	17	2	6.22	47	4.38
		3	15	1	6.35	38	3.32
Onondaga Crk.	1	1	6	0	9.56	20	0.3
		2	12	1	8.87	34	2.15
		3	10	1	8.93	30	1.8
	2	1	13	1	8.99	29	2.05
		2	9	1	8.83	31	1.8
		3	11	1	8.95	31	1.98
	3	1	8	0	9.31	25	0.88
		2	13	1	9.09	32	2.12
		3	11	0	9.15	29	1.45

Table 4. Wilcoxon Rank test scores for differences in metrics among streams (n=9).

Site	Mean score	P value
<b>Species Richness</b>		
Pine Creek	31.94	<.0001
Hoosic River	23.06	
Bloody Brook	13.17	
Onondaga Creek	5.83	
<b>EPT Richness</b>		
Pine Creek	32.00	<.0001
Hoosic River	23.00	
Bloody Brook	10.50	
Onondaga Creek	8.50	
<b>HBI</b>		
Pine Creek	5.00	<.0001
Hoosic River	14.00	
Bloody Brook	23.00	
Onondaga Creek	32.00	
<b>PMA</b>		
Pine Creek	32.00	<.0001
Hoosic River	20.61	
Bloody Brook	16.39	
Onondaga Creek	5.00	
<b>BAP</b>		
Pine Creek	32.00	<.0001
Hoosic River	23.00	
Bloody Brook	14.00	
Onondaga Creek	5.00	

Table 5. Results of Wilcoxon Rank test scores for differences in metrics among kick samples within sites (n=3 per metric, per site). SPP = Species Richness, EPT = Ephemeroptera, Plecoptera, Trichoptera richness, HBI = Hilsenhoff's Biotic Index, PMA = Percent Model Affinity, BAP = Biological Assessment Profile Score.

Metric	P value (< .05 Sig.)			
	Pine Creek	Hoosic River	Bloody Brook	Onondaga Creek
SPP	0.1235	0.9179	0.1585	0.7288
EPT	0.4383	0.6559	0.1517	0.2636
HBI	0.1767	0.9565	0.1741	0.2521
PMA	0.0752	0.1816	0.1051	0.9033
BAP	0.2253	0.7351	0.1585	0.7903

Table 6. Results of Tukey's Test for significant differences and similarities among sites based on all sub-samples. Duplicated letters signify no significant difference (n=9). SPP = Species Richness, EPT = Ephemeroptera, Plecoptera, Trichoptera richness, HBI = Hilsenhoff's Biotic Index, PMA = Percent Model Affinity, BAP = Biological Assessment Profile Score.

Metric	Min. Sig. Dif.	Site	Mean	Tukey Group
SPP	4.2507	Pine Creek	31.944	A
		Hoosic River	23.056	B
		Bloody Brook	13.167	C
		Onondaga Creek	5.8331	D
EPT	5.0309	Pine Creek	32.0	A
		Hoosic River	23.0	B
		Bloody Brook	10.5	C
		Onondaga Creek	8.5	C
HBI	3.4941	Pine Creek	32.0	A
		Hoosic River	23.0	B
		Bloody Brook	14.0	C
		Onondaga Creek	5.0	D
PMA	5.1052	Pine Creek	32.0	A
		Hoosic River	20.611	B
		Bloody Brook	16.389	B
		Onondaga Creek	5.0	C
BAP	3.4722	Pine Creek	32.0	A
		Hoosic River	23.0	B
		Bloody Brook	14.0	C
		Onondaga Creek	5.0	D

### 100 organism Subsample Family Level Bioassessment Profile Scores

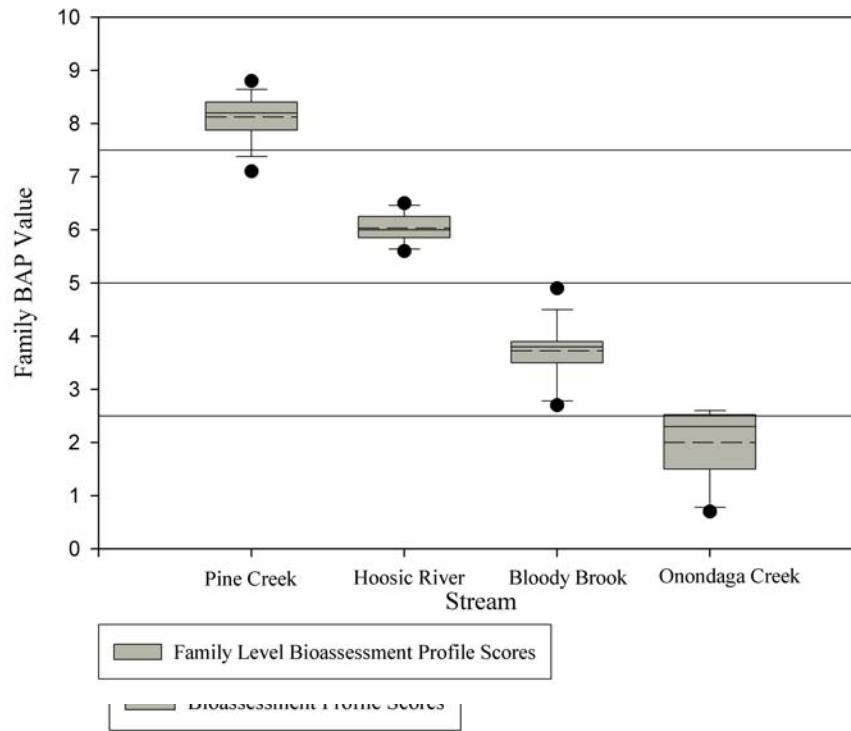


Figure 5. Box plot of BAP values from the nine subsamples from each site. Dashed lines represent the mean, solid lines represent the median, black circles signify outliers.

Figure 6. Box plots of Family Level BAP values from the nine subsamples from each site. Dashed lines represent the mean, solid lines represent the median, black circles signify outliers.

## **DISCUSSION**

The results generated by the Wilcoxon and Tukey's tests demonstrated that the metric values calculated from each stream were significantly different from each other. This establishes the fundamental ability of the methods to discriminate between four levels of water quality impact. If variation in metric results had been substantial within the subsamples from each stream, a clear separation between water quality classes would not be expected.

For single-habitat kick samples and fixed-count subsamples to be considered replicable and representative of in-stream macroinvertebrate communities, variability between kick samples within each site should be low. The results from this statistical analysis indicate significantly low variability among the three kick samples within each site (Table 5). The outcome from the Wilcoxon test supports the single habitat kick sample as a replicable method. It is suggested by our study as in several others that the single habitat method of sampling can be widely adopted for use in biological

monitoring. Chessman (1995) recommended against the use of multi-habitat data specifically when comparing sites, stating separate comparisons are necessary between each habitat to reduce the error derived from differences in habitats from different sites. Hewlett (2000), Parsons and Norris (1996), and Plafkin et al. (1989) found that single habitat sampling produced similar results to multiple habitat and concluded single habitat sampling provides adequate data for biomonitoring. We concur with such results, as our data suggest that a single kick sample from a single riffle area can be used to accurately assess water quality.

Although the Wilcoxon rank tests demonstrate the ability of the method to distinguish significant differences between the streams and also show identical results from kick samples within sites, the nature of the differences and similarities are not determined. These were provided by the Tukey's test results. Two metrics - EPT richness and PMA - were shown to be unable to discriminate between two of the impact categories (Table 6.). This outcome is understandable for the EPT metric, unable to distinguish moderately and severely impacted categories, since invertebrate communities from these high impacted sites have very low EPT values, allowing for little discrimination. The EPT metric is of greater benefit in discriminating between more diverse communities with less environmental impact (i.e., more EPT). PMA results from Tukey's Test show an inability to discriminate between the slightly and moderately impacted categories. This is because PMA is based on community similarities and differences mostly at the ordinal level. Communities in the slightly and moderately impacted categories may appear similar at the ordinal level, and result in similar PMA values, but may be very different at the levels of family, genus, and species. This indicates the necessity of this metric being combined with other metrics that require greater levels of taxonomic discrimination as was suggested in Novak and Bode, 1992. This is an example of the composition of a multimetric index compensating for the lack of discrimination of single metrics in certain situations.

Reynoldson et al. (1997) found multimetric data analysis to be much more inconsistent in determining water quality classifications when compared to two different multivariate techniques. We found our multimetric approach to be highly consistent in its assessments. However, large variation could result if the multimetric approach were to be dissected into its individual parts, or one of the four metrics were to be substituted by a different metric. Only when used together in a multimetric setting was the assessment consistently in agreement with predicted water quality. Examining the multimetric BAP score, which is based on the four metrics, Tukey's test results

indicate BAP scores from all subsamples within each site were significantly different from all other subsamples and had 100% placement in the predicted impact category. It was also shown that of the four metrics used, none was without shortcomings. None provided correct assessment of water quality for all levels of impact. This demonstrates that expectations for metric performance should not be judged individually, but within the multimetric context. Metrics should not be discarded based on failure to discriminate a particular type or level of impact. Using multiple metrics to calculate the BAP score provides the balance needed to accurately assess water quality over a range of impact types. These results also confirm that variability in the kick sampling method and the subsampling method is small enough to achieve the goal of correctly classifying impact within a four-tiered hierarchy of water quality. Norris (1995) states that most rapid assessment approaches lack within-site replication, implying that individual sites cannot be compared with confidence, since the differences may be the result of anything other than chance. Conversely, our data imply a small amount of variability, contradicting the concept of requiring replication to achieve a water quality assessment. It is demonstrated by the data-set that when using this multimetric method, a single 100-organism subsample of a single kick sample is robust enough to adequately represent the in-stream invertebrate community and accurately assess water quality.

Our findings are similar to those of Hewlett (2000) in which all levels of taxonomic resolution investigated were found to produce largely the same results. Plafkin et al. (1989) and Wright et al. (1995) also had similar results in which family level identification was sufficient enough to differentiate between different levels of impairment. The reduction of taxonomic resolution to family level in our samples caused a decrease in discrimination between the most impaired sites in comparison to genus/species identification. However, results did suggest that although 92% of subsamples diagnosed the proper level of impairment, family level BAP scores were least successful in correctly classifying subsamples from the severely impacted site. Even though finer degrees of identification suggest greater accuracy in assessing water quality, the results of this study indicate limited taxonomic identification can, in most cases, adequately assess impairment within New York State's 4 tiered impact regime.

In summary, our study found New York State's single 100-organism subsample of a single kick sample and the use of a multimetric analysis robust enough to adequately represent the in-stream invertebrate community and accurately assess water quality. The multimetric approach was found to be highly consistent in its assessments confirming that variability in these methods is small

enough to achieve the goal of correctly classifying impact within a four-tiered hierarchy of water quality.

As shown by this and previous studies, greater taxonomic resolution may not contribute greatly to the detection of water quality impacts. The greater value of increased taxonomic resolution may be in the diagnosis of causes of water quality impairment, as demonstrated by Riva-Murray et al. (2002). As ranges of environmental preferences become better defined for aquatic species, species level identifications will be crucial to the determination of type of stressors causing water quality impacts. The importance of this emerging discipline of water quality analysis supports the use of species level identifications in biological monitoring programs.

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#### **LITERATURE CITED**

Bailey, R. C., R. H. Norris, and T. B. Reynoldson. 2001. Taxonomic resolution of benthic macroinvertebrate communities in bioassessments. *Journal of the North American Benthological*

Society 20:280-286.

Barbour, M. T., J. Gerritsen, B. D. Snyder, and J. B. Stribling. 1999. Rapid bioassessment protocols for use in wadeable streams and rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

Barbour, M. T., and J. Gerritsen. 1996. Subsampling of benthic samples: a defense of the fixed-count method. *Journal of the North American Benthological Society* 15:386-391.

Bode, R. W., M. A. Novak, L. E. Abele, D. L. Heitzman, and A. J. Smith. 2002. Quality assurance work plan for biological stream monitoring in New York State. New York State Department of Environmental Conservation. Albany, New York.

Chessman, B. C. 1995. Rapid assessment of rivers using macroinvertebrates: A procedure based on habitat-specific sampling, family level identification and a biotic index. *Australian Journal of Ecology* 20:122-129.

Courtemanch, D. L. 1996. Commentary on the subsampling procedures used for rapid bioassessments. *Journal of the North American Benthological Society* 15:381-385.

Engel S. R. and J. R. Voshell, Jr. 2002. Volunteer Biological Monitoring: Can It Accurately Assess the Ecological Condition of Streams? *American Entomologist* 48:164-177.

Furse, M. T., J. F. Wright, P. D. Armitage, and D. Moss. 1981. An appraisal of pond-net samples for biological monitoring of lotic macro-invertebrates. *Water Research* 15:679-689.

Growns, J. E., B. C. Chessman, J. E. Jackson, and D. G. Ross. 1997. Rapid assessment of Australian rivers using macroinvertebrates: cost and efficiency of 6 methods of sample processing. *Journal of the North American Benthological Society* 16:682-693.

- Hewlett, R. 2000. Implications of Taxonomic resolution and sample habitat for stream classification at a broad geographic scale. *Journal of the North American Benthological Society* 19:352-361.
- Hilsenhoff, W. L..1987. An improved biotic index of organic stream pollution. *The Great Lakes Entomologist* 20:31-39.
- Hornig, C. E., and J. E. Pollard. 1978. Macroinvertebrate sampling techniques for streams in semi-arid regions. EPA- 600-4-78-040. U.S. Environmental Protection Agency; Environmental Monitoring and Support Laboratory, Las Vegas, Nevada.
- Karr, J. R., and E. W. Chu. 1999. Restoring life to running waters: Better biological monitoring. Island Press. Washington, D.C..
- Lenat, D. R.. 1988. Water quality assessment of streams using a qualitative collection method for benthic macroinvertebrates. *Journal of the North American Benthological Society* 7:222-233.
- Mackey, A. P., D. A. Cooling, and A. D. Berrie. 1984. An evaluation of sampling strategies for qualitative surveys of macroinvertebrates in rivers, using pond nets. *Journal of Applied Ecology* 21:515-534.
- Maxted, J. R., M. T. Barbour, J. Gerritsen, V. Poretti, N. Primrose, A. Silvia, D. Penrose, and R. Renfrow. 2000. Assessment framework for mid-Atlantic coastal plain streams using benthic macroinvertebrates. *Journal of the North American Benthological Society* 19:128-144.
- Nerbonne, J. F., and B. Vondracek. 2003. Volunteer macroinvertebrate monitoring: assessing training needs through examining error and bias in untrained volunteers. *Journal of the North American Benthological Society* 22:152-163.
- New York State Department of Environmental Conservation. 1996a. The 1996 Priority Waterbodies List for the Upper Hudson River Basin. Albany, NY. 120 pages.

- New York State Department of Environmental Conservation. 1996b. The 1996 Priority Waterbodies List for the Oswego-Seneca-Oneida River Basin. Albany, NY. 196 pages.
- Norris, R. H. 1995. Biological monitoring: the dilemma of data analysis. *Journal of the North American Benthological Society* 14:440-450.
- Novak, M. A., and R. W. Bode. 1992. Percent model affinity, a new measure of macroinvertebrate community composition. *Journal of the North American Benthological Society* 11:80-85.
- Parsons, M., and R. H. Norris. 1996. The effect of habitat-specific sampling on biological assessment of water quality using a predictive model. *Freshwater Biology* 36:419-434.
- Penrose, D. and S. M. Call. 1995. Volunteer monitoring of benthic macroinvertebrates: regulatory biologists' perspectives. *Journal of the North American Benthological Society* 14:203-209.
- Plafkin, J. L., M. T. Barbour, K. D. Porter, S. K. Gross, and R. M. Hughes. 1989. Rapid Bioassessment Protocols For Use In Streams And Rivers: Benthic Macroinvertebrates And Fish. EPA 440-4-89-001. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.
- Resh, V. H., and J. K. Jackson. 1993. Rapid assessment approaches to biomonitoring using benthic macroinvertebrates. Pages 195-233 in: D. M. Rosenberg and V. H. Resh (editors). *Freshwater Biomonitoring and Benthic Macroinvertebrates*. Chapman and Hall, New York.
- Resh, V. H., and J. D. Unzicker. 1975. Water quality monitoring and aquatic organisms: the importance of species identification. *Journal of the Water Pollution Control Federation* 47:9-19.
- Reynoldson, T. B., R. H. Norris, V. H. Resh, K. E. Day, and D. M. Rosenberg. 1997. The reference condition: a comparison of multimetric and multivariate approaches to assess water quality impairment using benthic macroinvertebrates. *Journal of the North American Benthological Society* 16:833-852.

Riva-Murray, K., R. W. Bode, P. J. Phillips, and G. L. Wall. 2002. Impact source determination with biomonitoring data in New York State: concordance with environmental data. *Northeastern Naturalist* 9:127-162.

Somers, K. M., R. A. Reid, and S. M. David. 1998. Rapid biological assessments: how many animals are enough? *Journal of the North American Benthological Society* 17:348-358.

Storey, A. W., D. H. D. Edward, and P. Gazey. 1991. Surber kick sampling: a comparison for the assessment of macroinvertebrate community structure in streams of south-western Australia. *Hydrobiologia* 211:111-121.

Vinson, M. R., and C. P. Hawkins. 1996. Effects of sampling area and subsampling procedure on comparisons of taxa richness among streams. *Journal of the North American Benthological Society* 15:392-399.

Wright, I. A., B. C. Chessman, P. G. Fairweather, and L. J. Benson. 1995. Measuring the impact of sewage effluent on the macroinvertebrate community of an upland stream: the effect of different levels of taxonomic resolution and quantification. *Australian Journal of Ecology* 20:142-149.

Yuan, L. L., and S. B. Norton. 2003. Comparing responses of macroinvertebrate metrics to increasing stress. *Journal of the North American Benthological Society* 22(2):308-322.

## Appendix I. BIOLOGICAL METHODS FOR KICK SAMPLING

A. Rationale. The use of the standardized kick sampling method provides a biological assessment technique that lends itself to rapid assessments of stream water quality.

B. Site Selection. Sampling sites are selected based on these criteria: (1) The sampling location should be a riffle with a substrate of rubble, gravel, and sand. Depth should be one meter or less, and current speed should be at least 0.4 meters per second. (2) The site should have comparable current speed, substrate type, embeddedness, and canopy cover to both upstream and downstream sites to the degree possible. (3) Sites are chosen to have a safe and convenient access.

C. Sampling. Macroinvertebrates are sampled using the standardized traveling kick method. An aquatic net is positioned in the water at arms' length downstream and the stream bottom is disturbed by foot, so that the dislodged organisms are carried into the net. Sampling is continued for a specified time and for a specified distance in the stream. Rapid assessment sampling specifies sampling 5 minutes for a distance of 5 meters. The net contents are emptied into a pan of stream water. The contents are then examined, and the major groups of organisms are recorded, usually on the ordinal level (e.g., stoneflies, mayflies, caddisflies). Larger rocks, sticks, and plants may be removed from the sample if organisms are first removed from them. The contents of the pan are poured into a U.S. No. 30 sieve and transferred to a quart jar. The sample is then preserved by adding 95% ethyl alcohol.

D. Sample Sorting and Subsampling. In the laboratory the sample is rinsed with tap water in a U.S. No. 40 standard sieve to remove any fine particles left in the residues from field sieving. The sample is transferred to an enamel pan and distributed homogeneously over the bottom of the pan. A small amount of the sample is randomly removed with a spatula, rinsed with water, and placed in a petri dish. This portion is examined under a dissecting stereomicroscope and 100 organisms are randomly removed from the debris. As they are removed, they are sorted into major groups, placed in vials containing 70 percent alcohol, and counted. The total number of organisms in the sample is estimated by weighing the residue from the picked subsample and determining its proportion of the total sample weight.

E. Organism Identification. All organisms are identified to the species level whenever possible. Chironomids and oligochaetes are slide-mounted and viewed through a compound microscope; most other organisms are identified as whole specimens using a dissecting stereomicroscope. The number of individuals in each species, and the total number of individuals in the subsample is recorded on a data sheet. All organisms from the subsample are archived, either slide-mounted or preserved in alcohol. Following identification of a subsample, if the results are ambiguous, suspected of being spurious, or do not yield a clear water quality assessment, additional subsampling may be required.

## Appendix II. MACROINVERTEBRATE COMMUNITY PARAMETERS

1. Species richness. This is the total number of species or taxa found in the sample. Expected ranges for 100-specimen subsamples of kick samples in most streams in New York State are: greater than 26, non-impacted; 19-26, slightly impacted; 11-18, moderately impacted; less than 11, severely impacted.
2. EPT value. EPT denotes the total number of species of mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddisflies (Trichoptera) found in an average 100-organism subsample. These are considered to be mostly clean-water organisms, and their presence generally is correlated with good water quality (Lenat, 1987). Expected ranges from most streams in New York State are: greater than 10, non-impacted; 6-10, slightly impacted; 2-5, moderately impacted; and 0-1, severely impacted.
3. Biotic index. The Hilsenhoff Biotic Index is a measure of the tolerance of the organisms in the sample to organic pollution (sewage effluent, animal wastes) and low dissolved oxygen levels. It is calculated by multiplying the number of individuals of each species by its assigned tolerance value, summing these products, and dividing by the total number of individuals. On a 0-10 scale, tolerance values range from intolerant (0) to tolerant (10). For purposes of characterizing species' tolerance, intolerant = 0-4, facultative = 5-7, and tolerant = 8-10. Values are listed in Hilsenhoff (1987); additional values are assigned by the NYS Stream Biomonitoring Unit. The most recent values for each species are listed in the Quality Assurance document (Bode et al., 1996). Ranges for the levels of impact are: 0-4.50, non-impacted; 4.51-6.50, slightly impacted; 6.51-8.50, moderately impacted; and 8.51-10.00, severely impacted.
4. Percent Model Affinity is a measure of similarity to a model non-impacted community based on percent abundance in 7 major groups (Novak and Bode, 1992). Percentage similarity is used to measure similarity to a community of 40% Ephemeroptera, 5% Plecoptera, 10% Trichoptera, 10% Coleoptera, 20% Chironomidae, 5% Oligochaeta, and 10% Other. Ranges for the levels of impact are: >64, non-impacted; 50-64, slightly impacted; 35-49, moderately impacted; and <35, severely impacted.

Bode, R.W., M.A. Novak, and L.E. Abele. 1996. Quality assurance work plan for biological stream monitoring in New York State. NYS DEC technical report, 89 pp.

Hilsenhoff, W. L. 1987. An improved biotic index of organic stream pollution. *The Great Lakes Entomologist* 20(1): 31-39.

Lenat, D. R. 1987. Water quality assessment using a new qualitative collection method for freshwater benthic macroinvertebrates. North Carolina DEM Tech. Report. 12 pp.

Novak, M.A., and R.W. Bode. 1992. Percent model affinity: a new measure of macroinvertebrate community composition. *J. N. Am. Benthol. Soc.* 11(1):80-85.

## Appendix III. LEVELS OF WATER QUALITY IMPACT IN STREAMS

The description of overall stream water quality based on biological parameters uses a four-tiered system of classification. Level of impact is assessed for each individual parameter, and then combined for all parameters to form a consensus determination. Four parameters are used: species richness, EPT value, biotic index, and percent model affinity. The consensus is based on the determination of the majority of the parameters; since parameters measure different aspects of the community, they cannot be expected to always form unanimous assessments. The ranges given for each parameter are based on 100-organism subsamples of macroinvertebrate riffle kick samples, and also apply to most multiplate samples, with the exception of percent model affinity.

### 1. Non-impacted

Indices reflect very good water quality. The macroinvertebrate community is diverse, usually with at least 27 species in riffle habitats. Mayflies, stoneflies, and caddisflies are well-represented; the EPT value is greater than 10. The biotic index value is 4.50 or less. Percent model affinity is greater than 64. Water quality should not be limiting to fish survival or propagation. This level of water quality includes both pristine habitats and those receiving discharges which minimally alter the biota.

### 2. Slightly impacted

Indices reflect good water quality. The macroinvertebrate community is slightly but significantly altered from the pristine state. Species richness usually is 19-26. Mayflies and stoneflies may be restricted, with EPT values of 6-10. The biotic index value is 4.51-6.50. Percent model affinity is 50-64. Water quality is usually not limiting to fish survival, but may be limiting to fish propagation.

### 3. Moderately impacted

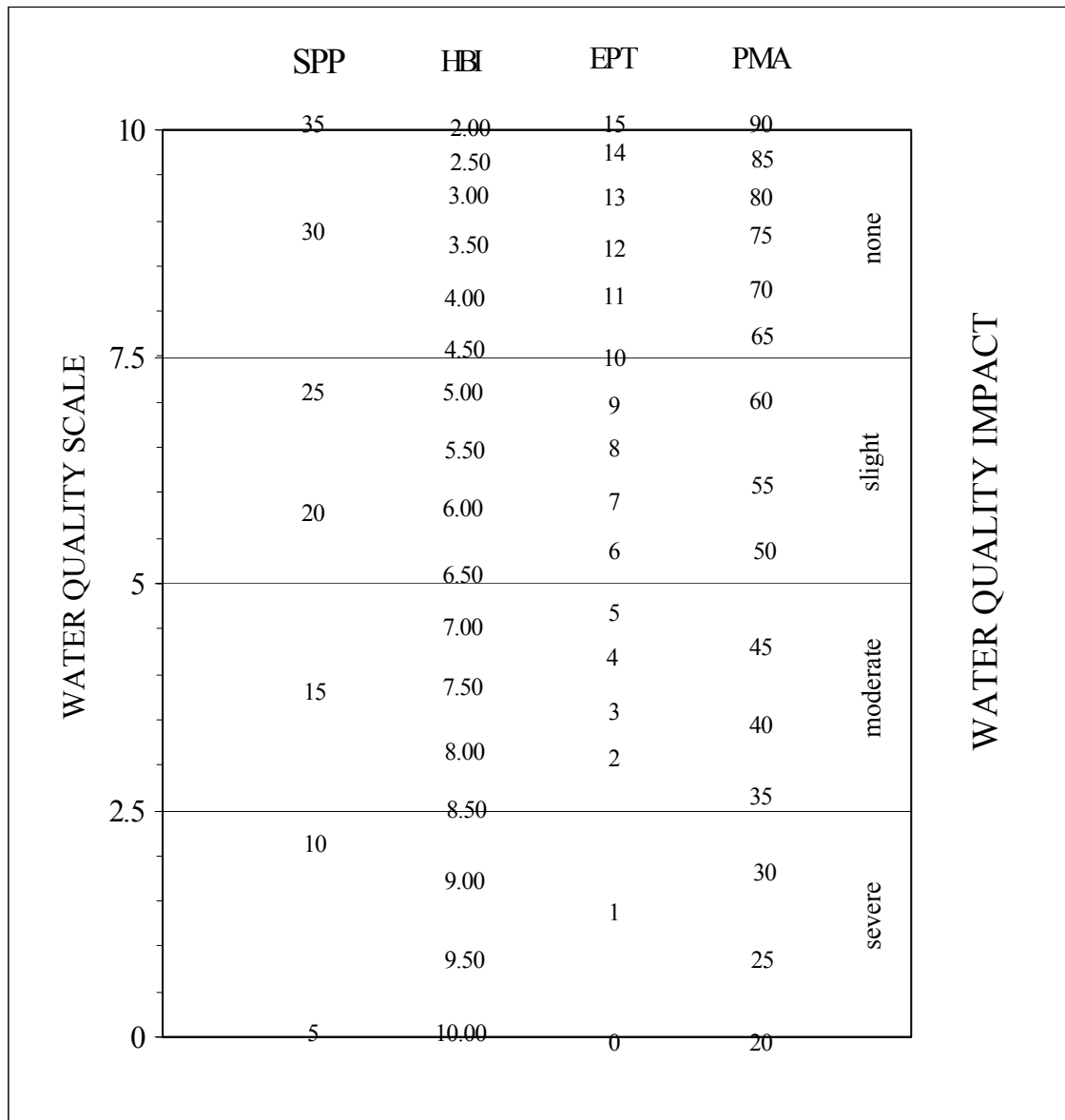
Indices reflect poor water quality. The macroinvertebrate community is altered to a large degree from the pristine state. Species richness usually is 11-18 species. Mayflies and stoneflies are rare or absent, and caddisflies are often restricted; the EPT value is 2-5. The biotic index value is 6.51-8.50. The percent model affinity value is 35-49. Water quality often is limiting to fish propagation, but usually not to fish survival.

### 4. Severely impacted

Indices reflect very poor water quality. The macroinvertebrate community is limited to a few tolerant species. Species richness is 10 or less. Mayflies, stoneflies, and caddisflies are rare or absent; EPT value is 0-1. The biotic index value is greater than 8.50. Percent model affinity is less than 35. The dominant species are almost all tolerant, and are usually midges and worms. Often 1-2 species are very abundant. Water quality is often limiting to both fish propagation and fish survival.

#### Appendix IV. BIOLOGICAL ASSESSMENT PROFILE OF INDEX VALUES

The Biological Assessment Profile of index values, developed by Mr. Phil O'Brien, Division of Water, NYS DEC, is a method of plotting biological index values on a common scale of water quality impact. Values from the four indices defined in Appendix II are converted to a common 0-10 scale as shown in the figure below. To plot survey data, each site is positioned on the x-axis according to river miles from the mouth, and the scaled values for the four indices are plotted on the common scale. The mean scale value of the four indices represents the assessed impact for each site.



Appendix V.  
WATER QUALITY ASSESSMENT CRITERIA

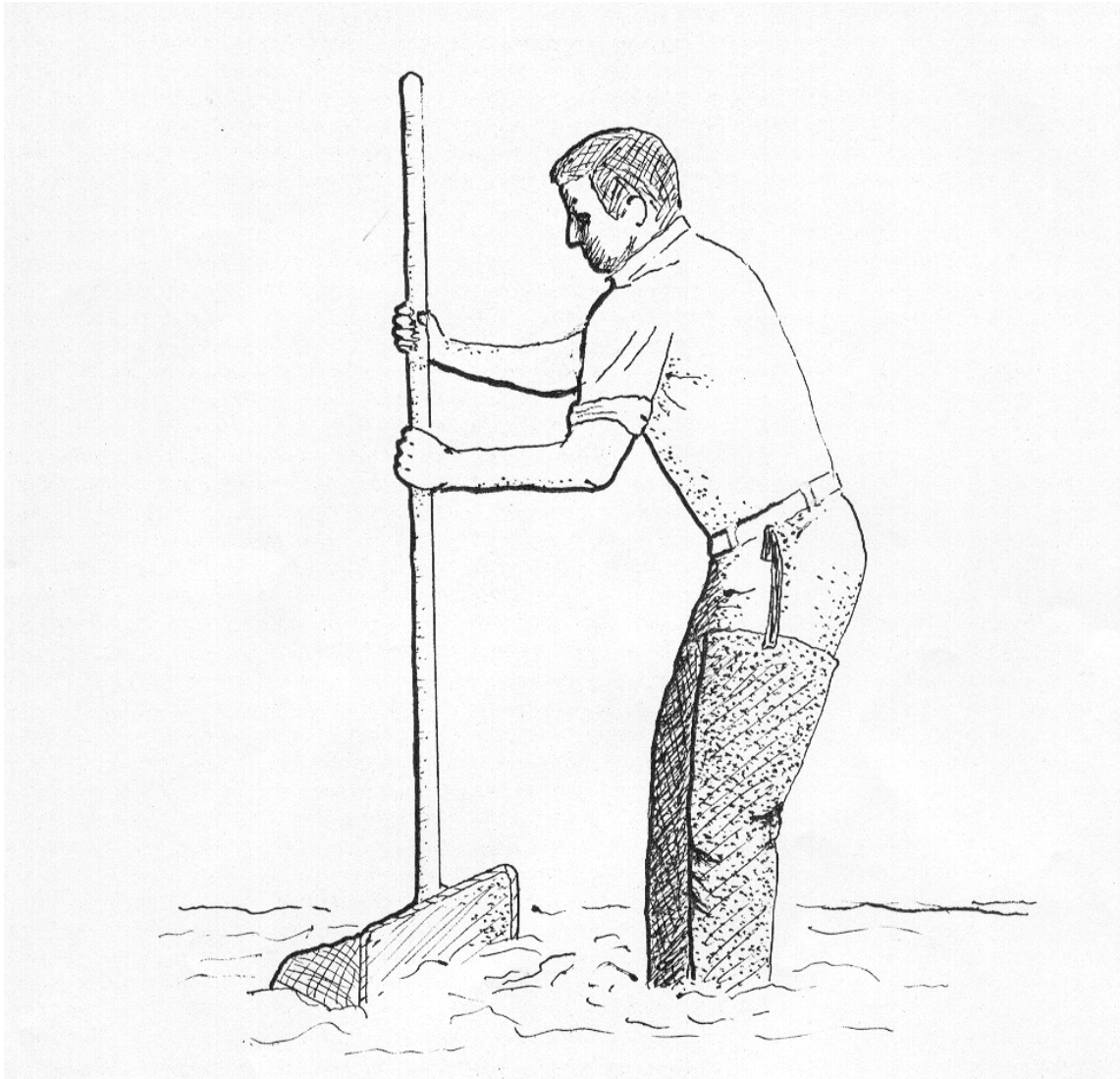
for non-navigable flowing waters

	Species Richness	Hilsenhoff Biotic Index	EPT Value	Percent Model Affinity#	Diversity*
Non-Impacted	>26	0.00-4.50	>10	>64	>4
Slightly Impacted	19-26	4.51-6.50	6-10	50-64	3.01-4.00
Moderately Impacted	11-18	6.51-8.50	2-5	35-49	2.01-3.00
Severely Impacted	0-10	8.51-10.00	0-1	<35	0.00-2.00

# Percent model affinity criteria are used for traveling kick samples but not for multiplate samples.

\* Diversity criteria are used for multiplate samples but not for traveling kick samples.

Appendix VI.  
THE TRAVELING KICK SAMPLE



current

Rocks and sediment in the riffle are dislodged by foot upstream of a net; organisms dislodged are carried by the current into the net. Sampling is continued for five minutes, as the sampler gradually moves downstream to cover a distance of five meters.

Appendix VII. A.  
AQUATIC MACROINVERTEBRATES THAT USUALLY INDICATE GOOD  
WATER QUALITY

**Mayfly** nymphs are often the most numerous organisms found in clean streams. They are sensitive to most types of pollution, including low dissolved oxygen (less than 5 ppm), chlorine, ammonia, metals, pesticides, and acidity. Most mayflies are found clinging to the undersides of rocks.



*MAYFLIES*

**Stonefly** nymphs are mostly limited to cool, well-oxygenated streams. They are sensitive to most of the same pollutants as mayflies, except acidity. They are usually much less numerous than mayflies. The presence of even a few stoneflies in a stream suggests that good water quality has been maintained for several months.



*STONEFLIES*

**Caddisfly** larvae often build a portable case of sand, stones, sticks, or other debris. Many caddisfly larvae are sensitive to pollution, although a few are tolerant. One family spins nets to catch drifting plankton, and is often numerous in nutrient-enriched stream segments.



*CADDISFLIES*

The most common **beetles** in streams are riffle beetles and water pennies. Most of these require a swift current and an adequate supply of oxygen, and are generally considered clean-water indicators.



*BEETLES*



Appendix VII. B.  
AQUATIC MACROINVERTEBRATES THAT USUALLY INDICATE POOR  
WATER QUALITY

**Midges** are the most common aquatic flies. The larvae occur in almost any aquatic situation. Many species are very tolerant to pollution. Large, red midge larvae called “bloodworms” indicate organic enrichment. Other midge larvae filter plankton, indicating nutrient enrichment when numerous.



**MIDGES**

**Black fly larvae** have specialized structures for filtering plankton and bacteria from the water, and require a strong current. Some species are tolerant of organic enrichment and toxic contaminants, while others are intolerant of pollutants.



**BLACK FLIES**



The segmented **worms** include the leeches and the small aquatic earthworms. The latter are more common, though usually unnoticed. They burrow in the substrate and feed on bacteria in the sediment. They can thrive under conditions of severe pollution and very low oxygen levels, and are thus valuable pollution indicators. Many leeches are also tolerant of poor water quality.



**WORMS**



Aquatic **sowbugs** are crustaceans that are often numerous in situations of high organic content and low oxygen levels. They are classic indicators of sewage pollution, and can also thrive in toxic situations. *Digital images by Larry Abele, New York State Department of Environmental Conservation, Stream Biomonitoring Unit.*



**SOWBUGS**

## Appendix VIII. THE RATIONALE OF BIOLOGICAL MONITORING

Biological monitoring as applied here refers to the use of resident benthic macroinvertebrate communities as indicators of water quality. Macroinvertebrates are larger-than-microscopic invertebrate animals that inhabit aquatic habitats; freshwater forms are primarily aquatic insects, worms, clams, snails, and crustaceans.

### Concept

Nearly all streams are inhabited by a community of benthic macroinvertebrates. The species comprising the community each occupy a distinct niche defined and limited by a set of environmental requirements. The composition of the macroinvertebrate community is thus determined by many factors, including habitat, food source, flow regime, temperature, and water quality. The community is presumed to be controlled primarily by water quality if the other factors are determined to be constant or optimal. Community components which can change with water quality include species richness, diversity, balance, abundance, and presence/absence of tolerant or intolerant species. Various indices or metrics are used to measure these community changes. Assessments of water quality are based on metric values of the community, compared to expected metric values.

### Advantages

The primary advantages to using macroinvertebrates as water quality indicators are:

- 1) they are sensitive to environmental impacts
- 2) they are less mobile than fish, and thus cannot avoid discharges
- 3) they can indicate effects of spills, intermittent discharges, and lapses in treatment
- 4) they are indicators of overall, integrated water quality, including synergistic effects and substances lower than detectable limits
- 5) they are abundant in most streams and are relatively easy and inexpensive to sample
- 6) they are able to detect non-chemical impacts to the habitat, e.g. siltation or thermal changes
- 7) they are vital components of the aquatic ecosystem and important as a food source for fish
- 8) they are more readily perceived by the public as tangible indicators of water quality
- 9) they can often provide an on-site estimate of water quality
- 10) they can often be used to identify specific stresses or sources of impairment
- 11) they can be preserved and archived for decades, allowing for direct comparison of specimens
- 12) they bioaccumulate many contaminants, so that analysis of their tissues is a good monitor of toxic substances in the aquatic food chain

### Limitations

Biological monitoring is not intended to replace chemical sampling, toxicity testing, or fish surveys. Each of these measurements provides information not contained in the others. Similarly, assessments based on biological sampling should not be taken as being representative of chemical sampling. Some substances may be present in levels exceeding ambient water quality criteria, yet have no apparent adverse community impact.

## Appendix X. MACROINVERTEBRATE RAW DATA TABLES

Pine Creek Macroinvertebrate Raw Data									
Genus species	Sample #, Replicate #, Subsample #								
	Sa.1, Rep. 1, Sub. 1	Sa.1, Rep. 1, Sub. 2	Sa.1, Rep. 1, Sub. 3	Sa.1, Rep. 2, Sub. 1	Sa.1, Rep. 2, Sub. 2	Sa.1, Rep. 2, Sub. 3	Sa.1, Rep.3, Sub1	Sa.1, Rep.3, Sub.2	Sa.1, Rep.3, Sub.3
Undetermined Enchytraeidae	1					1			
Undef. Tubificidae w/ cap. setae					1	1		1	
Isonychia bicolor	6	11	7	10	11	5	9	14	20
Acentrella sp.				1			2		2
Baetis intercalaris		2	3		3	1	1	1	
Epeorus (Iron) sp.	1	4	3	8	2	5	2		5
Leucrocota sp.	2	3	3	2	5	6	5	4	6
Stenacron interpunctatum	7								
Stenonema sp.	6	6	12	13	7	10	20	20	13
Paraleptophlebia sp.	1			1					
Tricorythodes sp.			1				1		
Undetermined Leuctridae		1				1	1		
Acroneuria abnormis		1	3	4	2			3	5
Neoperla sp.									1
Paragnetina immarginata			1		1		1		
Paragnetina media			2	1	1		1		
Ophiogomphus sp.						1			
Undetermined Gomphidae		2		2	1		2		2
Ectopria nervosa					2	1		2	2
Psephenus herricki	2	1	1	1					
Optioservus fastiditus		11	5	4	3	9	1	4	4
Optioservus sp.	4								
Stenelmis crenata		8	9		5	4	10	11	5
Stenelmis sp.	10			3					
Nigronia serricornis		1				1		1	
Sialis sp.						1			
Chimarra socia	8	5	10	7	2	4	8	5	3
Cheumatopsyche sp.	2	2	2	3	2	2	5	2	
Hydropsyche bronta	12	7	12	9	11	10	6	6	6
Hydropsyche morosa				3	2	4	3	3	2
Hydropsyche sparna	4	7	4	4	6	5	2	4	6
Hydropsyche walkeri	5	4	3	3	9	6	1	1	4
Macrostemum carolina		2	1		2	1		1	
Rhyacophila carolina?				3					
Rhyacophila sp.							2		1
Undetermined Rhyacophilidae	1								
Brachycentrus appalachia					1	1			2
Undetermined Brachycentridae								1	
Psilotreta sp.			1		1	1	2		

Antocha sp.						1			
Hexatoma sp.	5	2	4	5	3	4		5	1
Undetermined Tipulidae				1					
Undetermined Ceratopogonidae			1						
Atherix sp.		3		2	2	6	3	3	2
Parachaetocladus sp.		1			1	2	1		1
Parametrioctonus lundbecki	2	1	1				1		
Rheocricotopus robacki								1	
Tvetenia bavarica gr.	1	1		1			2		
Tvetenia vitracies	3		2	2	2	1	1		2
Microtendipes pedellus gr.		1	1	1					1
Nilthauma sp.					2				
Polypedilum aviceps	2		2	1		1	1	1	1
Polypedilum flavum	1								1
Polypedilum ontario	1								
Micropsectra dives gr.	1			3	9	2			
Neostempellina reissi	10	5	5	2	1	1	5	4	1
Rheotanytarsus exiguus gr.	1	1	1						
Rheotanytarsus pellucidus	1	7				1	1	2	1

**Hoosic River Macroinvertebrate Raw Data**

Genus species	Sample #, Replicate #, Subsample #								
	Sa.1, Rep. 1, Sub. 1	Sa.1, Rep. 1, Sub. 2	Sa.1, Rep. 1, Sub. 3	Sa.1, Rep. 2, Sub. 1	Sa.1, Rep. 2, Sub. 2	Sa.1, Rep. 2, Sub. 3	Sa.1, Rep.3, Sub1	Sa.1, Rep.3, Sub.2	Sa.1, Rep.3, Sub.3
Prostoma graecense				1					
Undetermined Turbellaria	1			1	1				
Undetermined Lumbriculidae	7	1	2	2	1	5	1	4	3
Acentrella sp.	2		5		1	1		3	3
Baetis intercalaris			1						
Stenacron interpunctatum								2	
Ephemerella sp.	2	2	1	5	5	2	2	5	1
Alloperla sp.		1	1						
Paragnetina media	2				2	1	1	1	
Psephenus herricki								1	
Dubiraphia sp.							1		
Optioservus fastiditus					1			1	
Optioservus trivittatus	14	23	26		25	17	20	23	19
Optioservus sp.				16					
Stenelmis crenata					2	3	9		6
Stenelmis sp.	1	4	7	1				2	
Chimarra aterrima?	3	1		2	7	4	4	3	1
Chimarra obscura	6	4	9	3	4	1	2		1
Psychomyia flavida		2	2	7	5	1	1	1	3
Undetermined Polycentropodidae	1								
Cheumatopsyche sp.	18	15	23	11	7	20	11	13	4
Hydropsyche bronta	13	19	6	19	17	17	13	15	20
Hydropsyche morosa	4	2	1	2	3	3	1	5	6
Hydropsyche sparna	4	6		6	4	5	3	1	5
Undetermined Hydroptilidae							1		
Antocha sp.	1	3	4	2	3	2	7	3	6
Atherix sp.	1		1	1		1			
Hemerodromia sp.	3	1	1	1					3
Thienemannimyia gr. spp.				1					1
Cardiocladius obscurus	6	4	3	9	1	3	9	5	2
Cricotopus tremulus gr.		8	4	5	6	7	7	4	12



**Bloody Brook Macroinvertebrate Raw Data**

Genus species	Sample #, Replicate #, Subsample #								
	Sa.1, Rep. 1, Sub. 1	Sa.1, Rep. 1, Sub. 2	Sa.1, Rep. 1, Sub. 3	Sa.1, Rep. 2, Sub. 1	Sa.1, Rep. 2, Sub. 2	Sa.1, Rep. 2, Sub. 3	Sa.1, Rep.3, Sub1	Sa.1, Rep.3, Sub.2	Sa.1, Rep.3, Sub.3
Undetermined Turbellaria	1	2	4	3	3	1	2	5	5
Undetermined Lumbricina									1
Potamothrix moldaviensis							1		
Undet. Tubificidae w/ cap. setae	2			3	3		2	2	1
Undet. Tubificidae w/o cap. setae	4	7	6	4			4		
Limnodrilus hoffmeisteri					3	3		6	11
Nais variabilis							1		
Physella sp.					1			1	
Pisidium sp.					1			1	1
Sphaerium sp.						1		1	4
Caecidotea sp.	3	5	4	2	3	3	4	4	
Gammarus sp.	30	20	27	8	32	34	28	26	
Stenelmis crenata	37	48	39	41	21	30	36	32	30
Cheumatopsyche sp.	1			4	3	1		1	
Hydropsyche betteni	2				4	2			1
Undetermined Hydropsychidae		1							
Hydroptila sp.					1			3	
Antocha sp.	1								
Tipula sp.					1				
Undetermined Tipulidae		1							
Undetermined Ceratopogonidae		1							
Simulium tuberosum				1					
Simulium vittatum									1
Hemerodromia sp.									2
Procladius sublettei			1						
Thienemannimyia gr. spp.				2		2		1	
Cricotopus bicinctus	9	10	4	7	4	3	5	5	7
Cricotopus tremulus gr.	8	4	6	16	15	11	15	9	4
Eukiefferiella claripennis gr.			2	1		1			

Parametricnemus lundbecki						1			
Rheocricotopus robacki			1	2	1	4	1	1	
Thienemanniella xena			3					1	
Thienemanniella sp.	1						1		
Chironomus sp.		1							
Polypedilum flavum	1		2	4	4	3		1	1
Paratanytarsus sp.	1		1	1					
Rheotanytarsus pellucidus	1								
Tanytarsus glabrescens gr.	1			1					

**Onondaga Creek Macroinvertebrate Raw Data**

Genus species	Sample #, Replicate #, Subsample #								
	Sa.1, Rep. 1, Sub. 1	Sa.1, Rep. 1, Sub. 2	Sa.1, Rep. 1, Sub. 3	Sa.1, Rep. 2, Sub. 1	Sa.1, Rep. 2, Sub. 2	Sa.1, Rep. 2, Sub. 3	Sa.1, Rep.3, Sub1	Sa.1, Rep.3, Sub.2	Sa.1, Rep.3, Sub.3
Prostoma graecense	1								
Undetermined Turbellaria			2	1	4	2	1	3	2
Undetermined Lumbriculidae						5			
Undetermined Enchytraeidae		2	2	2	1	1		4	5
Limnodrilus hoffmeisteri				4			7		
Limnodrilus udekemianus				1			1		
Undet. Tubificidae w/o cap. setae	9	5	8		2	7		7	13
Nais elinguis	75	58	54	63	60	54	70	58	55
Ophidonais serpentina		5	1	5		3	2	3	3
Physella sp.						2		1	1
Caecidotea sp.		2	2	1	1			1	2
Stenelmis crenata		1							
Stenelmis sp.									1
Hydroptila sp.		3	1	1	1	1		2	
Undetermined Psychodidae						1			
Pericoma sp.				1					
Cricotopus bicinctus	9	1	19	11	23	21	13	11	13
Cricotopus intersectus gr.				4			2		
Cricotopus tremulus gr.	5	11	7	5	6	3	4	4	4
Cricotopus trifascia gr.					2			1	
Eukiefferiella claripennis gr.		6							
Chironomus sp.	1		1	1				3	
Phaenopsectra dyari?								1	1
Polypedilum flavum		3	3						