

Hoko-Lyre Watershed (WRIA 19) Planning Unit

Benthic Index of Biotic Integrity Sampling Program Field Report

July 2005



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BENTHIC INDEX OF BIOTIC INTEGRITY
SAMPLING PROGRAM FIELD REPORT

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INTRODUCTION

BACKGROUND

Biological indicators are especially useful in a comprehensive water quality program as they reflect the habitat conditions in the aquatic system as well as water quality conditions over a longer term than non-continuous water quality sampling. Several types of organisms can be used in biological monitoring such as fish (Karr 1981, Fausch et al. 1984, Karr et al. 1986), invertebrates (Karr and Chu 1999), and periphyton (Bahls 1993), however, the Benthic Index of Biotic Integrity (BIBI) uses benthic invertebrates (or invertebrates found in stream substrate) and has been successfully and cost-effectively incorporated into many water quality monitoring programs in the Pacific Northwest. The several hundred identifiable invertebrates found in streams of the Pacific Northwest can be used to determine different levels of effect on habitat caused by human activities (Fore et al. 1996).

An understanding of the rationale for using the BIBI as part of a comprehensive water quality monitoring program, requires definitions of the terms “biological integrity” and “index”. The use of the phrase “biological integrity” stems from the Clean Water Act section 101(a) which states “the objective of this Act is to restore and maintain the chemical, physical, and biological integrity of the nation’s waters.” The term “biological integrity” is further defined by Karr and Dudley (1981) and Karr et al. (1986) as “the ability to support and maintain a balanced, integrated adaptive assemblage of organisms having species composition, diversity, and functional organization comparable to that of natural habitat in the region.” In this sense, the biological integrity of benthic organisms found in freshwater streams of WRIA 19 can be compared to reference conditions in the region that are considered to be in a natural state. Systems in a natural state are defined as having the ability to respond and adapt to the level of natural disturbance expected within a given region, as suggested by the term “integrated adaptive assemblage” used above.

The index component of the BIBI description indicates that several types of data are synthesized into a single number that depicts overall biological condition. The BIBI is a multi-metric index in which several metrics of the invertebrate community are calculated, and given a score, and then those scores are combined to give the index value. Metrics such as the number of pollution tolerant taxa, the total number of taxa, and population attributes such as the number of long-lived taxa or predator taxa are used to assess the health of the community. These metrics, once combined into a single index score, indicate the relative health of the system and are correlated to ratings and descriptions. The ratings used for this analysis consist of the following: Healthy, Compromised, Impaired, Highly Impaired, and Critically Impaired. More details on the definitions of these ratings is provided in Discussion Section.

OBJECTIVE

This Benthic Index of Biological Integrity (B-IBI) survey will provide information about biological health within sites sampled in the Hoko, Clallam, Pysht, Sekiu, Lyre, East Twin and West Twin Rivers, and Deep Creek. These waterways are distributed across WRIA 19 and provide a geographically comprehensive sample of the major waterways in the inventory area. For a geo-referenced map of sample sites, see Figures 1 and 2. The data from this survey will serve as a "baseline" or an initial point from which to compare future water quality and habitat monitoring. Sampling for this study was conducted in accordance with the Streamkeepers of Clallam County Protocols (Streamkeepers 2005a). The Streamkeepers staff has been monitoring sites throughout Clallam County using specific protocols for several years. Staff members work with volunteers to monitor the physical, biological, and chemical health of streams, including monitoring of macroinvertebrates using the BIBI. This study adopted the Streamkeepers Protocol (Streamkeepers 2005b) to keep the data consistent with other BIBI data collected

in Clallam County. In accordance with these protocols, sampling took place between September 15, 2004 and October 15, 2004.

Samples were collected in pairs with one set of samples collected from a site higher in the watershed of each waterway and the other set of samples collected lower in the watershed of each waterway. The initial hypothesis of the study was that sites higher in the watershed would be less affected by human activities than sites lower in the watershed. Development has occurred to a great extent along the major highways lower in the watershed, and many of the areas upstream have lower levels of human activity. Additionally, some effects from upstream activities may propagate downstream resulting in higher levels of disturbance in the downstream sites. In this proposed study design, the upstream sites were designed to serve as reference sites for the downstream sample sites. The null hypothesis (H_0) of the study was that there would be no difference between downstream and upstream sample sites, and any pattern of difference would indicate a need for further investigation into the factors affecting the ecological health of the system.

In the actual implementation of the study, some sites were not accessible during the limited sampling period for collecting the BIBI samples. For consistent data collection, all samples are collected in the area between September 15 and October 15 so that data are comparable across years and across sites. Within this narrow timeframe, samples were collected from sites where permission to sample had been granted and where the sampling location was feasible to physically access. These initial sample points provide a context for future sampling efforts. Future efforts at the same sites will establish trends in ecological health through time. Additional sampling sites can be added as appropriate in other areas in each watershed if other reference sites are desired and land access is granted.

SITE SELECTION

As described above the process for selecting sample sites was based on the concept of having one site higher in the watershed and one site lower in the watershed to provide a reference site for the lower sites, and place the scores for each waterway in the context of the watershed. In general sites were collected according to this design, but site selection was constrained by land access permission, as well as physical access to sites in the upper watersheds. On the Hoko and the Pysht River, an additional sample was collected as there were more than two sample sites that could be accessed during the sampling period. Figures 1 and 2 show the specific site locations.

FIELD PROCEDURE

Sample Locations

Once a site was located, the specific sample location was determined using the following criteria from Streamkeepers (2005b) to select the site to place the Surber sampler (Photo 1) within the stream channel at each site. At each site, the Surber sampler was placed nine times in sets of three to produce three replicate samples for each site. Each sample was composed of the organic material and invertebrates collected from three square feet, or three placements of the Surber sampler. Details on specific sampling procedures are in Attachment A, Sample Protocols.

Ideal conditions at each site are as follows (from Streamkeepers 2005b):

- Riffles within the main flow and near the middle of the stream, from 4-16" deep (Photo 2).
- Substrate should be 2-4" rocks, with smaller pebbles underneath (avoid substrates with rocks larger than 12" in diameter).

- Thickest overhead canopy and riparian vegetation within riffle.

Ideally, the riffle should be large enough to accommodate all nine placements of the Surber sampler. (The three placements of the sampler for any given replicate should be close together, but the different replicates should each be at least 6' apart.) If there is no single riffle that is large enough, it may be necessary to sample from adjacent riffles. Depth, flow, and substrate type should be similar for all sampling locations (Streamkeepers 2005b).



Photo 1. Surber Sampler Used to Collect Invertebrates.



Photo 2. Measuring Depth at Sample Site

DATA ANALYSIS AND RESULTS

Once samples were collected, they were packaged and shipped to Aquatic Biology Associates, Corvallis, OR, for professional analysis and identification of invertebrates. Specific steps for identification of invertebrates are described in Appendix B, Quality Assurance / Quality Control Laboratory Guidelines. Invertebrates were identified to the “lowest practical taxonomic level” and ten summary parameters or metrics were calculated. These metrics were then summed to provide the index score for the BIBI, or the BIBI Score. A description of each metric is provided below (Streamkeepers, 2005).

- Total Taxa Richness: The total number of unique taxa identified in each replicate. The numbers from the three replicates are then averaged for this metric.
- Ephemeroptera Taxa Richness: The total number of unique mayfly (Ephemeroptera) taxa identified in each replicate. The numbers from the three replicates are then averaged for this metric.
- Plecoptera Taxa Richness: The total number of unique stonefly (Plecoptera) taxa identified in each replicate. The numbers from the three replicates are then averaged for this metric.
- Trichoptera Taxa Richness: The total number of unique caddisfly (Trichoptera) taxa identified in each replicate. The numbers from the three replicates are then averaged for this metric.
- Number of Long-lived Taxa: The total number of unique long-lived taxa identified in each replicate. The numbers from the three replicates are then averaged for this metric.
- Number of Intolerant Taxa: The total number of unique intolerant taxa identified in each replicate. The numbers from the three replicates are then averaged for this metric.
- Percent Tolerant Individuals: The total number of tolerant individuals counted in each replicate, divided by the total number of individuals in that replicate, multiplied by 100. The numbers from the three replicates are then averaged for this metric.
- Number of Clinger Taxa: The total number of unique clinger taxa identified in each replicate. The numbers from the three replicates are then averaged for this metric.
- Percent Predator Individuals: The total number of predator individuals counted in each replicate, divided by the total number of individuals in that replicate, multiplied by 100. The numbers from the three replicates are then averaged for this metric.
- Percent Dominance: The sum of individuals in the three most abundant taxa in each replicate, divided by the total number of individuals in that replicate, multiplied by 100. The numbers from the three replicates are then averaged for this metric.

The value of each metric is calculated for each replicate, and then the average of the metrics is used to determine the index score. The sum of the index scores for each of the 10 metrics is the BIBI Score, used to determine the health rating for the sample reach. Table 2 identifies the boundaries for the index scores for each metric.

TABLE 2. SCORING CRITERIA FOR BIBI METRICS			
Metrics	Scoring Criteria – Index Scores		
Taxa Richness and Composition	1	3	5
Total Taxa Richness	0-<14	14-28	>28
Ephemeroptera Taxa Richness	0-<3.5	3.5-7	>7
Plecoptera Taxa Richness	0-,2.7	2.7-5.3	>5.3
Trichoptera Taxa Richness	0-<2.7	2.7-5.3	>5.3
Number of Long-lived Taxa	0-<4	4-8	>8
Number of Intolerant Taxa	0-<2	2-4	>4
Percent Tolerant Individuals	>44	27<44	<27
Number of Clinger Taxa	0-<8	8-16	>16
Percent Predator Individuals	0-<4.5	4.5-9	>9
Percent Dominance	>74	55-74	0-<55
Source: http://www.clallam.net/streamkeepers/html/benthic_index.html			

The sum of each index score produces the BIBI Score. The maximum BIBI Score is 50, if each metric were scored a 5 for all ten metrics. A value near 50 indicates that the sampled stream is close to the maximum potential for streams in a natural state in that area. The minimum value for a BIBI Score is 10, which would indicate that the sampled stream’s biological health is in poor condition. Descriptions for score ratings are provided in Table 3.

TABLE 3. “GRADING” SYSTEM FOR BIBI SCORES		
Total BIBI Score	Grade	Definition
50-46	Healthy	Ecologically intact, supporting the most sensitive life forms.
44-36	Compromised	Showing signs of ecological degradation. Impacts expected to one or more salmon life stages.
34-28	Impaired	Healthy ecosystem functions demonstrably impaired. Cannot support self-sustaining salmon populations.
26-18	Highly Impaired	Highly adverse to salmon and various other life forms.
18-10	Critically Impaired	Unable to support a large population of once-native life forms.
Source: http://www.clallam.net/streamkeepers/html/benthic_index.html		

These definitions are more detailed than the original grading system from Karr (1999) that has labels of Excellent, Good, Fair, Poor, and Very Poor without descriptions. Even streams rated as Good under the old system were showing signs of impairment, and this element was not conveyed by the original labels from Karr (1999). Additionally, Poor and Very Poor did not convey the risk to salmon and other native life forms that may be present in streams with these ratings. The descriptions provided by the Clallam County Streamkeepers provide a more integrative interpretation of the BIBI Scores and have been reviewed and accepted by Karr and others (Streamkeepers 2005).

RESULTS

BIBI Scores for each sample site are shown in Table 4 and the corresponding rating for each BIBI Score is also shown.

TABLE 4. SUMMARY OF BIBI SCORES FOR WRIA 19			
Site ID	River	IBI Score	Rating
Lower Hoko	Hoko	30	Impaired
Upper Hoko	Hoko	38	Compromised
Upper Hoko2	Hoko	40	Compromised
Upper Clallam	Clallam	42	Compromised
Lower Clallam	Clallam	36	Compromised
Upper Pysht	Pysht	44	Compromised
Lower Pysht1	Pysht	38	Compromised
Lower Pysht2	Pysht	32	Impaired
Upper Sekiu	Sekiu	40	Compromised
Lower Sekiu	Sekiu	40	Compromised
Upper Lyre	Lyre	32	Impaired
Lower Lyre	Lyre	34	Impaired
Upper Deep Creek	Deep Creek	46	Healthy
Lower Deep Creek	Deep Creek	44	Compromised
Upper East Twin	East Twin	46	Healthy
Lower East Twin	East Twin	44	Compromised
Upper West Twin	West Twin	46	Healthy
Lower West Twin	West Twin	44	Compromised

Additional information from each sample site is available from examining the individual metrics that compose the BIBI Score. The following pages present the actual data from each sample site that were used to generate the BIBI Score.

LOWER HOKO



Downstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	28	31	25	3
Ephemeroptera Taxa Richness	5	5	4	3
Plecoptera Taxa Richness	5	3	4	3
Trichoptera Taxa Richness	4	3	2	3
Number of Long-lived Taxa (Cumulative)	1			1
Number of Intolerant Taxa (Cumulative)	2			3
Percent Tolerant Individuals	66.94	64.92	57.2	1
Number of Clinger Taxa	20	18	16	5
Percent Predator Individuals	10.46	12.42	13.25	5
Percent Dominance	66.94	64.92	57.2	3
BIBI Score				30
1. Score is derived from the average value of the three replicates.				

Average depth at the Lower Hoko sampling site was 7.87 inches. Substrate at the site is shown below.



Substrate at sample site.

UPPER HOKO



Downstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	36	41	34	5
Ephemeroptera Taxa Richness	6	5	6	3
Plecoptera Taxa Richness	7	4	6	3
Trichoptera Taxa Richness	5	9	5	5
Number of Long-lived Taxa (Cumulative)	2			1
Number of Intolerant Taxa (Cumulative)	3			3
Percent Tolerant Individuals	24.92	38.5	48.04	3
Number of Clinger Taxa	27	27	27	5
Percent Predator Individuals	14.16	10.43	7.32	5
Percent Dominance	57.07	58.54	69.7	3
BIBI Score				38
1. Score is derived from the average value of the three replicates.				

Average depth at the Upper Hoko sampling site was 5.91 inches. Substrate at the site is shown below.



Substrate at sample site.

UPPER HOKO 2



Downstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	27	37	36	5
Ephemeroptera Taxa Richness	7	8	9	5
Plecoptera Taxa Richness	4	6	6	3
Trichoptera Taxa Richness	3	8	9	5
Number of Long-lived Taxa (Cumulative)	3			1
Number of Intolerant Taxa (Cumulative)	3			3
Percent Tolerant Individuals	27.78	23.22	11.53	5
Number of Clinger Taxa	20	19	31	5
Percent Predator Individuals	7.90	7.43	12.56	5
Percent Dominance	64.46	68.63	59.32	3
BIBI Score				40
1. Score is derived from the average value of the three replicates.				

Average depth at the Upper Hoko 2 sampling site was 5.77 inches. Substrate at the site is shown below.



Substrate at sample site.

UPPER CLALLAM



Upstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	52	47	54	5
Ephemeroptera Taxa Richness	10	8	9	5
Plecoptera Taxa Richness	7	8	8	5
Trichoptera Taxa Richness	4	5	6	3
Number of Long-lived Taxa (Cumulative)	3			1
Number of Intolerant Taxa (Cumulative)	5			5
Percent Tolerant Individuals	27.46	42.12	32.51	3
Number of Clinger Taxa	29	29	35	5
Percent Predator Individuals	16.15	9.86	11.26	5
Percent Dominance	42.56	52.32	42.00	5
BIBI Score				42
<p>1. Score is derived from the average value of the three replicates.</p>				

Average depth at the Upper Clallam sampling site was 4.24 inches. Substrate at the site is shown below.



Substrate at sample site.

LOWER CLALLAM



Downstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	36	37	42	5
Ephemeroptera Taxa Richness	7	6	7	3
Plecoptera Taxa Richness	5	5	7	5
Trichoptera Taxa Richness	4	0	1	1
Number of Long-lived Taxa (Cumulative)	2			1
Number of Intolerant Taxa (Cumulative)	0			1
Percent Tolerant Individuals	31.30	25.76	18.71	5
Number of Clinger Taxa	23	19	24	5
Percent Predator Individuals	24.49	18.56	8.67	5
Percent Dominance	46.96	35.23	37.09	5
BIBI Score				36
1. Score is derived from the average value of the three replicates.				

Average depth at the Lower Clallam sampling site was 6.56 inches. Substrate at the site is shown below.



Substrate at sample site.

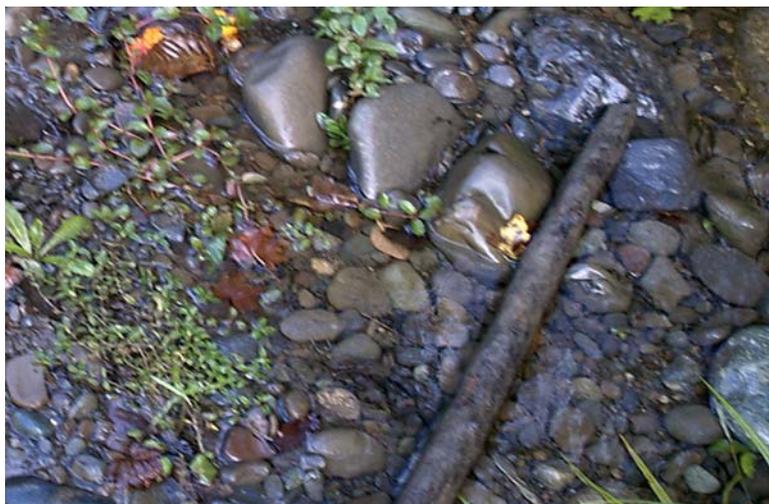
UPPER PYSHT



Upstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	39	37	50	5
Ephemeroptera Taxa Richness	8	8	9	5
Plecoptera Taxa Richness	8	5	11	5
Trichoptera Taxa Richness	3	6	6	3
Number of Long-lived Taxa (Cumulative)	5			3
Number of Intolerant Taxa (Cumulative)	7			5
Percent Tolerant Individuals	24.51	20.60	16.90	5
Number of Clinger Taxa	24	23	32	5
Percent Predator Individuals	9.85	8.72	10.76	5
Percent Dominance	58.86	60.75	50.71	5
BIBI Score				44
1. Score is derived from the average value of the three replicates.				

Average depth at the Upper Pysht sampling site was 9.84 inches. Substrate at the site is shown below.



Substrate at sample site.

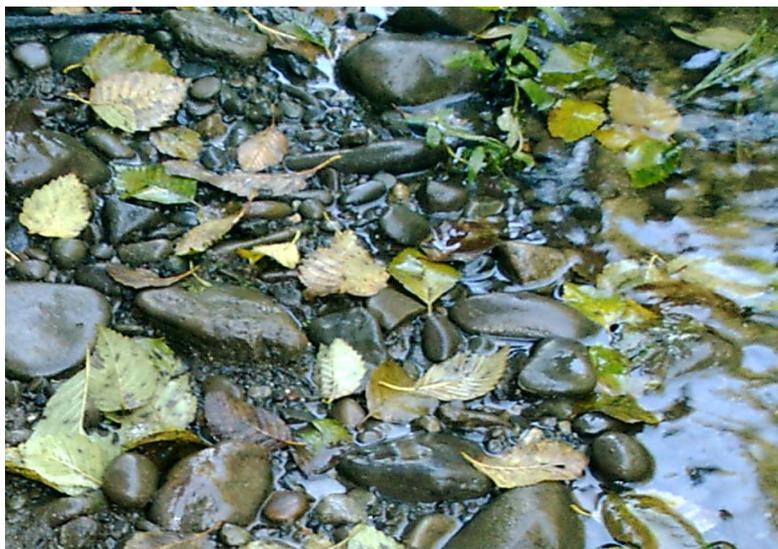
LOWER PYSHT 1



Downstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	39	42	38	5
Ephemeroptera Taxa Richness	10	12	11	5
Plecoptera Taxa Richness	6	7	5	5
Trichoptera Taxa Richness	1	2	0	1
Number of Long-lived Taxa (Cumulative)	2			1
Number of Intolerant Taxa (Cumulative)	4			1
Percent Tolerant Individuals	13.81	13.23	14.11	5
Number of Clinger Taxa	20	26	22	5
Percent Predator Individuals	3.74	5.64	5.29	3
Percent Dominance	39.59	44.26	47.26	5
BIBI Score				38
1. Score is derived from the average value of the three replicates.				

Average depth at the Lower Pysht 1 sampling site was 8.27 inches. Substrate at the site is shown below.



Substrate at sample site.

LOWER PYSHT 2



Upstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	27	24	30	3
Ephemeroptera Taxa Richness	7	5	4	3
Plecoptera Taxa Richness	6	6	4	3
Trichoptera Taxa Richness	0	2	1	1
Number of Long-lived Taxa (Cumulative)	2			1
Number of Intolerant Taxa (Cumulative)	3			3
Percent Tolerant Individuals	33.47	18.72	14.90	5
Number of Clinger Taxa	16	16	15	3
Percent Predator Individuals	15.38	13.29	10.55	5
Percent Dominance	56.56	40.39	37.26	5
BIBI Score				32
1. Score is derived from the average value of the three replicates.				

Average depth at the Lower Pysht 2 sampling site was 8.27 inches. Substrate at the site is shown below.



Substrate at sample site.

UPPER SEKIU



Downstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	39	38	38	5
Ephemeroptera Taxa Richness	7	7	7	3
Plecoptera Taxa Richness	5	7	7	5
Trichoptera Taxa Richness	5	7	8	5
Number of Long-lived Taxa (Cumulative)	3			1
Number of Intolerant Taxa (Cumulative)	4			3
Percent Tolerant Individuals	11.34	10.85	11.43	5
Number of Clinger Taxa	24	26	26	5
Percent Predator Individuals	22.13	27.34	22.34	5
Percent Dominance	60.61	64.18	52.19	3
BIBI Score				40
1. Score is derived from the average value of the three replicates.				

Average depth at the Upper Sekiu sampling site was 6.04 inches. Substrate at the site is shown below.



Substrate at sample site.

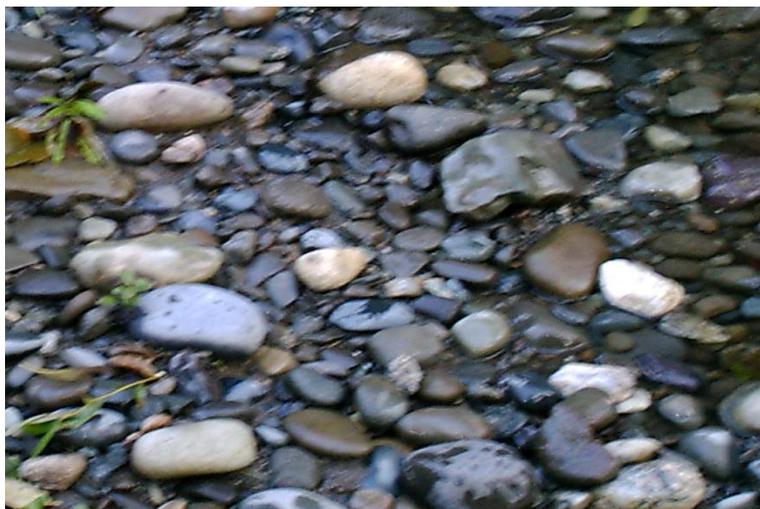
LOWER SEKIU



Upstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	27	32	34	5
Ephemeroptera Taxa Richness	8	6	6	3
Plecoptera Taxa Richness	4	7	7	5
Trichoptera Taxa Richness	1	3	4	3
Number of Long-lived Taxa (Cumulative)	3			1
Number of Intolerant Taxa (Cumulative)	2			3
Percent Tolerant Individuals	33.07	14.98	17.73	5
Number of Clinger Taxa	17	23	23	5
Percent Predator Individuals	9.46	18.51	11.76	5
Percent Dominance	44.89	61.84	55.88	5
BIBI Score				40
1. Score is derived from the average value of the three replicates.				

Average depth at the Lower Sekiu sampling site was 7.83 inches. Substrate at the site is shown below.



Substrate at sample site.

UPPER LYRE



Upstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	26	31	22	3
Ephemeroptera Taxa Richness	5	6	5	3
Plecoptera Taxa Richness	5	4	5	3
Trichoptera Taxa Richness	5	5	4	3
Number of Long-lived Taxa (Cumulative)	4			3
Number of Intolerant Taxa (Cumulative)	2			3
Percent Tolerant Individuals	37.73	43.93	27.78	3
Number of Clinger Taxa	20	23	16	5
Percent Predator Individuals	8.70	4.78	10.95	3
Percent Dominance	65.41	71.12	76.77	3
BIBI Score				32
<p>1. Score is derived from the average value of the three replicates.</p>				

Average depth at the Upper Lyre sampling site was 6.47 inches. Substrate at the site is shown below.



Substrate at sample site.

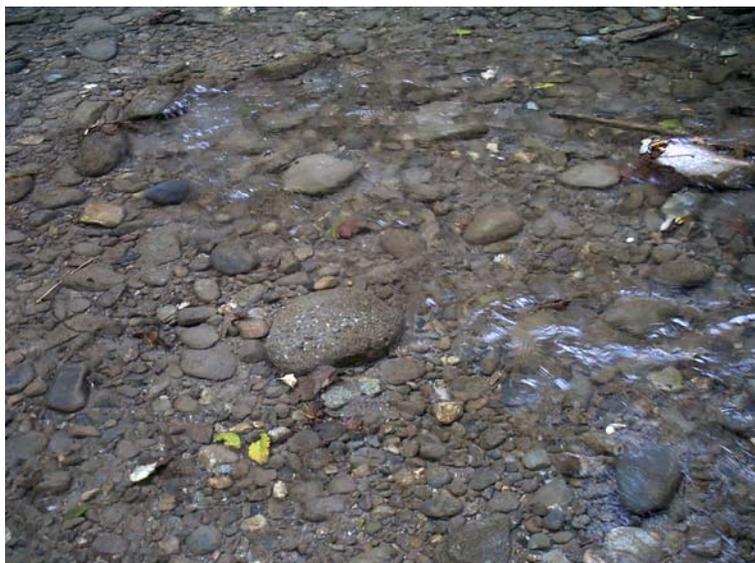
LOWER LYRE



Downstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	43	42	31	5
Ephemeroptera Taxa Richness	4	4	3	3
Plecoptera Taxa Richness	4	5	6	3
Trichoptera Taxa Richness	7	7	4	5
Number of Long-lived Taxa (Cumulative)	4			3
Number of Intolerant Taxa (Cumulative)	0			1
Percent Tolerant Individuals	32.87	26.23	27.96	3
Number of Clinger Taxa	25	24	21	5
Percent Predator Individuals	7.35	6.53	9.77	3
Percent Dominance	60.35	61.09	68.27	3
BIBI Score				34
1. Score is derived from the average value of the three replicates.				

Average depth at the Lower Lyre sampling site was 7.09 inches. Substrate at the site is shown below.



Substrate at sample site.

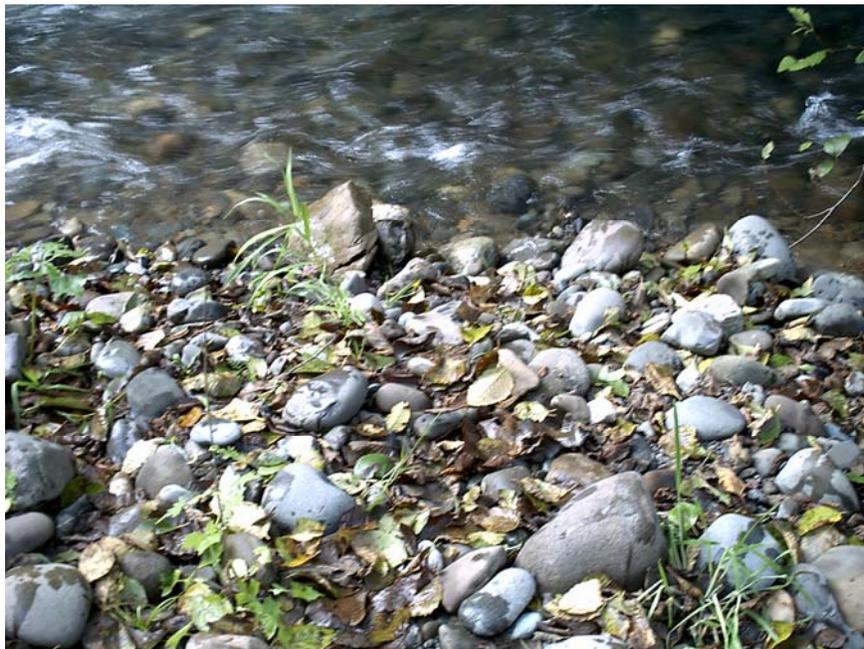
LOWER DEEP CREEK



Upstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	23	43	51	5
Ephemeroptera Taxa Richness	6	10	10	5
Plecoptera Taxa Richness	4	6	7	5
Trichoptera Taxa Richness	2	3	8	3
Number of Long-lived Taxa (Cumulative)	4			3
Number of Intolerant Taxa (Cumulative)	7			5
Percent Tolerant Individuals	4.42	10.56	5.45	5
Number of Clinger Taxa	17	28	34	5
Percent Predator Individuals	11.05	16.90	10.03	5
Percent Dominance	77.29	41.55	67.59	3
BIBI Score				44
1. Score is derived from the average value of the three replicates.				

Average depth at the Lower Deep Creek sampling site was 8.49 inches. Substrate at the site is shown below.



Substrate at sample site.

UPPER DEEP CREEK



Upstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	38	53	36	5
Ephemeroptera Taxa Richness	10	10	9	5
Plecoptera Taxa Richness	7	9	6	5
Trichoptera Taxa Richness	4	7	3	3
Number of Long-lived Taxa (Cumulative)	4			3
Number of Intolerant Taxa (Cumulative)	5			5
Percent Tolerant Individuals	11.07	21.30	22.11	5
Number of Clinger Taxa	26	34	23	5
Percent Predator Individuals	13.93	15.45	15.96	5
Percent Dominance	55.34	44.50	43.20	5
BIBI Score				46
<p>1. Score is derived from the average value of the three replicates.</p>				

Average depth at the Upper Deep Creek sampling site was 5.95 inches. Substrate at the site is shown below.



Substrate at sample site.

UPPER EAST TWIN



Downstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	40	35	40	5
Ephemeroptera Taxa Richness	9	9	9	5
Plecoptera Taxa Richness	7	7	7	5
Trichoptera Taxa Richness	8	8	7	5
Number of Long-lived Taxa (Cumulative)	5			3
Number of Intolerant Taxa (Cumulative)	10			5
Percent Tolerant Individuals	5.98	22.49	16.49	5
Number of Clinger Taxa	30	27	27	5
Percent Predator Individuals	9.75	8.76	10.91	5
Percent Dominance	53.54	70.74	62.84	3
BIBI Score				46
1. Score is derived from the average value of the three replicates.				

Average depth at the Upper East Twin sampling site was 5.47 inches. Substrate at the site is shown below.



Substrate at sample site.

LOWER EAST TWIN



Downstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	27	43	42	5
Ephemeroptera Taxa Richness	6	8	8	3
Plecoptera Taxa Richness	5	6	7	5
Trichoptera Taxa Richness	4	9	6	5
Number of Long-lived Taxa (Cumulative)	3			1
Number of Intolerant Taxa (Cumulative)	6			5
Percent Tolerant Individuals	8.77	8.47	5.33	5
Number of Clinger Taxa	19	30	24	5
Percent Predator Individuals	21.04	14.37	12.90	5
Percent Dominance	57.98	50.98	42.44	5
BIBI Score				44

1. Score is derived from the average value of the three replicates.

Average depth at the Lower East Twin sampling site was 6.26 inches. Substrate at the site is shown below.



Substrate at sample site.

UPPER WEST TWIN



Downstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	49	36	50	5
Ephemeroptera Taxa Richness	9	10	11	5
Plecoptera Taxa Richness	9	7	6	5
Trichoptera Taxa Richness	10	7	8	5
Number of Long-lived Taxa (Cumulative)	6			3
Number of Intolerant Taxa (Cumulative)	4			3
Percent Tolerant Individuals	20.68	26.99	25.85	5
Number of Clinger Taxa	33	29	32	5
Percent Predator Individuals	18.15	12.78	24.02	5
Percent Dominance	47.80	57.96	43.99	5
BIBI Score				46
1. Score is derived from the average value of the three replicates.				

Average depth at the Upper West Twin sampling site was 8.22 inches. Substrate at the site is shown below.



Substrate at sample site.

LOWER WEST TWIN



Upstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	36	40	45	5
Ephemeroptera Taxa Richness	7	8	9	5
Plecoptera Taxa Richness	7	5	6	5
Trichoptera Taxa Richness	3	6	3	3
Number of Long-lived Taxa (Cumulative)	2			1
Number of Intolerant Taxa (Cumulative)	6			5
Percent Tolerant Individuals	29.77	27.68	15.28	5
Number of Clinger Taxa	23	27	25	5
Percent Predator Individuals	17.99	13.76	13.17	5
Percent Dominance	60.33	55.74	45.18	5
BIBI Score				44
1. Score is derived from the average value of the three replicates.				

Average depth at the Lower West Twin sampling site was 7.66 inches. Substrate at the site is shown below.



Substrate at sample site.

DISCUSSION

The BIBI Score has been described as “one of the most direct ways to address the Clean Water Act’s biological standards for aquatic life” (Karr and Chu 1999). Background information on the BIBI Scores indicates that the existence of living organism in itself integrates the environmental conditions within a system, suggesting that BIBI Scores provide a holistic health rating for aquatic systems. Multi-metric indices, such as BIBI, build on the efforts of earlier monitoring work by applying empirical knowledge of how biological attributes respond to human influence. Metrics are selected because they have predictable responses to changes in landscape condition, such as physical, chemical, and biological factors that stress biological systems (Karr and Chu 1999). Metrics are also selected because they are easy to measure and interpret.

Specific metrics can be used to further refine the information provided by the BIBI Score. For example, Karr and Chu (1999) identify that changes in the total number of taxa are shown to track changes in ecosystem processes such as rates of leaf litter processing and storage of organic matter. They also found that percent predators within a sample reflected the complexity of the invertebrate trophic structure, and the stability of the invertebrate community. The number of Ephemeroptera taxa in a sample are generally reduced when toxic chemicals such as mine wastes are present (Kiffney and Clements 1994). The number of Plecoptera taxa in a sample disappear as riparian vegetation is lost and sediment clogs the interstitial spaces among cobbles. The number of Plecoptera taxa tends to decline at less intense levels of human influence than the number of Trichoptera or Ephemeroptera taxa. Using these concepts, each metric value can be translated into words to describe how high scoring sites differ from medium or low scoring sites (Karr and Chu 1999). Simple graphs and basic statistical analysis are often one of the best ways of looking at and interpreting data from a variety of sample sites, once multiple years of data have been collected, or if sites are able to be segregated into disturbed and undisturbed (Fore et al. 1996).

The examination of individual metrics can give us clues to the activities that may be affecting the health of an aquatic system, but typically, multiple human activities influence watershed simultaneously. Collecting data for biological monitoring is not a goal in itself, but should be conducted to answer specific questions relevant to environmental management (Fore et al. 1996). Biological monitoring is a means of documenting divergence from expected baseline conditions, and allows scientists to associate those divergences with knowledge of human activities. The goal of this survey is to track conditions through time and find out where conditions have moved away from biological integrity. BIBI Scores can provide a measuring device to rank sites that would be the best for restoration. Scores from sites across the WRIA 19 region provide a context for interpreting each score and identifying trends. Additionally, at a smaller scale, individual metrics are available to make site specific assessments and help focus on potential sources of degradation. The eventual goal of monitoring and restoration efforts is to determine why conditions have moved away from biological integrity and to design a restoration plan to address those reasons.

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ATTACHMENT A. SAMPLE PROTOCOLS

EQUIPMENT AND MATERIALS

- 1 complete Surber sampler
- 2 buckets, marked “clean” and “dirty”
- 2 500-micron sieves, also “clean” and “dirty”
- 2 rubber dishpans
- weeding fork to disturb substrate
- *timepiece with second hand
- decanter with handle
- 2 angled-spout wash bottles (one for water, one for alcohol)
- 2 squirt bottles (one water, one alcohol)
- .. plastic spatula
- .. forceps (tweezers)
- .. magnifying glasses
- .. spoons
- .. eye droppers
- .. paintbrushes
- sample jars with screwtop lids
- alcohol
- electrical tape
- shallow white trays
- Field Key to Macroinvertebrate Identification
- pre-printed labels
- ziplock bags—small and large
- 3 washers with flagging tape attached
- permanent marker
- *reach map for each reach
- *100’ tape
- camera with photo log
- tarp
- *data sheet, clipboard, pencil

COLLECTING SAMPLES

Three replicates will be collected at each of the two sites per stream.

Sampling will begin downstream and move upstream to avoid disturbing terrestrial vegetation overhead or upstream of the sampling site, and to avoid getting terrestrial insects in the sample.

- 1.) Frame out the Surber sampler, and place it on the selected spot with the opening of the nylon net facing upstream and the collection cup stretched out behind. Hold the frame firmly on the stream bottom, allowing the current to move directly into the net.
- 2.) Lift the larger rocks resting within or beneath the frame and, holding them in the water in front of the net, brush off any crawling or loosely attached organisms so that they drift into the net. After “cleaning” the rocks, place them in a dishpan. Once these rocks have been removed, the frame should be squarely on the stream bottom. At this point, note the water depth in inches, using the marked notches in the Surber’s frame.
- 3.) Once the larger rocks are removed, disturb the substrate vigorously with the weeding fork for 60 seconds, to a depth of about 4 inches. Organisms and detritus should wash into the net.
- 4.) Lift the sampler out of the water: keeping the open end pointing upstream, tilt it up out of the water, to help wash organisms into the collection cup.
- 5.) Without emptying the cup, repeat the sampling procedure twice more at nearby spots. These three sampling efforts, combined into the collection cup, constitute a single replicate. Three replicates will be collected at each of the two sites per stream.
- 6.) Mark the area of this replicate’ sampling with one of the flagged washers. Among the three “digs,” mark the spot:
 - Furthest upstream, and
 - Laterally at the middle of the 3 digs
- 7.) Put a small amount of isopropyl alcohol in the sample collection jar and begin examining the large rocks collected in the dishpan, using a magnifying glass.
- 8.) Using a brush or forceps, gently move any organisms found into the sample jar.
- 9.) After examining each rock, wash it over the pan with filtered water, and set it on the bank. When all rocks have been cleaned, pour the water from the dishpan through the clean sieve. Rinse the pan, agitate and pour again, filtering out any invertebrates that washed off of the rocks. Return the rocks to the stream in the area of the sampling site.
- 10.) Meanwhile, other samplers should attend to the Surber sampler. Wash all objects caught on the inside of the net into the collection cup:
 - i) With the opening out of the water, rotate the net around in the water so that most of the objects inside wash into the cup.
 - ii) On the bank, finish rinsing the contents of the net into the cup. Use the decanter or bucket to pour unfiltered water into the net from the outside, or pour filtered water down the sides of the net from the inside.
 - iii.) Examine the net to make sure no insects are left in it. When the net is clean, empty the contents of the collection cup into the 2nd dishpan.

-
- iv.) Clean the neck and collar of the sampler over the dishpan to collect any insects that may remain inside.
- v.) Rinse the cup and empty again, continuing until you have emptied it completely. (To rinse, pour clean water inside the cup; or dip the cup into the stream, holding it upright, and let the stream water filter in through the mesh on the side of the cup.)
- 11.) Pick out large debris (sticks and leaves) from the material in the sieve. Using a magnifying glass and squirt bottle or tools, pick off any organisms and return them to the sieve or sample jar before discarding these pieces.
- 12.) Pour some clean water into the dishpan and swirl the sample around in it. While the water is still agitated, pour it off into the clean sieve. Most of the organic matter should enter the sieve with the water, while the rocks stay at the bottom. Repeat this decanting procedure until the water is completely clear and there are no invertebrates still crawling around in the debris in the dishpan.
- 13.) Pick through the contents of the dishpan with a magnifying glass before discarding. If the insects will not separate from the sand, decant and then put the sand in jars too.
- [Alternate means of separating insects from sand: Decant the water out of the dishpan, and then put in enough alcohol to cover the sand. Swirl and see if insects start releasing from the sand particles. If so, decant them into the sieve, catching the alcohol in another dishpan. If there are insects in that waste alcohol too, save that as well, marking it "Through sieve from sand-float."]
- 14.) Transfer the remaining contents of the clean sieve into the sample jar. To best get most of the contents of the sieve down at one end, dip the sieve at an angle in clean water in one of the dishpans. Use gentle forceps, a spatula, and/or a squirt bottle to move the remaining contents of the clean sieve into the sample jar.
- 15.) Fill the jar no more than halfway with contents from the sieve then fill to near the top with alcohol. Complete a label with the date, stream, reach number, replicate number, first initials and last names of samplers.
- 16.) Place inside the jar, ideally so that the writing can be seen from the outside. Close the jar tightly and wrap the seal 2-3 times with electrical tape. On the lid write date, stream, reach number, and replicate number as follows:
- | | |
|------------------|---------------------|
| SAMPLE JAR LID: | SAMPLE ZIPLOCK BAG: |
| 9/15/2000 | 9/15/2000 |
| Peabody 2, Rep 1 | Peabody 2, Rep 1 |
| Jar 1 of 2 | 2 Jars |
- (If the material will not fit in one jar, put it into two or more jars, and add "Jar 1 of 2," etc. to the slips of paper inside the jars and the jar lids.) Place the jar(s) from a single replicate in a single small ziplock bag, labeled with the same information as the lid. See Appendix A, Quality Control Plan, for more detail on the treatment of samples.
- 17.) Collect two more replicates, following the same procedure as above.
- 18.) Measure and record the following information about the area in which you collected each replicate:
- The average water depth at the spots where you dug that replicate (with the rocks removed), to the nearest number of inches.
 - The width of the riffle in the area where you dug, to the nearest number of feet.

- The length of the riffle in the area where you dug, to the nearest number of feet.
- 19.) Photograph the sampling sites in the following manner:
- a) If all three replicates were taken from the same riffle or riffle sequence, one set of photos will suffice.
 - b) Replicates that were taken far apart or from areas that look very different should have separate sets of photos.
 - c) A set of photos consists of the following:
 - A photograph of the riffle area itself, ideally showing some of the substrate; if the gravel is visible, try to hold a familiar object near it to help gauge its size.
 - Photographs of the riparian corridor taken upstream and down stream from the sampling area.
 - If possible, take a photo of the team actually doing the sampling.
 - d Complete the photo log for each photo.
- 20.) Clean and store the equipment. Make sure the net and sieves are clean.

ATTACHMENT B. QUALITY ASSURANCE/QUALITY CONTROL LABORATORY GUIDELINES

(From Aquatic Biology Associates, Corvallis Oregon, <http://www.aquaticbio.com/>)

The following quality assurance/quality control (QA/QC) procedures are routinely followed at Aquatic Biology Associates, Inc. in processing benthic macroinvertebrate samples. Procedures will be altered to fit the needs of the client for specific projects. Alterations in QA/QC procedure may add to the per sample cost.

1. Samples are unpacked upon receipt and preservative levels checked. Labels are checked to make sure they are intelligible and that the experimental design is understandable (e.g. sites & replicates). Non-smear labels are made that go on the inside of sample jars. The client is called if samples have been damaged in shipping and/or if the labeling system is not understandable.
2. The entire sample is floated in water in a white plastic tray. Large debris is rinsed and removed. The sample is then elutriated until all organic matter and invertebrates are floated off the mineral residue. Sieves of a pore size specified by the client are used in this process (500 micron is the most common). The mineral residue remaining in the white pan after elutriation is searched for stone-cased caddisflies and molluscs that have not floated off.
3. Unless otherwise specified by the client, a portion of the sample will be sorted that contains 500-600 organisms. The Caton Tray is normally used to randomly obtain a fraction of the total sample containing 500-600 organisms. Sample data is converted to a full sample basis. Other methodologies may be used to split some sample types, such as lake benthic samples. If densities are low, Surber and Hess samples are usually processed in their entirety. If a sample is subsampled, our normal procedure is to archive the unused sample portion until the project is completed. Unused sample fractions will be returned to the client if requested (shipping charges will be billed to the client). If requested, Aquatic Biology Associates, Inc. will archive unused sample fractions for 1 year at no charge.
4. Experienced technicians are used to remove all invertebrates from the sample residue using dissecting scopes at 6X or 12X power. For small projects, a single technician is assigned. For larger projects, several technicians are given the responsibility for sorting. All invertebrates removed from a sample are placed in a single sorting vial and given directly to Robert W. Wisseman, Senior Scientist of Aquatic Biology Associates, Inc. Logs are kept by each technician to record label data, fraction sorted, hours required to complete sorting, and any comments on sample matrix or problems. Our sorting efficacy is well above EPA requirements, as has been determined by an independent lab. Detailed sorting procedures followed by Aquatic Biology Associates, Inc. can be sent upon request.
5. The entire sample residue is saved after sorting to check for sorting efficacy. Sorting efficacy of 95% or better is required on all samples. A 20% aliquot of each residue is thoroughly re-sorted to determine efficacy. The entire residue is re-sorted if 95% or better sorting efficacy has not been achieved, as estimated from the 20% aliquot re-sort.

All sample residues can be returned to the client for independent checks. The client will be charged for shipping and sample containers.

6. Invertebrate identifications are performed by Robert W. Wisseman and associates. For standard level identifications, Robert W. Wisseman performs the initial identifications and counts on all samples, and then determines which specialists will be required to assure accurate identifications to levels specified for a project. He has over 15 years of experience in the identification of freshwater invertebrates. Aquatic Biology Associates, Inc. uses specialists from throughout North America for performing more detailed taxonomy, or to verify questionable identifications.
7. The choices for archiving invertebrate material for QA/QC checks by other experts are as follows:
 - You can trust Aquatic Biology Associates, Inc. to do a competent job, and let us pull out material that we think is significant...e.g. for verification by specialists, to be incorporated into museum collections, or to save for educational purposes. This is our preferred method of operating.
 - Save a reference/synoptic series of specimens of each taxa identified. There will be nominal charge for this service. All invertebrate material can be saved by each individual sample for archiving or QA/QC checks by another lab. An additional charge per sample will be added for this service, since it greatly slows sample processing.
 - The client can request that specific taxonomic groups be archived by individual sample for possible future taxonomic analysis (e.g. all the oligochaete worms). There is usually no charge if one or a few groups are involved.
 - Aquatic Biology Associates, Inc. requests permission to remove material from samples that may be of interest to specialists or that we feel would be a valuable addition to museum collections.
8. Identifications and counts are recorded on bench-sheets and then transferred to electronic files. Standardized bench-sheets reduce data entry errors. Robert W. Wisseman and Mary Jo Wevers (Aquatic Biology Associates, Inc. senior scientists) perform all data entry and analysis.

The following sample preservation methods are recommended.

- Use 95 or 99% alcohol to preserve most field samples. Organic residues will be holding a lot of water. If too dilute of a alcohol/water mixture is used, it will not effectively preserve the sample.
- If the sample residue is mostly coarse mineral material, then dilute the alcohol to about 80% with stream water. Coarse, woody organic material will "consume" less alcohol; but fine, leafy material requires a lot of alcohol.
- Use copious amounts of alcohol; at least twice the volume of the sample residue.
- For best results, let the field-applied alcohol sit in the sample jars for a few hours to a day, then decant off most of the original alcohol, add fresh 80% alcohol, and stir/shake gently.
- Be reasonably gentle with the samples, so that invertebrates don't break into pieces. When a large amount of fine organic matter or silt is present (e.g. lentic benthos samples), then make sure the alcohol gets well mixed into the residue. For this sample

type, you may want to consider spiking the alcohol with formalin (about 4 cc formalin per liter jar). **If you do use formalin, please write "Formalin" on the outside label of the sample jar.** Please avoid formalin if at all possible. Re-preserving the field collected sample with fresh alcohol (as described above) will be adequate in most cases.

- Do not allow samples to sit around for long without preserving (especially in direct sunlight). Invertebrates will die and deteriorate very rapidly. Preserve samples shortly after collection. Never leave unpreserved samples out in the hot sun. Also, try to keep preserved samples from sitting in the hot sun for too long.

TREATMENT OF SAMPLES

Shipping of Benthic Invertebrate Samples

Coolers are the best containers for shipping. These can be rented from Aquatic Biology Associates, Inc. Your coolers will be returned to you. For shipping by UPS, coolers that exceed a combined girth + length of 130 inches will be charged as an oversize package. Cooler weight should not exceed 70 pounds. UPS may charge \$2 extra for coolers with handles on them, since they can't place them on conveyor belts.

Make sure sample jar lids are screwed on tightly! Vibration during transport can quickly loosen lids. Lids of Nalgene® jars supplied by Aquatic Biology Associates, Inc. will not vibrate loose. See the supplies section for leak-proof sample jar suppliers. When in doubt whether jar lids will vibrate loose, secure them with electrical tape. Please use electrical tape, since it can be easily stripped from the jars.

UPS and Federal Express are preferred carriers. They treat packages much more gently than the U.S. Postal Service, are faster, usually cheaper, and will deliver samples to the door of our lab.

List on any manifest, that you are sending river sediment samples for scientific analysis. If you are shipping samples preserved only with formalin, you will have to check with UPS on packaging requirements. Formalin is classified as a hazardous substance. Small amounts of formalin added to the alcohol, to insure fixing of invertebrates, do not warrant calling attention to. If you use any formalin, then you must use leak-proof jars & ship in sealed coolers. Please line coolers with plastic garbage bags. Place sample jars in the bag and seal with a twist ties. This lining adds an extra layer of protection in case some preservative leaks. Make sure the cooler drain-cock is closed and taped shut. If you enclose documents in the coolers, please seal them in large zip-lock bags. Secure cooler lids with reinforced strapping tape. When shipping by UPS or Federal Express, please do not request that the carrier obtain a signature from our lab.

Labeling of Benthic Invertebrate Samples

Place an interior label into each sample jar! Information recorded on the interior label has priority over the exterior label. Include whatever information is needed to positively identify the sample and tie it back to field notes or sample collection forms.

Use Rite-in-the-Rain paper and a soft lead pencil. Include at least this information on the interior label:

Client/Project:	This can be abbreviated, e.g. CLNP for Crater Lake National Park
Waterbody:	e.g. Sun Creek
Site:	e.g. Site 1, 5800'
Replicate:	if applicable
Sample type:	e.g. Erosional Sample
Date:	I prefer month, day, year e.g. 4-28-93. Write out or abbreviate the month if you think there will be any confusion.
Collector initials:	e.g. RWW

If a sample is so large that it must be divided between two or more sample jars, then please use this convention on the label:

e.g. when divided between 3 jars:

Site 1 Replicate 1 (1 of 3); S1 R1 (2 of 3); S1 R1 (3 of 3).

Exterior labels are not to be trusted to remain legible. They are used only for basic project inventory purposes in the field and lab. Use a ring of "label tape" around the outside of the sample jar to record abbreviated project/site/rep./date information. Label tape is available from scientific supply houses (see Supplies & Equipment). This tape stays on the jars well, but peels off cleanly, so jars can be recycled. Use permanent ink or "Sharpie" to record sample information on the exterior label.

Do not write directly on sample jars supplied by Aquatic Biology Associates, Inc. and use only label tape on the outside.

**ATTACHMENT C.
GPS COORDINATES FOR EACH SAMPLE SITE**

TABLE C-1. GPS COORDINATES FOR SAMPLE SITES		
Site Name	Lat (N) (°, ', ")	Long(W) (°, ', ")
Lower Hoko	48,15,30.5	124,21,7.7
Upper Hoko	48,12,13.7	124,25,37.8
Upper Hoko2	48,8,16.3	124,23,6.3
Upper Clallam	48,13,4.3	124,15,11.2
Lower Clallam	48,14,52.7	124,15,8.3
Upper Pysht	48,10,7.5	124,12,39.9
Lower Pysht1	48,11,12.6	124,10,39.2
Lower Pysht2	48,11,23.0	124,9,4.1
Upper Sekiu	48,16,31.9	124,30,5.9
Lower Sekiu	48,17,0.7	124,25,46.5
Upper Lyre	48,6,0.6	123,49,3.4
Lower Lyre	48,9,0.9	123,50,8.6
Lower Deep Creek	48,9,48.5	124,1,54.4
Upper Deep Creek	48,9,29.5	124,2,10.2
Upper East Twin	48,8,31.9	123,56,9.3
Lower East Twin	48,9,2.7	123,56,10.5
Upper West Twin	48,9,11.5	123,57,0.2
Lower West Twin	48,9,43.4	123,57,13.2

Hoko-Lyre Watershed (WRIA 19) Planning Unit

Benthic Index of Biotic Integrity Sampling Program Field Report

July 2005



TETRA TECH/KCM

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BENTHIC INDEX OF BIOTIC INTEGRITY
SAMPLING PROGRAM FIELD REPORT

JULY 2005

Prepared for:

WRIA 19 Planning Unit

Lead Agency:

Clallam County

Department of Community Development

Prepared by:



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Project #3440023

**BENTHIC INDEX OF BIOTIC INTEGRITY
SAMPLING PROGRAM FIELD REPORT
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INTRODUCTION

BACKGROUND

Biological indicators are especially useful in a comprehensive water quality program as they reflect the habitat conditions in the aquatic system as well as water quality conditions over a longer term than non-continuous water quality sampling. Several types of organisms can be used in biological monitoring such as fish (Karr 1981, Fausch et al. 1984, Karr et al. 1986), invertebrates (Karr and Chu 1999), and periphyton (Bahls 1993), however, the Benthic Index of Biotic Integrity (BIBI) uses benthic invertebrates (or invertebrates found in stream substrate) and has been successfully and cost-effectively incorporated into many water quality monitoring programs in the Pacific Northwest. The several hundred identifiable invertebrates found in streams of the Pacific Northwest can be used to determine different levels of effect on habitat caused by human activities (Fore et al. 1996).

An understanding of the rationale for using the BIBI as part of a comprehensive water quality monitoring program, requires definitions of the terms “biological integrity” and “index”. The use of the phrase “biological integrity” stems from the Clean Water Act section 101(a) which states “the objective of this Act is to restore and maintain the chemical, physical, and biological integrity of the nation’s waters.” The term “biological integrity” is further defined by Karr and Dudley (1981) and Karr et al. (1986) as “the ability to support and maintain a balanced, integrated adaptive assemblage of organisms having species composition, diversity, and functional organization comparable to that of natural habitat in the region.” In this sense, the biological integrity of benthic organisms found in freshwater streams of WRIA 19 can be compared to reference conditions in the region that are considered to be in a natural state. Systems in a natural state are defined as having the ability to respond and adapt to the level of natural disturbance expected within a given region, as suggested by the term “integrated adaptive assemblage” used above.

The index component of the BIBI description indicates that several types of data are synthesized into a single number that depicts overall biological condition. The BIBI is a multi-metric index in which several metrics of the invertebrate community are calculated, and given a score, and then those scores are combined to give the index value. Metrics such as the number of pollution tolerant taxa, the total number of taxa, and population attributes such as the number of long-lived taxa or predator taxa are used to assess the health of the community. These metrics, once combined into a single index score, indicate the relative health of the system and are correlated to ratings and descriptions. The ratings used for this analysis consist of the following: Healthy, Compromised, Impaired, Highly Impaired, and Critically Impaired. More details on the definitions of these ratings is provided in Discussion Section.

OBJECTIVE

This Benthic Index of Biological Integrity (B-IBI) survey will provide information about biological health within sites sampled in the Hoko, Clallam, Pysht, Sekiu, Lyre, East Twin and West Twin Rivers, and Deep Creek. These waterways are distributed across WRIA 19 and provide a geographically comprehensive sample of the major waterways in the inventory area. For a geo-referenced map of sample sites, see Figures 1 and 2. The data from this survey will serve as a "baseline" or an initial point from which to compare future water quality and habitat monitoring. Sampling for this study was conducted in accordance with the Streamkeepers of Clallam County Protocols (Streamkeepers 2005a). The Streamkeepers staff has been monitoring sites throughout Clallam County using specific protocols for several years. Staff members work with volunteers to monitor the physical, biological, and chemical health of streams, including monitoring of macroinvertebrates using the BIBI. This study adopted the Streamkeepers Protocol (Streamkeepers 2005b) to keep the data consistent with other BIBI data collected

in Clallam County. In accordance with these protocols, sampling took place between September 15, 2004 and October 15, 2004.

Samples were collected in pairs with one set of samples collected from a site higher in the watershed of each waterway and the other set of samples collected lower in the watershed of each waterway. The initial hypothesis of the study was that sites higher in the watershed would be less affected by human activities than sites lower in the watershed. Development has occurred to a great extent along the major highways lower in the watershed, and many of the areas upstream have lower levels of human activity. Additionally, some effects from upstream activities may propagate downstream resulting in higher levels of disturbance in the downstream sites. In this proposed study design, the upstream sites were designed to serve as reference sites for the downstream sample sites. The null hypothesis (H_0) of the study was that there would be no difference between downstream and upstream sample sites, and any pattern of difference would indicate a need for further investigation into the factors affecting the ecological health of the system.

In the actual implementation of the study, some sites were not accessible during the limited sampling period for collecting the BIBI samples. For consistent data collection, all samples are collected in the area between September 15 and October 15 so that data are comparable across years and across sites. Within this narrow timeframe, samples were collected from sites where permission to sample had been granted and where the sampling location was feasible to physically access. These initial sample points provide a context for future sampling efforts. Future efforts at the same sites will establish trends in ecological health through time. Additional sampling sites can be added as appropriate in other areas in each watershed if other reference sites are desired and land access is granted.

SITE SELECTION

As described above the process for selecting sample sites was based on the concept of having one site higher in the watershed and one site lower in the watershed to provide a reference site for the lower sites, and place the scores for each waterway in the context of the watershed. In general sites were collected according to this design, but site selection was constrained by land access permission, as well as physical access to sites in the upper watersheds. On the Hoko and the Pysht River, an additional sample was collected as there were more than two sample sites that could be accessed during the sampling period. Figures 1 and 2 show the specific site locations.

FIELD PROCEDURE

Sample Locations

Once a site was located, the specific sample location was determined using the following criteria from Streamkeepers (2005b) to select the site to place the Surber sampler (Photo 1) within the stream channel at each site. At each site, the Surber sampler was placed nine times in sets of three to produce three replicate samples for each site. Each sample was composed of the organic material and invertebrates collected from three square feet, or three placements of the Surber sampler. Details on specific sampling procedures are in Attachment A, Sample Protocols.

Ideal conditions at each site are as follows (from Streamkeepers 2005b):

- Riffles within the main flow and near the middle of the stream, from 4-16" deep (Photo 2).
- Substrate should be 2-4" rocks, with smaller pebbles underneath (avoid substrates with rocks larger than 12" in diameter).

- Thickest overhead canopy and riparian vegetation within riffle.

Ideally, the riffle should be large enough to accommodate all nine placements of the Surber sampler. (The three placements of the sampler for any given replicate should be close together, but the different replicates should each be at least 6' apart.) If there is no single riffle that is large enough, it may be necessary to sample from adjacent riffles. Depth, flow, and substrate type should be similar for all sampling locations (Streamkeepers 2005b).



Photo 1. Surber Sampler Used to Collect Invertebrates.



Photo 2. Measuring Depth at Sample Site

DATA ANALYSIS AND RESULTS

Once samples were collected, they were packaged and shipped to Aquatic Biology Associates, Corvallis, OR, for professional analysis and identification of invertebrates. Specific steps for identification of invertebrates are described in Appendix B, Quality Assurance / Quality Control Laboratory Guidelines. Invertebrates were identified to the “lowest practical taxonomic level” and ten summary parameters or metrics were calculated. These metrics were then summed to provide the index score for the BIBI, or the BIBI Score. A description of each metric is provided below (Streamkeepers, 2005).

- Total Taxa Richness: The total number of unique taxa identified in each replicate. The numbers from the three replicates are then averaged for this metric.
- Ephemeroptera Taxa Richness: The total number of unique mayfly (Ephemeroptera) taxa identified in each replicate. The numbers from the three replicates are then averaged for this metric.
- Plecoptera Taxa Richness: The total number of unique stonefly (Plecoptera) taxa identified in each replicate. The numbers from the three replicates are then averaged for this metric.
- Trichoptera Taxa Richness: The total number of unique caddisfly (Trichoptera) taxa identified in each replicate. The numbers from the three replicates are then averaged for this metric.
- Number of Long-lived Taxa: The total number of unique long-lived taxa identified in each replicate. The numbers from the three replicates are then averaged for this metric.
- Number of Intolerant Taxa: The total number of unique intolerant taxa identified in each replicate. The numbers from the three replicates are then averaged for this metric.
- Percent Tolerant Individuals: The total number of tolerant individuals counted in each replicate, divided by the total number of individuals in that replicate, multiplied by 100. The numbers from the three replicates are then averaged for this metric.
- Number of Clinger Taxa: The total number of unique clinger taxa identified in each replicate. The numbers from the three replicates are then averaged for this metric.
- Percent Predator Individuals: The total number of predator individuals counted in each replicate, divided by the total number of individuals in that replicate, multiplied by 100. The numbers from the three replicates are then averaged for this metric.
- Percent Dominance: The sum of individuals in the three most abundant taxa in each replicate, divided by the total number of individuals in that replicate, multiplied by 100. The numbers from the three replicates are then averaged for this metric.

The value of each metric is calculated for each replicate, and then the average of the metrics is used to determine the index score. The sum of the index scores for each of the 10 metrics is the BIBI Score, used to determine the health rating for the sample reach. Table 2 identifies the boundaries for the index scores for each metric.

TABLE 2. SCORING CRITERIA FOR BIBI METRICS			
Metrics	Scoring Criteria – Index Scores		
Taxa Richness and Composition	1	3	5
Total Taxa Richness	0-<14	14-28	>28
Ephemeroptera Taxa Richness	0-<3.5	3.5-7	>7
Plecoptera Taxa Richness	0-,2.7	2.7-5.3	>5.3
Trichoptera Taxa Richness	0-<2.7	2.7-5.3	>5.3
Number of Long-lived Taxa	0-<4	4-8	>8
Number of Intolerant Taxa	0-<2	2-4	>4
Percent Tolerant Individuals	>44	27<44	<27
Number of Clinger Taxa	0-<8	8-16	>16
Percent Predator Individuals	0-<4.5	4.5-9	>9
Percent Dominance	>74	55-74	0-<55
Source: http://www.clallam.net/streamkeepers/html/benthic_index.html			

The sum of each index score produces the BIBI Score. The maximum BIBI Score is 50, if each metric were scored a 5 for all ten metrics. A value near 50 indicates that the sampled stream is close to the maximum potential for streams in a natural state in that area. The minimum value for a BIBI Score is 10, which would indicate that the sampled stream’s biological health is in poor condition. Descriptions for score ratings are provided in Table 3.

TABLE 3. “GRADING” SYSTEM FOR BIBI SCORES		
Total BIBI Score	Grade	Definition
50-46	Healthy	Ecologically intact, supporting the most sensitive life forms.
44-36	Compromised	Showing signs of ecological degradation. Impacts expected to one or more salmon life stages.
34-28	Impaired	Healthy ecosystem functions demonstrably impaired. Cannot support self-sustaining salmon populations.
26-18	Highly Impaired	Highly adverse to salmon and various other life forms.
18-10	Critically Impaired	Unable to support a large population of once-native life forms.
Source: http://www.clallam.net/streamkeepers/html/benthic_index.html		

These definitions are more detailed than the original grading system from Karr (1999) that has labels of Excellent, Good, Fair, Poor, and Very Poor without descriptions. Even streams rated as Good under the old system were showing signs of impairment, and this element was not conveyed by the original labels from Karr (1999). Additionally, Poor and Very Poor did not convey the risk to salmon and other native life forms that may be present in streams with these ratings. The descriptions provided by the Clallam County Streamkeepers provide a more integrative interpretation of the BIBI Scores and have been reviewed and accepted by Karr and others (Streamkeepers 2005).

RESULTS

BIBI Scores for each sample site are shown in Table 4 and the corresponding rating for each BIBI Score is also shown.

TABLE 4. SUMMARY OF BIBI SCORES FOR WRIA 19			
Site ID	River	IBI Score	Rating
Lower Hoko	Hoko	30	Impaired
Upper Hoko	Hoko	38	Compromised
Upper Hoko2	Hoko	40	Compromised
Upper Clallam	Clallam	42	Compromised
Lower Clallam	Clallam	36	Compromised
Upper Pysht	Pysht	44	Compromised
Lower Pysht1	Pysht	38	Compromised
Lower Pysht2	Pysht	32	Impaired
Upper Sekiu	Sekiu	40	Compromised
Lower Sekiu	Sekiu	40	Compromised
Upper Lyre	Lyre	32	Impaired
Lower Lyre	Lyre	34	Impaired
Upper Deep Creek	Deep Creek	46	Healthy
Lower Deep Creek	Deep Creek	44	Compromised
Upper East Twin	East Twin	46	Healthy
Lower East Twin	East Twin	44	Compromised
Upper West Twin	West Twin	46	Healthy
Lower West Twin	West Twin	44	Compromised

Additional information from each sample site is available from examining the individual metrics that compose the BIBI Score. The following pages present the actual data from each sample site that were used to generate the BIBI Score.

LOWER HOKO



Downstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	28	31	25	3
Ephemeroptera Taxa Richness	5	5	4	3
Plecoptera Taxa Richness	5	3	4	3
Trichoptera Taxa Richness	4	3	2	3
Number of Long-lived Taxa (Cumulative)	1			1
Number of Intolerant Taxa (Cumulative)	2			3
Percent Tolerant Individuals	66.94	64.92	57.2	1
Number of Clinger Taxa	20	18	16	5
Percent Predator Individuals	10.46	12.42	13.25	5
Percent Dominance	66.94	64.92	57.2	3
BIBI Score				30

1. Score is derived from the average value of the three replicates.

Average depth at the Lower Hoko sampling site was 7.87 inches. Substrate at the site is shown below.



Substrate at sample site.

UPPER HOKO



Downstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	36	41	34	5
Ephemeroptera Taxa Richness	6	5	6	3
Plecoptera Taxa Richness	7	4	6	3
Trichoptera Taxa Richness	5	9	5	5
Number of Long-lived Taxa (Cumulative)	2			1
Number of Intolerant Taxa (Cumulative)	3			3
Percent Tolerant Individuals	24.92	38.5	48.04	3
Number of Clinger Taxa	27	27	27	5
Percent Predator Individuals	14.16	10.43	7.32	5
Percent Dominance	57.07	58.54	69.7	3
BIBI Score				38

1. Score is derived from the average value of the three replicates.

Average depth at the Upper Hoko sampling site was 5.91 inches. Substrate at the site is shown below.



Substrate at sample site.

UPPER HOKO 2



Downstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	27	37	36	5
Ephemeroptera Taxa Richness	7	8	9	5
Plecoptera Taxa Richness	4	6	6	3
Trichoptera Taxa Richness	3	8	9	5
Number of Long-lived Taxa (Cumulative)	3			1
Number of Intolerant Taxa (Cumulative)	3			3
Percent Tolerant Individuals	27.78	23.22	11.53	5
Number of Clinger Taxa	20	19	31	5
Percent Predator Individuals	7.90	7.43	12.56	5
Percent Dominance	64.46	68.63	59.32	3
BIBI Score				40
<p>1. Score is derived from the average value of the three replicates.</p>				

Average depth at the Upper Hoko 2 sampling site was 5.77 inches. Substrate at the site is shown below.



Substrate at sample site.

UPPER CLALLAM



Upstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	52	47	54	5
Ephemeroptera Taxa Richness	10	8	9	5
Plecoptera Taxa Richness	7	8	8	5
Trichoptera Taxa Richness	4	5	6	3
Number of Long-lived Taxa (Cumulative)	3			1
Number of Intolerant Taxa (Cumulative)	5			5
Percent Tolerant Individuals	27.46	42.12	32.51	3
Number of Clinger Taxa	29	29	35	5
Percent Predator Individuals	16.15	9.86	11.26	5
Percent Dominance	42.56	52.32	42.00	5
BIBI Score				42
<p>1. Score is derived from the average value of the three replicates.</p>				

Average depth at the Upper Clallam sampling site was 4.24 inches. Substrate at the site is shown below.



Substrate at sample site.

LOWER CLALLAM



Downstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	36	37	42	5
Ephemeroptera Taxa Richness	7	6	7	3
Plecoptera Taxa Richness	5	5	7	5
Trichoptera Taxa Richness	4	0	1	1
Number of Long-lived Taxa (Cumulative)	2			1
Number of Intolerant Taxa (Cumulative)	0			1
Percent Tolerant Individuals	31.30	25.76	18.71	5
Number of Clinger Taxa	23	19	24	5
Percent Predator Individuals	24.49	18.56	8.67	5
Percent Dominance	46.96	35.23	37.09	5
BIBI Score				36

1. Score is derived from the average value of the three replicates.

Average depth at the Lower Clallam sampling site was 6.56 inches. Substrate at the site is shown below.



Substrate at sample site.

UPPER PYSHT



Upstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	39	37	50	5
Ephemeroptera Taxa Richness	8	8	9	5
Plecoptera Taxa Richness	8	5	11	5
Trichoptera Taxa Richness	3	6	6	3
Number of Long-lived Taxa (Cumulative)	5			3
Number of Intolerant Taxa (Cumulative)	7			5
Percent Tolerant Individuals	24.51	20.60	16.90	5
Number of Clinger Taxa	24	23	32	5
Percent Predator Individuals	9.85	8.72	10.76	5
Percent Dominance	58.86	60.75	50.71	5
BIBI Score				44
<p>1. Score is derived from the average value of the three replicates.</p>				

Average depth at the Upper Pysht sampling site was 9.84 inches. Substrate at the site is shown below.



Substrate at sample site.

LOWER PYSHT 1



Downstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	39	42	38	5
Ephemeroptera Taxa Richness	10	12	11	5
Plecoptera Taxa Richness	6	7	5	5
Trichoptera Taxa Richness	1	2	0	1
Number of Long-lived Taxa (Cumulative)	2			1
Number of Intolerant Taxa (Cumulative)	4			1
Percent Tolerant Individuals	13.81	13.23	14.11	5
Number of Clinger Taxa	20	26	22	5
Percent Predator Individuals	3.74	5.64	5.29	3
Percent Dominance	39.59	44.26	47.26	5
BIBI Score				38
<p>1. Score is derived from the average value of the three replicates.</p>				

Average depth at the Lower Pysht 1 sampling site was 8.27 inches. Substrate at the site is shown below.



Substrate at sample site.

LOWER PYSHT 2



Upstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	27	24	30	3
Ephemeroptera Taxa Richness	7	5	4	3
Plecoptera Taxa Richness	6	6	4	3
Trichoptera Taxa Richness	0	2	1	1
Number of Long-lived Taxa (Cumulative)	2			1
Number of Intolerant Taxa (Cumulative)	3			3
Percent Tolerant Individuals	33.47	18.72	14.90	5
Number of Clinger Taxa	16	16	15	3
Percent Predator Individuals	15.38	13.29	10.55	5
Percent Dominance	56.56	40.39	37.26	5
BIBI Score				32
<p>1. Score is derived from the average value of the three replicates.</p>				

Average depth at the Lower Pysht 2 sampling site was 8.27 inches. Substrate at the site is shown below.



Substrate at sample site.

UPPER SEKIU



Downstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	39	38	38	5
Ephemeroptera Taxa Richness	7	7	7	3
Plecoptera Taxa Richness	5	7	7	5
Trichoptera Taxa Richness	5	7	8	5
Number of Long-lived Taxa (Cumulative)	3			1
Number of Intolerant Taxa (Cumulative)	4			3
Percent Tolerant Individuals	11.34	10.85	11.43	5
Number of Clinger Taxa	24	26	26	5
Percent Predator Individuals	22.13	27.34	22.34	5
Percent Dominance	60.61	64.18	52.19	3
BIBI Score				40

1. Score is derived from the average value of the three replicates.

Average depth at the Upper Sekiu sampling site was 6.04 inches. Substrate at the site is shown below.



Substrate at sample site.

LOWER SEKIU



Upstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	27	32	34	5
Ephemeroptera Taxa Richness	8	6	6	3
Plecoptera Taxa Richness	4	7	7	5
Trichoptera Taxa Richness	1	3	4	3
Number of Long-lived Taxa (Cumulative)	3			1
Number of Intolerant Taxa (Cumulative)	2			3
Percent Tolerant Individuals	33.07	14.98	17.73	5
Number of Clinger Taxa	17	23	23	5
Percent Predator Individuals	9.46	18.51	11.76	5
Percent Dominance	44.89	61.84	55.88	5
BIBI Score				40
1. Score is derived from the average value of the three replicates.				

Average depth at the Lower Sekiu sampling site was 7.83 inches. Substrate at the site is shown below.



Substrate at sample site.

UPPER LYRE



Upstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	26	31	22	3
Ephemeroptera Taxa Richness	5	6	5	3
Plecoptera Taxa Richness	5	4	5	3
Trichoptera Taxa Richness	5	5	4	3
Number of Long-lived Taxa (Cumulative)	4			3
Number of Intolerant Taxa (Cumulative)	2			3
Percent Tolerant Individuals	37.73	43.93	27.78	3
Number of Clinger Taxa	20	23	16	5
Percent Predator Individuals	8.70	4.78	10.95	3
Percent Dominance	65.41	71.12	76.77	3
BIBI Score				32

1. Score is derived from the average value of the three replicates.

Average depth at the Upper Lyre sampling site was 6.47 inches. Substrate at the site is shown below.



Substrate at sample site.

LOWER LYRE



Downstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	43	42	31	5
Ephemeroptera Taxa Richness	4	4	3	3
Plecoptera Taxa Richness	4	5	6	3
Trichoptera Taxa Richness	7	7	4	5
Number of Long-lived Taxa (Cumulative)	4			3
Number of Intolerant Taxa (Cumulative)	0			1
Percent Tolerant Individuals	32.87	26.23	27.96	3
Number of Clinger Taxa	25	24	21	5
Percent Predator Individuals	7.35	6.53	9.77	3
Percent Dominance	60.35	61.09	68.27	3
BIBI Score				34

1. Score is derived from the average value of the three replicates.

Average depth at the Lower Lyre sampling site was 7.09 inches. Substrate at the site is shown below.



Substrate at sample site.

LOWER DEEP CREEK



Upstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	23	43	51	5
Ephemeroptera Taxa Richness	6	10	10	5
Plecoptera Taxa Richness	4	6	7	5
Trichoptera Taxa Richness	2	3	8	3
Number of Long-lived Taxa (Cumulative)	4			3
Number of Intolerant Taxa (Cumulative)	7			5
Percent Tolerant Individuals	4.42	10.56	5.45	5
Number of Clinger Taxa	17	28	34	5
Percent Predator Individuals	11.05	16.90	10.03	5
Percent Dominance	77.29	41.55	67.59	3
BIBI Score				44

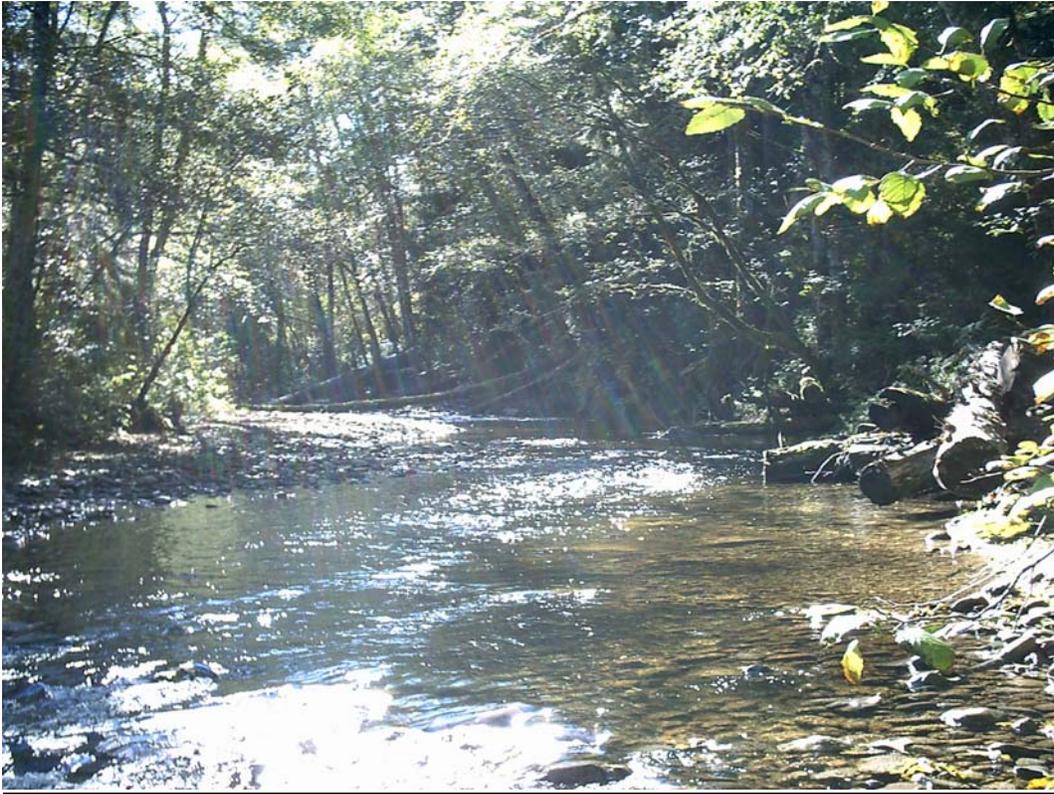
1. Score is derived from the average value of the three replicates.

Average depth at the Lower Deep Creek sampling site was 8.49 inches. Substrate at the site is shown below.



Substrate at sample site.

UPPER DEEP CREEK



Upstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	38	53	36	5
Ephemeroptera Taxa Richness	10	10	9	5
Plecoptera Taxa Richness	7	9	6	5
Trichoptera Taxa Richness	4	7	3	3
Number of Long-lived Taxa (Cumulative)	4			3
Number of Intolerant Taxa (Cumulative)	5			5
Percent Tolerant Individuals	11.07	21.30	22.11	5
Number of Clinger Taxa	26	34	23	5
Percent Predator Individuals	13.93	15.45	15.96	5
Percent Dominance	55.34	44.50	43.20	5
BIBI Score				46
<p>1. Score is derived from the average value of the three replicates.</p>				

Average depth at the Upper Deep Creek sampling site was 5.95 inches. Substrate at the site is shown below.



Substrate at sample site.

UPPER EAST TWIN



Downstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	40	35	40	5
Ephemeroptera Taxa Richness	9	9	9	5
Plecoptera Taxa Richness	7	7	7	5
Trichoptera Taxa Richness	8	8	7	5
Number of Long-lived Taxa (Cumulative)	5			3
Number of Intolerant Taxa (Cumulative)	10			5
Percent Tolerant Individuals	5.98	22.49	16.49	5
Number of Clinger Taxa	30	27	27	5
Percent Predator Individuals	9.75	8.76	10.91	5
Percent Dominance	53.54	70.74	62.84	3
BIBI Score				46
1. Score is derived from the average value of the three replicates.				

Average depth at the Upper East Twin sampling site was 5.47 inches. Substrate at the site is shown below.



Substrate at sample site.

LOWER EAST TWIN



Downstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	27	43	42	5
Ephemeroptera Taxa Richness	6	8	8	3
Plecoptera Taxa Richness	5	6	7	5
Trichoptera Taxa Richness	4	9	6	5
Number of Long-lived Taxa (Cumulative)	3			1
Number of Intolerant Taxa (Cumulative)	6			5
Percent Tolerant Individuals	8.77	8.47	5.33	5
Number of Clinger Taxa	19	30	24	5
Percent Predator Individuals	21.04	14.37	12.90	5
Percent Dominance	57.98	50.98	42.44	5
BIBI Score				44

1. Score is derived from the average value of the three replicates.

Average depth at the Lower East Twin sampling site was 6.26 inches. Substrate at the site is shown below.



Substrate at sample site.

UPPER WEST TWIN



Downstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	49	36	50	5
Ephemeroptera Taxa Richness	9	10	11	5
Plecoptera Taxa Richness	9	7	6	5
Trichoptera Taxa Richness	10	7	8	5
Number of Long-lived Taxa (Cumulative)	6			3
Number of Intolerant Taxa (Cumulative)	4			3
Percent Tolerant Individuals	20.68	26.99	25.85	5
Number of Clinger Taxa	33	29	32	5
Percent Predator Individuals	18.15	12.78	24.02	5
Percent Dominance	47.80	57.96	43.99	5
BIBI Score				46
<p>1. Score is derived from the average value of the three replicates.</p>				

Average depth at the Upper West Twin sampling site was 8.22 inches. Substrate at the site is shown below.



Substrate at sample site.

LOWER WEST TWIN



Upstream view from sample site.

Metric	Metric Values			Index Score ¹
	Replicate 1	Replicate 2	Replicate 3	
Total Taxa Richness	36	40	45	5
Ephemeroptera Taxa Richness	7	8	9	5
Plecoptera Taxa Richness	7	5	6	5
Trichoptera Taxa Richness	3	6	3	3
Number of Long-lived Taxa (Cumulative)	2			1
Number of Intolerant Taxa (Cumulative)	6			5
Percent Tolerant Individuals	29.77	27.68	15.28	5
Number of Clinger Taxa	23	27	25	5
Percent Predator Individuals	17.99	13.76	13.17	5
Percent Dominance	60.33	55.74	45.18	5
BIBI Score				44
<p>1. Score is derived from the average value of the three replicates.</p>				

Average depth at the Lower West Twin sampling site was 7.66 inches. Substrate at the site is shown below.



Substrate at sample site.

DISCUSSION

The BIBI Score has been described as “one of the most direct ways to address the Clean Water Act’s biological standards for aquatic life” (Karr and Chu 1999). Background information on the BIBI Scores indicates that the existence of living organism in itself integrates the environmental conditions within a system, suggesting that BIBI Scores provide a holistic health rating for aquatic systems. Multi-metric indices, such as BIBI, build on the efforts of earlier monitoring work by applying empirical knowledge of how biological attributes respond to human influence. Metrics are selected because they have predictable responses to changes in landscape condition, such as physical, chemical, and biological factors that stress biological systems (Karr and Chu 1999). Metrics are also selected because they are easy to measure and interpret.

Specific metrics can be used to further refine the information provided by the BIBI Score. For example, Karr and Chu (1999) identify that changes in the total number of taxa are shown to track changes in ecosystem processes such as rates of leaf litter processing and storage of organic matter. They also found that percent predators within a sample reflected the complexity of the invertebrate trophic structure, and the stability of the invertebrate community. The number of Ephemeroptera taxa in a sample are generally reduced when toxic chemicals such as mine wastes are present (Kiffney and Clements 1994). The number of Plecoptera taxa in a sample disappear as riparian vegetation is lost and sediment clogs the interstitial spaces among cobbles. The number of Plecoptera taxa tends to decline at less intense levels of human influence than the number of Trichoptera or Ephemeroptera taxa. Using these concepts, each metric value can be translated into words to describe how high scoring sites differ from medium or low scoring sites (Karr and Chu 1999). Simple graphs and basic statistical analysis are often one of the best ways of looking at and interpreting data from a variety of sample sites, once multiple years of data have been collected, or if sites are able to be segregated into disturbed and undisturbed (Fore et al. 1996).

The examination of individual metrics can give us clues to the activities that may be affecting the health of an aquatic system, but typically, multiple human activities influence watershed simultaneously. Collecting data for biological monitoring is not a goal in itself, but should be conducted to answer specific questions relevant to environmental management (Fore et al. 1996). Biological monitoring is a means of documenting divergence from expected baseline conditions, and allows scientists to associate those divergences with knowledge of human activities. The goal of this survey is to track conditions through time and find out where conditions have moved away from biological integrity. BIBI Scores can provide a measuring device to rank sites that would be the best for restoration. Scores from sites across the WRIA 19 region provide a context for interpreting each score and identifying trends. Additionally, at a smaller scale, individual metrics are available to make site specific assessments and help focus on potential sources of degradation. The eventual goal of monitoring and restoration efforts is to determine why conditions have moved away from biological integrity and to design a restoration plan to address those reasons.

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ATTACHMENT A. SAMPLE PROTOCOLS

EQUIPMENT AND MATERIALS

- 1 complete Surber sampler
- 2 buckets, marked “clean” and “dirty”
- 2 500-micron sieves, also “clean” and “dirty”
- 2 rubber dishpans
- weeding fork to disturb substrate
- *timepiece with second hand
- decanter with handle
- 2 angled-spout wash bottles (one for water, one for alcohol)
- 2 squirt bottles (one water, one alcohol)
- .. plastic spatula
- .. forceps (tweezers)
- .. magnifying glasses
- .. spoons
- .. eye droppers
- .. paintbrushes
- sample jars with screwtop lids
- alcohol
- electrical tape
- shallow white trays
- Field Key to Macroinvertebrate Identification
- pre-printed labels
- ziplock bags—small and large
- 3 washers with flagging tape attached
- permanent marker
- *reach map for each reach
- *100’ tape
- camera with photo log
- tarp
- *data sheet, clipboard, pencil

COLLECTING SAMPLES

Three replicates will be collected at each of the two sites per stream.

Sampling will begin downstream and move upstream to avoid disturbing terrestrial vegetation overhead or upstream of the sampling site, and to avoid getting terrestrial insects in the sample.

- 1.) Frame out the Surber sampler, and place it on the selected spot with the opening of the nylon net facing upstream and the collection cup stretched out behind. Hold the frame firmly on the stream bottom, allowing the current to move directly into the net.
- 2.) Lift the larger rocks resting within or beneath the frame and, holding them in the water in front of the net, brush off any crawling or loosely attached organisms so that they drift into the net. After “cleaning” the rocks, place them in a dishpan. Once these rocks have been removed, the frame should be squarely on the stream bottom. At this point, note the water depth in inches, using the marked notches in the Surber’s frame.
- 3.) Once the larger rocks are removed, disturb the substrate vigorously with the weeding fork for 60 seconds, to a depth of about 4 inches. Organisms and detritus should wash into the net.
- 4.) Lift the sampler out of the water: keeping the open end pointing upstream, tilt it up out of the water, to help wash organisms into the collection cup.
- 5.) Without emptying the cup, repeat the sampling procedure twice more at nearby spots. These three sampling efforts, combined into the collection cup, constitute a single replicate. Three replicates will be collected at each of the two sites per stream.
- 6.) Mark the area of this replicate’ sampling with one of the flagged washers. Among the three “digs,” mark the spot:
 - Furthest upstream, and
 - Laterally at the middle of the 3 digs
- 7.) Put a small amount of isopropyl alcohol in the sample collection jar and begin examining the large rocks collected in the dishpan, using a magnifying glass.
- 8.) Using a brush or forceps, gently move any organisms found into the sample jar.
- 9.) After examining each rock, wash it over the pan with filtered water, and set it on the bank. When all rocks have been cleaned, pour the water from the dishpan through the clean sieve. Rinse the pan, agitate and pour again, filtering out any invertebrates that washed off of the rocks. Return the rocks to the stream in the area of the sampling site.
- 10.) Meanwhile, other samplers should attend to the Surber sampler. Wash all objects caught on the inside of the net into the collection cup:
 - i) With the opening out of the water, rotate the net around in the water so that most of the objects inside wash into the cup.
 - ii) On the bank, finish rinsing the contents of the net into the cup. Use the decanter or bucket to pour unfiltered water into the net from the outside, or pour filtered water down the sides of the net from the inside.
 - iii.) Examine the net to make sure no insects are left in it. When the net is clean, empty the contents of the collection cup into the 2nd dishpan.

-
- iv.) Clean the neck and collar of the sampler over the dishpan to collect any insects that may remain inside.
 - v.) Rinse the cup and empty again, continuing until you have emptied it completely. (To rinse, pour clean water inside the cup; or dip the cup into the stream, holding it upright, and let the stream water filter in through the mesh on the side of the cup.)
- 11.) Pick out large debris (sticks and leaves) from the material in the sieve. Using a magnifying glass and squirt bottle or tools, pick off any organisms and return them to the sieve or sample jar before discarding these pieces.
 - 12.) Pour some clean water into the dishpan and swirl the sample around in it. While the water is still agitated, pour it off into the clean sieve. Most of the organic matter should enter the sieve with the water, while the rocks stay at the bottom. Repeat this decanting procedure until the water is completely clear and there are no invertebrates still crawling around in the debris in the dishpan.
 - 13.) Pick through the contents of the dishpan with a magnifying glass before discarding. If the insects will not separate from the sand, decant and then put the sand in jars too.

[Alternate means of separating insects from sand: Decant the water out of the dishpan, and then put in enough alcohol to cover the sand. Swirl and see if insects start releasing from the sand particles. If so, decant them into the sieve, catching the alcohol in another dishpan. If there are insects in that waste alcohol too, save that as well, marking it "Through sieve from sand-float."]
 - 14.) Transfer the remaining contents of the clean sieve into the sample jar. To best get most of the contents of the sieve down at one end, dip the sieve at an angle in clean water in one of the dishpans. Use gentle forceps, a spatula, and/or a squirt bottle to move the remaining contents of the clean sieve into the sample jar.
 - 15.) Fill the jar no more than halfway with contents from the sieve then fill to near the top with alcohol. Complete a label with the date, stream, reach number, replicate number, first initials and last names of samplers.
 - 16.) Place inside the jar, ideally so that the writing can be seen from the outside. Close the jar tightly and wrap the seal 2-3 times with electrical tape. On the lid write date, stream, reach number, and replicate number as follows:

SAMPLE JAR LID:	SAMPLE ZIPLOCK BAG:
9/15/2000	9/15/2000
Peabody 2, Rep 1	Peabody 2, Rep 1
Jar 1 of 2	2 Jars

(If the material will not fit in one jar, put it into two or more jars, and add "Jar 1 of 2," etc. to the slips of paper inside the jars and the jar lids.) Place the jar(s) from a single replicate in a single small ziplock bag, labeled with the same information as the lid. See Appendix A, Quality Control Plan, for more detail on the treatment of samples.
 - 17.) Collect two more replicates, following the same procedure as above.
 - 18.) Measure and record the following information about the area in which you collected each replicate:
 - The average water depth at the spots where you dug that replicate (with the rocks removed), to the nearest number of inches.
 - The width of the riffle in the area where you dug, to the nearest number of feet.
-

- The length of the riffle in the area where you dug, to the nearest number of feet.
- 19.) Photograph the sampling sites in the following manner:
- a) If all three replicates were taken from the same riffle or riffle sequence, one set of photos will suffice.
 - b) Replicates that were taken far apart or from areas that look very different should have separate sets of photos.
 - c) A set of photos consists of the following:
 - A photograph of the riffle area itself, ideally showing some of the substrate; if the gravel is visible, try to hold a familiar object near it to help gauge its size.
 - Photographs of the riparian corridor taken upstream and down stream from the sampling area.
 - If possible, take a photo of the team actually doing the sampling.
 - d Complete the photo log for each photo.
- 20.) Clean and store the equipment. Make sure the net and sieves are clean.

ATTACHMENT B. QUALITY ASSURANCE/QUALITY CONTROL LABORATORY GUIDELINES

(From Aquatic Biology Associates, Corvallis Oregon, <http://www.aquaticbio.com/>)

The following quality assurance/quality control (QA/QC) procedures are routinely followed at Aquatic Biology Associates, Inc. in processing benthic macroinvertebrate samples. Procedures will be altered to fit the needs of the client for specific projects. Alterations in QA/QC procedure may add to the per sample cost.

1. Samples are unpacked upon receipt and preservative levels checked. Labels are checked to make sure they are intelligible and that the experimental design is understandable (e.g. sites & replicates). Non-smear labels are made that go on the inside of sample jars. The client is called if samples have been damaged in shipping and/or if the labeling system is not understandable.
2. The entire sample is floated in water in a white plastic tray. Large debris is rinsed and removed. The sample is then elutriated until all organic matter and invertebrates are floated off the mineral residue. Sieves of a pore size specified by the client are used in this process (500 micron is the most common). The mineral residue remaining in the white pan after elutriation is searched for stone-cased caddisflies and molluscs that have not floated off.
3. Unless otherwise specified by the client, a portion of the sample will be sorted that contains 500-600 organisms. The Caton Tray is normally used to randomly obtain a fraction of the total sample containing 500-600 organisms. Sample data is converted to a full sample basis. Other methodologies may be used to split some sample types, such as lake benthic samples. If densities are low, Surber and Hess samples are usually processed in their entirety. If a sample is subsampled, our normal procedure is to archive the unused sample portion until the project is completed. Unused sample fractions will be returned to the client if requested (shipping charges will be billed to the client). If requested, Aquatic Biology Associates, Inc. will archive unused sample fractions for 1 year at no charge.
4. Experienced technicians are used to remove all invertebrates from the sample residue using dissecting scopes at 6X or 12X power. For small projects, a single technician is assigned. For larger projects, several technicians are given the responsibility for sorting. All invertebrates removed from a sample are placed in a single sorting vial and given directly to Robert W. Wisseman, Senior Scientist of Aquatic Biology Associates, Inc. Logs are kept by each technician to record label data, fraction sorted, hours required to complete sorting, and any comments on sample matrix or problems. Our sorting efficacy is well above EPA requirements, as has been determined by an independent lab. Detailed sorting procedures followed by Aquatic Biology Associates, Inc. can be sent upon request.
5. The entire sample residue is saved after sorting to check for sorting efficacy. Sorting efficacy of 95% or better is required on all samples. A 20% aliquot of each residue is thoroughly re-sorted to determine efficacy. The entire residue is re-sorted if 95% or better sorting efficacy has not been achieved, as estimated from the 20% aliquot re-sort.

All sample residues can be returned to the client for independent checks. The client will be charged for shipping and sample containers.

6. Invertebrate identifications are performed by Robert W. Wisseman and associates. For standard level identifications, Robert W. Wisseman performs the initial identifications and counts on all samples, and then determines which specialists will be required to assure accurate identifications to levels specified for a project. He has over 15 years of experience in the identification of freshwater invertebrates. Aquatic Biology Associates, Inc. uses specialists from throughout North America for performing more detailed taxonomy, or to verify questionable identifications.
7. The choices for archiving invertebrate material for QA/QC checks by other experts are as follows:
 - You can trust Aquatic Biology Associates, Inc. to do a competent job, and let us pull out material that we think is significant...e.g. for verification by specialists, to be incorporated into museum collections, or to save for educational purposes. This is our preferred method of operating.
 - Save a reference/synoptic series of specimens of each taxa identified. There will be nominal charge for this service. All invertebrate material can be saved by each individual sample for archiving or QA/QC checks by another lab. An additional charge per sample will be added for this service, since it greatly slows sample processing.
 - The client can request that specific taxonomic groups be archived by individual sample for possible future taxonomic analysis (e.g. all the oligochaete worms). There is usually no charge if one or a few groups are involved.
 - Aquatic Biology Associates, Inc. requests permission to remove material from samples that may be of interest to specialists or that we feel would be a valuable addition to museum collections.
8. Identifications and counts are recorded on bench-sheets and then transferred to electronic files. Standardized bench-sheets reduce data entry errors. Robert W. Wisseman and Mary Jo Wevers (Aquatic Biology Associates, Inc. senior scientists) perform all data entry and analysis.

The following sample preservation methods are recommended.

- Use 95 or 99% alcohol to preserve most field samples. Organic residues will be holding a lot of water. If too dilute of a alcohol/water mixture is used, it will not effectively preserve the sample.
- If the sample residue is mostly coarse mineral material, then dilute the alcohol to about 80% with stream water. Coarse, woody organic material will "consume" less alcohol; but fine, leafy material requires a lot of alcohol.
- Use copious amounts of alcohol; at least twice the volume of the sample residue.
- For best results, let the field-applied alcohol sit in the sample jars for a few hours to a day, then decant off most of the original alcohol, add fresh 80% alcohol, and stir/shake gently.
- Be reasonably gentle with the samples, so that invertebrates don't break into pieces. When a large amount of fine organic matter or silt is present (e.g. lentic benthos samples), then make sure the alcohol gets well mixed into the residue. For this sample

type, you may want to consider spiking the alcohol with formalin (about 4 cc formalin per liter jar). **If you do use formalin, please write "Formalin" on the outside label of the sample jar.** Please avoid formalin if at all possible. Re-preserving the field collected sample with fresh alcohol (as described above) will be adequate in most cases.

- Do not allow samples to sit around for long without preserving (especially in direct sunlight). Invertebrates will die and deteriorate very rapidly. Preserve samples shortly after collection. Never leave unpreserved samples out in the hot sun. Also, try to keep preserved samples from sitting in the hot sun for too long.

TREATMENT OF SAMPLES

Shipping of Benthic Invertebrate Samples

Coolers are the best containers for shipping. These can be rented from Aquatic Biology Associates, Inc. Your coolers will be returned to you. For shipping by UPS, coolers that exceed a combined girth + length of 130 inches will be charged as an oversize package. Cooler weight should not exceed 70 pounds. UPS may charge \$2 extra for coolers with handles on them, since they can't place them on conveyor belts.

Make sure sample jar lids are screwed on tightly! Vibration during transport can quickly loosen lids. Lids of Nalgene® jars supplied by Aquatic Biology Associates, Inc. will not vibrate loose. See the supplies section for leak-proof sample jar suppliers. When in doubt whether jar lids will vibrate loose, secure them with electrical tape. Please use electrical tape, since it can be easily stripped from the jars.

UPS and Federal Express are preferred carriers. They treat packages much more gently than the U.S. Postal Service, are faster, usually cheaper, and will deliver samples to the door of our lab.

List on any manifest, that you are sending river sediment samples for scientific analysis. If you are shipping samples preserved only with formalin, you will have to check with UPS on packaging requirements. Formalin is classified as a hazardous substance. Small amounts of formalin added to the alcohol, to insure fixing of invertebrates, do not warrant calling attention to. If you use any formalin, then you must use leak-proof jars & ship in sealed coolers. Please line coolers with plastic garbage bags. Place sample jars in the bag and seal with a twist ties. This lining adds an extra layer of protection in case some preservative leaks. Make sure the cooler drain-cock is closed and taped shut. If you enclose documents in the coolers, please seal them in large zip-lock bags. Secure cooler lids with reinforced strapping tape. When shipping by UPS or Federal Express, please do not request that the carrier obtain a signature from our lab.

Labeling of Benthic Invertebrate Samples

Place an interior label into each sample jar! Information recorded on the interior label has priority over the exterior label. Include whatever information is needed to positively identify the sample and tie it back to field notes or sample collection forms.

Use Rite-in-the-Rain paper and a soft lead pencil. Include at least this information on the interior label:

Client/Project:	This can be abbreviated, e.g. CLNP for Crater Lake National Park
Waterbody:	e.g. Sun Creek
Site:	e.g. Site 1, 5800'
Replicate:	if applicable
Sample type:	e.g. Erosional Sample
Date:	I prefer month, day, year e.g. 4-28-93. Write out or abbreviate the month if you think there will be any confusion.
Collector initials:	e.g. RWW

If a sample is so large that it must be divided between two or more sample jars, then please use this convention on the label:

e.g. when divided between 3 jars:

Site 1 Replicate 1 (1 of 3); S1 R1 (2 of 3); S1 R1 (3 of 3).

Exterior labels are not to be trusted to remain legible. They are used only for basic project inventory purposes in the field and lab. Use a ring of "label tape" around the outside of the sample jar to record abbreviated project/site/rep./date information. Label tape is available from scientific supply houses (see Supplies & Equipment). This tape stays on the jars well, but peels off cleanly, so jars can be recycled. Use permanent ink or "Sharpie" to record sample information on the exterior label.

Do not write directly on sample jars supplied by Aquatic Biology Associates, Inc. and use only label tape on the outside.

**ATTACHMENT C.
GPS COORDINATES FOR EACH SAMPLE SITE**

TABLE C-1. GPS COORDINATES FOR SAMPLE SITES		
Site Name	Lat (N) (°, ', ")	Long(W) (°, ', ")
Lower Hoko	48,15,30.5	124,21,7.7
Upper Hoko	48,12,13.7	124,25,37.8
Upper Hoko2	48,8,16.3	124,23,6.3
Upper Clallam	48,13,4.3	124,15,11.2
Lower Clallam	48,14,52.7	124,15,8.3
Upper Pysht	48,10,7.5	124,12,39.9
Lower Pysht1	48,11,12.6	124,10,39.2
Lower Pysht2	48,11,23.0	124,9,4.1
Upper Sekiu	48,16,31.9	124,30,5.9
Lower Sekiu	48,17,0.7	124,25,46.5
Upper Lyre	48,6,0.6	123,49,3.4
Lower Lyre	48,9,0.9	123,50,8.6
Lower Deep Creek	48,9,48.5	124,1,54.4
Upper Deep Creek	48,9,29.5	124,2,10.2
Upper East Twin	48,8,31.9	123,56,9.3
Lower East Twin	48,9,2.7	123,56,10.5
Upper West Twin	48,9,11.5	123,57,0.2
Lower West Twin	48,9,43.4	123,57,13.2

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The BIBI Score has been described as “one of the most direct ways to address the Clean Water Act’s biological standards for aquatic life” (Karr and Chu 1999). Background information on the BIBI Scores indicates that the existence of living organism in itself integrates the environmental conditions within a system, suggesting that BIBI Scores provide a holistic health rating for aquatic systems. Multi-metric indices, such as BIBI, build on the efforts of earlier monitoring work by applying empirical knowledge of how biological attributes respond to human influence. Metrics are selected because they have predictable responses to changes in landscape condition, such as physical, chemical, and biological factors that stress biological systems (Karr and Chu 1999). Metrics are also selected because they are easy to measure and interpret.

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- 3.) Once the larger rocks are removed, disturb the substrate vigorously with the weeding fork for 60 seconds, to a depth of about 4 inches. Organisms and detritus should wash into the net.
- 4.) Lift the sampler out of the water: keeping the open end pointing upstream, tilt it up out of the water, to help wash organisms into the collection cup.
- 5.) Without emptying the cup, repeat the sampling procedure twice more at nearby spots. These three sampling efforts, combined into the collection cup, constitute a single replicate. Three replicates will be collected at each of the two sites per stream.
- 6.) Mark the area of this replicate’ sampling with one of the flagged washers. Among the three “digs,” mark the spot:
 - Furthest upstream, and
 - Laterally at the middle of the 3 digs
- 7.) Put a small amount of isopropyl alcohol in the sample collection jar and begin examining the large rocks collected in the dishpan, using a magnifying glass.
- 8.) Using a brush or forceps, gently move any organisms found into the sample jar.
- 9.) After examining each rock, wash it over the pan with filtered water, and set it on the bank. When all rocks have been cleaned, pour the water from the dishpan through the clean sieve. Rinse the pan, agitate and pour again, filtering out any invertebrates that washed off of the rocks. Return the rocks to the stream in the area of the sampling site.
- 10.) Meanwhile, other samplers should attend to the Surber sampler. Wash all objects caught on the inside of the net into the collection cup:
 - i) With the opening out of the water, rotate the net around in the water so that most of the objects inside wash into the cup.
 - ii) On the bank, finish rinsing the contents of the net into the cup. Use the decanter or bucket to pour unfiltered water into the net from the outside, or pour filtered water down the sides of the net from the inside.
 - iii.) Examine the net to make sure no insects are left in it. When the net is clean, empty the contents of the collection cup into the 2nd dishpan.

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- iv.) Clean the neck and collar of the sampler over the dishpan to collect any insects that may remain inside.
- v.) Rinse the cup and empty again, continuing until you have emptied it completely. (To rinse, pour clean water inside the cup; or dip the cup into the stream, holding it upright, and let the stream water filter in through the mesh on the side of the cup.)
- 11.) Pick out large debris (sticks and leaves) from the material in the sieve. Using a magnifying glass and squirt bottle or tools, pick off any organisms and return them to the sieve or sample jar before discarding these pieces.
- 12.) Pour some clean water into the dishpan and swirl the sample around in it. While the water is still agitated, pour it off into the clean sieve. Most of the organic matter should enter the sieve with the water, while the rocks stay at the bottom. Repeat this decanting procedure until the water is completely clear and there are no invertebrates still crawling around in the debris in the dishpan.
- 13.) Pick through the contents of the dishpan with a magnifying glass before discarding. If the insects will not separate from the sand, decant and then put the sand in jars too.
- [Alternate means of separating insects from sand: Decant the water out of the dishpan, and then put in enough alcohol to cover the sand. Swirl and see if insects start releasing from the sand particles. If so, decant them into the sieve, catching the alcohol in another dishpan. If there are insects in that waste alcohol too, save that as well, marking it "Through sieve from sand-float."]
- 14.) Transfer the remaining contents of the clean sieve into the sample jar. To best get most of the contents of the sieve down at one end, dip the sieve at an angle in clean water in one of the dishpans. Use gentle forceps, a spatula, and/or a squirt bottle to move the remaining contents of the clean sieve into the sample jar.
- 15.) Fill the jar no more than halfway with contents from the sieve then fill to near the top with alcohol. Complete a label with the date, stream, reach number, replicate number, first initials and last names of samplers.
- 16.) Place inside the jar, ideally so that the writing can be seen from the outside. Close the jar tightly and wrap the seal 2-3 times with electrical tape. On the lid write date, stream, reach number, and replicate number as follows:
- | | |
|------------------|---------------------|
| SAMPLE JAR LID: | SAMPLE ZIPLOCK BAG: |
| 9/15/2000 | 9/15/2000 |
| Peabody 2, Rep 1 | Peabody 2, Rep 1 |
| Jar 1 of 2 | 2 Jars |
- (If the material will not fit in one jar, put it into two or more jars, and add "Jar 1 of 2," etc. to the slips of paper inside the jars and the jar lids.) Place the jar(s) from a single replicate in a single small ziplock bag, labeled with the same information as the lid. See Appendix A, Quality Control Plan, for more detail on the treatment of samples.
- 17.) Collect two more replicates, following the same procedure as above.
- 18.) Measure and record the following information about the area in which you collected each replicate:
- The average water depth at the spots where you dug that replicate (with the rocks removed), to the nearest number of inches.
 - The width of the riffle in the area where you dug, to the nearest number of feet.
-

- The length of the riffle in the area where you dug, to the nearest number of feet.
- 19.) Photograph the sampling sites in the following manner:
- a) If all three replicates were taken from the same riffle or riffle sequence, one set of photos will suffice.
 - b) Replicates that were taken far apart or from areas that look very different should have separate sets of photos.
 - c) A set of photos consists of the following:
 - A photograph of the riffle area itself, ideally showing some of the substrate; if the gravel is visible, try to hold a familiar object near it to help gauge its size.
 - Photographs of the riparian corridor taken upstream and down stream from the sampling area.
 - If possible, take a photo of the team actually doing the sampling.
 - d Complete the photo log for each photo.
- 20.) Clean and store the equipment. Make sure the net and sieves are clean.

ATTACHMENT B. QUALITY ASSURANCE/QUALITY CONTROL LABORATORY GUIDELINES

(From Aquatic Biology Associates, Corvallis Oregon, <http://www.aquaticbio.com/>)

The following quality assurance/quality control (QA/QC) procedures are routinely followed at Aquatic Biology Associates, Inc. in processing benthic macroinvertebrate samples. Procedures will be altered to fit the needs of the client for specific projects. Alterations in QA/QC procedure may add to the per sample cost.

1. Samples are unpacked upon receipt and preservative levels checked. Labels are checked to make sure they are intelligible and that the experimental design is understandable (e.g. sites & replicates). Non-smear labels are made that go on the inside of sample jars. The client is called if samples have been damaged in shipping and/or if the labeling system is not understandable.
2. The entire sample is floated in water in a white plastic tray. Large debris is rinsed and removed. The sample is then elutriated until all organic matter and invertebrates are floated off the mineral residue. Sieves of a pore size specified by the client are used in this process (500 micron is the most common). The mineral residue remaining in the white pan after elutriation is searched for stone-cased caddisflies and molluscs that have not floated off.
3. Unless otherwise specified by the client, a portion of the sample will be sorted that contains 500-600 organisms. The Caton Tray is normally used to randomly obtain a fraction of the total sample containing 500-600 organisms. Sample data is converted to a full sample basis. Other methodologies may be used to split some sample types, such as lake benthic samples. If densities are low, Surber and Hess samples are usually processed in their entirety. If a sample is subsampled, our normal procedure is to archive the unused sample portion until the project is completed. Unused sample fractions will be returned to the client if requested (shipping charges will be billed to the client). If requested, Aquatic Biology Associates, Inc. will archive unused sample fractions for 1 year at no charge.
4. Experienced technicians are used to remove all invertebrates from the sample residue using dissecting scopes at 6X or 12X power. For small projects, a single technician is assigned. For larger projects, several technicians are given the responsibility for sorting. All invertebrates removed from a sample are placed in a single sorting vial and given directly to Robert W. Wisseman, Senior Scientist of Aquatic Biology Associates, Inc. Logs are kept by each technician to record label data, fraction sorted, hours required to complete sorting, and any comments on sample matrix or problems. Our sorting efficacy is well above EPA requirements, as has been determined by an independent lab. Detailed sorting procedures followed by Aquatic Biology Associates, Inc. can be sent upon request.
5. The entire sample residue is saved after sorting to check for sorting efficacy. Sorting efficacy of 95% or better is required on all samples. A 20% aliquot of each residue is thoroughly re-sorted to determine efficacy. The entire residue is re-sorted if 95% or better sorting efficacy has not been achieved, as estimated from the 20% aliquot re-sort.

All sample residues can be returned to the client for independent checks. The client will be charged for shipping and sample containers.

6. Invertebrate identifications are performed by Robert W. Wisseman and associates. For standard level identifications, Robert W. Wisseman performs the initial identifications and counts on all samples, and then determines which specialists will be required to assure accurate identifications to levels specified for a project. He has over 15 years of experience in the identification of freshwater invertebrates. Aquatic Biology Associates, Inc. uses specialists from throughout North America for performing more detailed taxonomy, or to verify questionable identifications.
7. The choices for archiving invertebrate material for QA/QC checks by other experts are as follows:
 - You can trust Aquatic Biology Associates, Inc. to do a competent job, and let us pull out material that we think is significant...e.g. for verification by specialists, to be incorporated into museum collections, or to save for educational purposes. This is our preferred method of operating.
 - Save a reference/synoptic series of specimens of each taxa identified. There will be nominal charge for this service. All invertebrate material can be saved by each individual sample for archiving or QA/QC checks by another lab. An additional charge per sample will be added for this service, since it greatly slows sample processing.
 - The client can request that specific taxonomic groups be archived by individual sample for possible future taxonomic analysis (e.g. all the oligochaete worms). There is usually no charge if one or a few groups are involved.
 - Aquatic Biology Associates, Inc. requests permission to remove material from samples that may be of interest to specialists or that we feel would be a valuable addition to museum collections.
8. Identifications and counts are recorded on bench-sheets and then transferred to electronic files. Standardized bench-sheets reduce data entry errors. Robert W. Wisseman and Mary Jo Wevers (Aquatic Biology Associates, Inc. senior scientists) perform all data entry and analysis.

The following sample preservation methods are recommended.

- Use 95 or 99% alcohol to preserve most field samples. Organic residues will be holding a lot of water. If too dilute of a alcohol/water mixture is used, it will not effectively preserve the sample.
- If the sample residue is mostly coarse mineral material, then dilute the alcohol to about 80% with stream water. Coarse, woody organic material will "consume" less alcohol; but fine, leafy material requires a lot of alcohol.
- Use copious amounts of alcohol; at least twice the volume of the sample residue.
- For best results, let the field-applied alcohol sit in the sample jars for a few hours to a day, then decant off most of the original alcohol, add fresh 80% alcohol, and stir/shake gently.
- Be reasonably gentle with the samples, so that invertebrates don't break into pieces. When a large amount of fine organic matter or silt is present (e.g. lentic benthos samples), then make sure the alcohol gets well mixed into the residue. For this sample

type, you may want to consider spiking the alcohol with formalin (about 4 cc formalin per liter jar). **If you do use formalin, please write "Formalin" on the outside label of the sample jar.** Please avoid formalin if at all possible. Re-preserving the field collected sample with fresh alcohol (as described above) will be adequate in most cases.

- Do not allow samples to sit around for long without preserving (especially in direct sunlight). Invertebrates will die and deteriorate very rapidly. Preserve samples shortly after collection. Never leave unpreserved samples out in the hot sun. Also, try to keep preserved samples from sitting in the hot sun for too long.

TREATMENT OF SAMPLES

Shipping of Benthic Invertebrate Samples

Coolers are the best containers for shipping. These can be rented from Aquatic Biology Associates, Inc. Your coolers will be returned to you. For shipping by UPS, coolers that exceed a combined girth + length of 130 inches will be charged as an oversize package. Cooler weight should not exceed 70 pounds. UPS may charge \$2 extra for coolers with handles on them, since they can't place them on conveyor belts.

Make sure sample jar lids are screwed on tightly! Vibration during transport can quickly loosen lids. Lids of Nalgene® jars supplied by Aquatic Biology Associates, Inc. will not vibrate loose. See the supplies section for leak-proof sample jar suppliers. When in doubt whether jar lids will vibrate loose, secure them with electrical tape. Please use electrical tape, since it can be easily stripped from the jars.

UPS and Federal Express are preferred carriers. They treat packages much more gently than the U.S. Postal Service, are faster, usually cheaper, and will deliver samples to the door of our lab.

List on any manifest, that you are sending river sediment samples for scientific analysis. If you are shipping samples preserved only with formalin, you will have to check with UPS on packaging requirements. Formalin is classified as a hazardous substance. Small amounts of formalin added to the alcohol, to insure fixing of invertebrates, do not warrant calling attention to. If you use any formalin, then you must use leak-proof jars & ship in sealed coolers. Please line coolers with plastic garbage bags. Place sample jars in the bag and seal with a twist ties. This lining adds an extra layer of protection in case some preservative leaks. Make sure the cooler drain-cock is closed and taped shut. If you enclose documents in the coolers, please seal them in large zip-lock bags. Secure cooler lids with reinforced strapping tape. When shipping by UPS or Federal Express, please do not request that the carrier obtain a signature from our lab.

Labeling of Benthic Invertebrate Samples

Place an interior label into each sample jar! Information recorded on the interior label has priority over the exterior label. Include whatever information is needed to positively identify the sample and tie it back to field notes or sample collection forms.

Use Rite-in-the-Rain paper and a soft lead pencil. Include at least this information on the interior label:

Client/Project:	This can be abbreviated, e.g. CLNP for Crater Lake National Park
Waterbody:	e.g. Sun Creek
Site:	e.g. Site 1, 5800'
Replicate:	if applicable
Sample type:	e.g. Erosional Sample
Date:	I prefer month, day, year e.g. 4-28-93. Write out or abbreviate the month if you think there will be any confusion.
Collector initials:	e.g. RWW

If a sample is so large that it must be divided between two or more sample jars, then please use this convention on the label:

e.g. when divided between 3 jars:

Site 1 Replicate 1 (1 of 3); S1 R1 (2 of 3); S1 R1 (3 of 3).

Exterior labels are not to be trusted to remain legible. They are used only for basic project inventory purposes in the field and lab. Use a ring of "label tape" around the outside of the sample jar to record abbreviated project/site/rep./date information. Label tape is available from scientific supply houses (see Supplies & Equipment). This tape stays on the jars well, but peels off cleanly, so jars can be recycled. Use permanent ink or "Sharpie" to record sample information on the exterior label.

Do not write directly on sample jars supplied by Aquatic Biology Associates, Inc. and use only label tape on the outside.

**ATTACHMENT C.
GPS COORDINATES FOR EACH SAMPLE SITE**

TABLE C-1. GPS COORDINATES FOR SAMPLE SITES		
Site Name	Lat (N) (°, ', ")	Long(W) (°, ', ")
Lower Hoko	48,15,30.5	124,21,7.7
Upper Hoko	48,12,13.7	124,25,37.8
Upper Hoko2	48,8,16.3	124,23,6.3
Upper Clallam	48,13,4.3	124,15,11.2
Lower Clallam	48,14,52.7	124,15,8.3
Upper Pysht	48,10,7.5	124,12,39.9
Lower Pysht1	48,11,12.6	124,10,39.2
Lower Pysht2	48,11,23.0	124,9,4.1
Upper Sekiu	48,16,31.9	124,30,5.9
Lower Sekiu	48,17,0.7	124,25,46.5
Upper Lyre	48,6,0.6	123,49,3.4
Lower Lyre	48,9,0.9	123,50,8.6
Lower Deep Creek	48,9,48.5	124,1,54.4
Upper Deep Creek	48,9,29.5	124,2,10.2
Upper East Twin	48,8,31.9	123,56,9.3
Lower East Twin	48,9,2.7	123,56,10.5
Upper West Twin	48,9,11.5	123,57,0.2
Lower West Twin	48,9,43.4	123,57,13.2