Greater Lake Washington and Green-Duwamish River Watersheds Wadeable Freshwater Streams Benthic Macroinvertebrate Sampling and Analysis Plan

December 2002

Prepared by:

King County
Department of Natural Resources and Parks
Water and Land Resources Division

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1 INTRODUCTION

The primary objective of this project is to characterize aquatic macroinvertebrate populations to assess the biological conditions within King County watersheds. The secondary objective is to consolidate and update the existing macroinvertebrate sampling programs within King County Water and Land Resources Division (WLRD). This Sampling and Analysis Plan (SAP) is for the 2002 and 2003 sampling seasons and will be updated for the 2004 sampling season to accommodate any changes in scope, budget and/or protocols. The study will attempt to determine the current status of aquatic macroinvertebrate health in wadeable stream sub-basins within the Greater Lake Washington (Figure 2) and Green-Duwamish River (Figure 3) Watersheds. The same data collected over time will prove useful in detecting long-term macroinvertebrate population trends.

This chapter details the basis for using benthic macroinvertebrates as an index of stream health, the history of benthic macroinvertebrate sampling in King County WLRD, the goals and objectives of the proposed sampling program, and the scope of the study.

1.1 Project Background

This section explains the rationale behind benthic macroinvertebrate sampling, explains the Benthic Index of Biotic Integrity (B-IBI), and describes previous sampling efforts within WLRD.

1.1.1 Benthic Macroinvertebrates

Aquatic macroinvertebrates are aquatic animals without backbones that are visible to the naked eye, including insects, crustaceans, worms, snails, and clams. Benthic macroinvertebrates spend all or most of their lives living in or on the bottom of the streambed and other substrates such as logs or plants in the stream channel. Benthic macroinvertebrates are monitored because they are good indicators of the biological health of stream systems and play a crucial role in the stream ecosystem (Karr and Chu, 1999). Since they complete most or all of their life cycle in the aquatic environment and they are relatively sedentary, benthic communities are reflective of local sediment, water quality, hydrologic and habitat conditions (Booth et al., *in review*). The monitoring of macroinvertebrate populations provides a relatively inexpensive and powerful tool to assess the short and long-term effects of a wide range of environmental disturbances.

1.1.2 Benthic-Index for Biotic Integrity (B-IBI)

The Benthic Index for Biotic Integrity (B-IBI) that King County uses was developed specifically for Puget Sounds lowland stream systems (Karr, 1998, 1999; Fore et al., 2001; Morley and Karr, 2002). It is composed of ten metrics that measure different aspects of stream biology, including taxonomic richness and composition, tolerance and intolerance, habit, reproductive strategy, feeding ecology, and population structure (Appendix A). Each metric describes some aspect of the community that responds to degradation. The raw value

of each metric is calculated, and from the raw value, a score of 1, 3, or 5 is assigned to the metric. The ten metric scores are then added to produce the overall B-IBI score that ranges from 10 to 50. Based on this score the streams are rated on a qualitative scale as excellent, good, fair, poor or very poor (Table 1).

Table 1. Benthic-Index of Biotic Integrity stream ratings.

B-IBI Score	Condition of Biological Integrity
46-50	Excellent
38-44	Good
28-36	Fair
18-26	Poor
10-16	Very Poor

1.1.3   WLRD Benthic Sampling History

King County Water and Land Resources Division (WLRD) is charged with monitoring the water quality and overall ecological health of stream systems within the County's jurisdiction. Benthic macroinvertebrates have been monitored under two distinct programs within WLRD, one wastewater funded and one surface water funded.

The wastewater related benthic monitoring program was initiated in the mid 1970s. The primary objective was to monitor streams that were, or could potentially be, impacted by untreated wastewater, treated effluent and the system of pipes and pumps that make up the wastewater collection and transfer system. This program continues today relatively unchanged and is part of a regional water quality monitoring program that includes lakes, mainstem rivers and streams.

In the mid 1990s Basin Plans were created for six King County watersheds including: Lower Cedar River, Soos Creek, Bear Creek, Issaquah Creek, May Creek, and East Lake Sammamish. The goal of the Basin Management Evaluation Program (BMEP) macroinvertebrate-monitoring program was to provide data to evaluate the success of the basin plans and, when possible, to make specific recommendations for improved management. Macroinvertebrate samples have been collected in these basins since 1995.

The wastewater funded and surface water funded programs were designed, and are currently implemented to address different, but closely related and complimentary water quality issues. The purpose of this SAP is to combine the benthic macroinvertebrate programs within WLRD as part of a larger effort to consolidate the County's freshwater monitoring programs. In addition, changes in project design will allow for long-term trend detection on a larger scale than was previously possible.

1.2 Study Objectives

This study has two major components. The first involves consolidating the two existing
benthic invertebrate sampling programs within WRLD. To this end we will sample all of the
wastewater sites and BMEP sites in 2002 using sample collection and analysis method as
described in this SAP. In 2002/2003 we will evaluate these sites individually to determine if
they should be retained in the new monitoring program or eliminated. They may be
eliminated if they fail to meet the standards of the statistical design or they are found
unsuitable.

The second major component of this study involves developing a comprehensive monitoring
plan within the greater Lake Washington and Green-Duwamish River Watersheds. This aspect
of the program involves establishing 8 to 10 randomly selected sampling sites (Fore, 2001) per
sub-basin in order to establish baseline watershed conditions and detect trends in stream
biological health over time. Random site selection allows the results from a few sites to be
extrapolated to the entire sub-basin.

The objectives of this SAP are to:

1. Characterize existing aquatic macroinvertebrate conditions of wadeable stream
    sub-basins located within the Greater Lake Washington and Green-Duwamish
    River Watersheds.

2. Identify differences in macroinvertebrate communities between sub-basins in the
    Greater Lake Washington and Green-Duwamish River Watersheds.

3. Collect data that can be used as a baseline for detecting long-term trends in
    benthic macroinvertebrate communities.

4. Consolidate the two existing benthic macroinvertebrate monitoring programs.

5. Supply data for a future study relating benthic macroinvertebrate data to land use
    conditions in the sub-basins or contributing watersheds.

1.3 Study Area

The study area is comprised of wadeable streams located in the Greater Lake Washington and
Green-Duwamish River Watersheds (Figures 1, 2 and 3), but does not include the mainstem
portion of the Cedar, Sammamish or Green-Duwamish Rivers.

Figure 1. Watershed locations.

1.3.1   Greater Lake Washington Watershed Study Area

The project study area encompasses the Cedar/Sammamish/ Lake Washington Watershed but does not include the area above the Landsburg Dam. This includes the Ship Canal, Lake Union and Portage Bay, Lake Washington, Lake Sammamish and the tributary rivers and streams. The watershed encompasses about 692 square miles, from its mouth at the Ballard Locks to the Cedar River below the Landsburg Dam, and into Snohomish County including the headwaters of Little Bear, Swamp, North and Bear Creeks. Salt water drainages are excluded from this study. The drainages within the greater Lake Washington Watershed are divided into ten watershed sub-basins (Figure 2) as follows:

- West Lake Washington Tributaries
- East Lake Washington Tributaries
- North Creek and Swamp Creek
- Little Bear Creek
- Bear Creek
- Evans Creek
- Sammamish River Tributaries
- Lake Sammamish Tributaries

- Issaquah Creek
- Lower Cedar River Tributaries
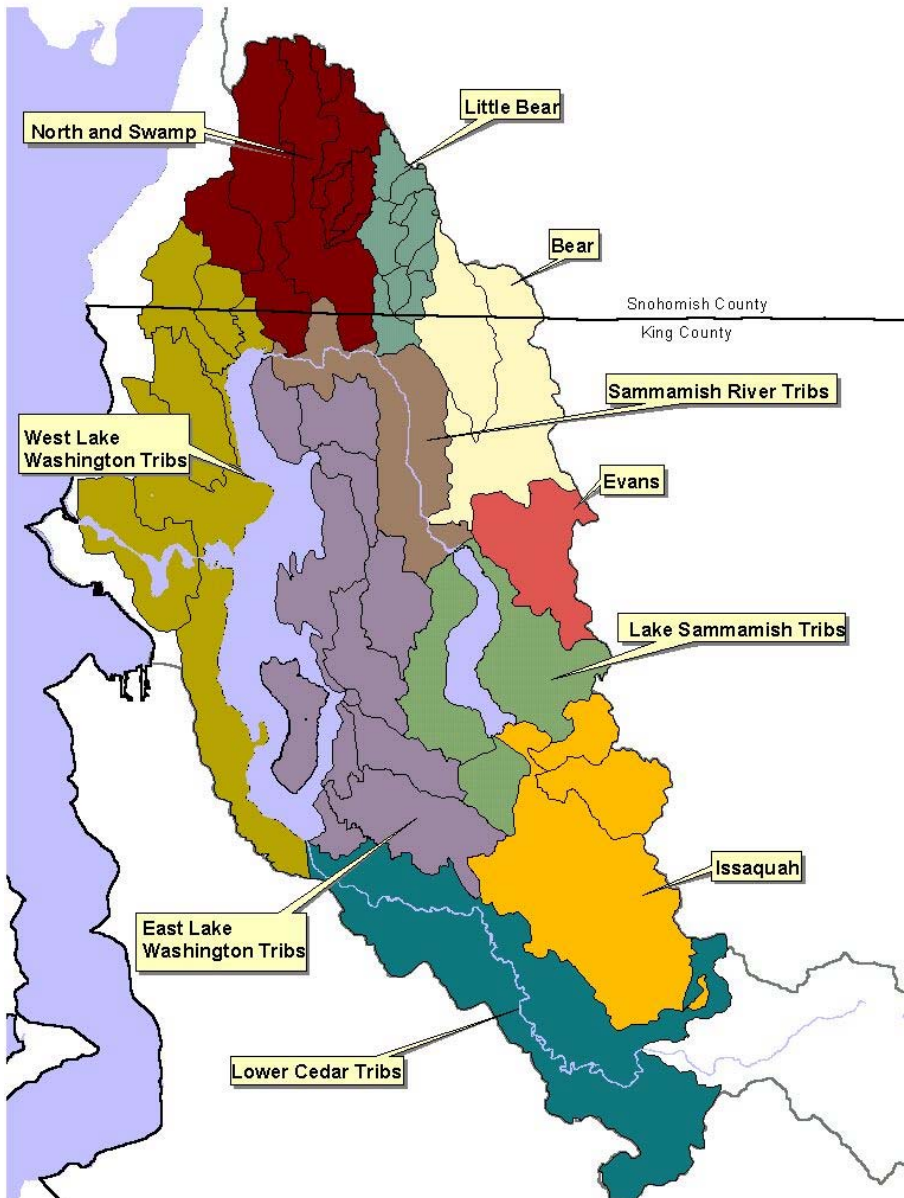
Figure 2. Greater Lake Washington Watershed sub-basins.

1.3.2 Green-Duwamish River Watershed Study Area

The Green-Duwamish Watershed has a drainage area of approximately 492 square miles. Terrain and land use vary from forested headwater areas at the crest of the Cascade Mountains to industrial facilities in the Duwamish estuary. The project study area encompasses all freshwater wadeable streams in the Green-Duwamish Watershed from below the Tacoma Diversion Dam at river mile 61 to the mouth of the Duwamish River at Elliott Bay (Figure 1 and Figure 3), approximately 261 square miles. The Duwamish River and

Estuary, the mainstem Green River and the upper Green River Basin (231 square miles) are not included in the study area.

For the purpose of this study, the streams located within the Green-Duwamish Watershed were divided into eight watershed sub-basins. For sampling logistics, several of the smaller stream basins have been grouped together to form a larger basin area. The sub-basins that will be sampled are identified as:

- Black River
- Deep/Coal Creek
- Jenkins/Covington Creek
- Duwamish River
- Lower Green River/Mill Creek
- Middle Green River
- Newaukum Creek
- Soos Creek

Figure 3. Green-Duwamish River Watershed sub-basins.

2 STUDY DESIGN

The following section states the study questions and hypotheses, and details the methods used
for selection of sampling sites.

2.1 Study Questions and Hypotheses

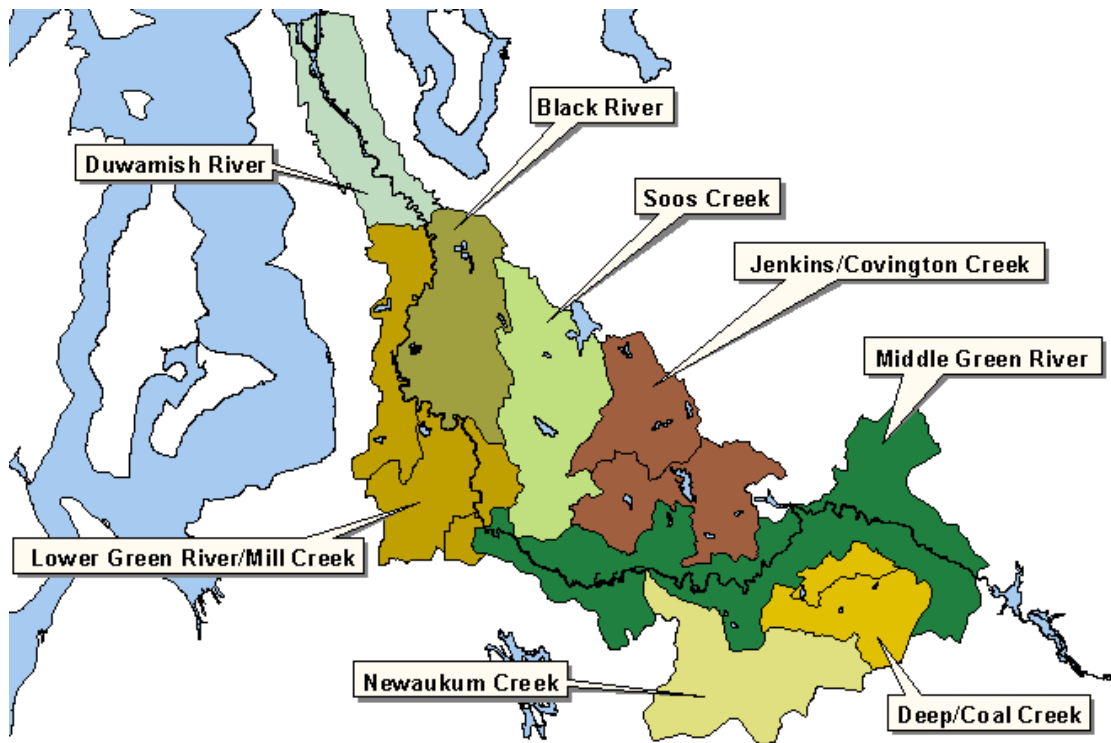The study questions and hypotheses for the Greater Lake Washington and Green-Duwamish
River Watersheds are designed to test for differences across sub-basins, through time and in
response to different types of land use. Comparisons will be based on the benthic index of
biotic integrity derived from stream macroinvertebrate samples.

1. Question: Do different watershed sub-basins within the Greater Lake
Washington Watershed (e.g. Bear Creek, Issaquah Creek, Lower Cedar
Tributaries Creek, etc.) differ in terms of biological condition?

    Null hypothesis: B-IBI scores from different sub-basins in the Greater Lake
    Washington River Watershed do not differ significantly.

2. Question: Do different watershed sub-basins within the Greater Green-
Duwamish (e.g. Soos Creek, Newaukum Creek, Jenkins/Covington Creek,
etc.) differ in terms of biological condition?

    Null hypothesis: B-IBI scores from different sub-basins in the Greater Green-
    Duwamish River Watershed do not differ significantly.

3. Question: Is the biological condition improving (or declining) over time? Is
the trend significant?

    Null hypothesis: B-IBI scores for watershed sub-basins do not change
    significantly over time.

4. Question: Do different land use patterns measured at the sub-basin level
affect biological conditions differently within the watershed?

    Null hypothesis: B-IBI scores for watershed sub-basins do not differ
    significantly with different land use regimes.

2.2 Site Selection

To reduce statistical bias while selecting sampling sites, a multi-step approach using
randomly selected sites will be employed. The process involves using the Arc-View GIS
program and a random number generator to select possible sites, inspecting the sites to ensure
they meet specific criteria, and obtaining property access. When sampling commences, the

sites will be sampled in order of their selection (i.e., #1 will be sampled first, #2 sampled second, etc.) until eight to ten samples are collected.[1]

2.2.1   Geographic Information System (GIS)

The Arc-View GIS computer program will be used to identify suitable land and stream characteristics. For the Greater Lake Washington and Green-Duwamish River Watersheds, a grid comprised of 0.33 mi^2 squares will be overlaid on a map of each watershed. Grid squares on the map will be assigned a unique identification number. Squares that do not contain a section of stream will be eliminated from the pool of suitable sites while the remaining squares will be listed as potential sampling sites

2.2.2   Random Site Selection

A random number generator will be used to select thirty sites from the pool of potential sites. These sites will be inspected to ensure they meet specific criteria necessary for B-IBI sampling (Section 2.2.3) until a list of ten suitable sites is identified. If ten suitable sites are not identified during site visits from the initial thirty random sites, additional sites will be randomly selected, and inspected until there are a total of ten sites.

2.2.3   Site Reconnaissance

Each potential sampling site will be visited prior to sampling to determine if it is suitable for the sampling methods described in Section 4. Sampling site suitability is defined as a stream reach with a riffle[2] (in the absence of well-defined riffles, choose the fastest flowing, most turbulent, non-depositional location possible). The site should have sandy, pebbly, gravely or rocky substrate (e.g., no concrete culverts or subsurface flow), a minimum channel width of one foot, and a water depth ranging from one inch to one foot. Sites without suitable flow, depth, width or substrate characteristics will be eliminated and the next potential site on the list will be visited.

2.2.4   Property Access

Right of entry permission will be obtained from the landowner(s) adjacent to the stream prior to accessing the stream and sampling sites. It may be necessary to use road or public utility easements for access to some sites. If access is denied by the landowner or the landowner can not be contacted, then the next randomly selected site on the list will be used. A record of permission or description of how access was obtained will be maintained.

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[1] Some sub-basins may have less than eight sampling sites due to lack of acceptable sampling locations (e.g., the majority of the streams in a sub-basin run underground, dominated by low gradient streams without riffles or are ephemeral streams).

[2] A riffle is defined as a fast-flowing area of a stream where shallow water races over stones and gravel. (McCafferty 1998).

2.3 Statistical Analysis

The sampling design for this study is based on recommendations from an analysis of historic BMEP data (Fore, 2001). Based on this analysis we should be able to detect a 3.5% change per year in B-IBI scores after five years of annual sampling with an estimated confidence level of 95% (α=0.05) and 80% power (0.80) for a one-sided test. We will use random site selection to ensure representativeness with and sample the same sites annually the first two years. The following is the list of study questions previously mentioned in section 2.1 and the corresponding statistical tests:

1. <u>Question</u>: Do different watershed sub-basins within the Greater Lake Washington Watershed (e.g. Bear Creek, Issaquah Creek, Lower Cedar Tributaries Creek, etc.) differ in terms of biological condition?

    Statistics: An ANOVA test will be used for normally distributed data with sub-basins as the categories. If the data are non-parametric a Kruskal-Wallis test will be used.

2. <u>Question</u>: Do different watershed sub-basins within the Greater Green-Duwamish (e.g. Soos Creek, Newaukum Creek, Jenkins/Covington Creek, etc.) differ in terms of biological condition?

    Statistics: Same statistical tests used to answer question 1.

3. <u>Question</u>: Is the biological condition improving (or declining) over time? Is the trend significant?

    Statistics: To determine if the scores are changing over time a t-test will be used for parametric data and the Mann-Whitney test for non-parametric data. To determine trends over time regression or the Seasonal Kendall trend test will be used.

4. <u>Question:</u> Do different land use patterns measured at the sub-basin level affect biological conditions differently within the watershed?

    Statistics: A nested ANOVA will be used if the data are parametric and a Kruskal-Wallis test if the data are non-parametric.

3 PROJECT MANAGEMENT

The following section summarizes the project personnel and their responsibilities, provides the project timeline and project deliverables.

3.1 Project Personnel

The following table outlines the project personnel and responsibilities.

Table 2. Project roles and responsibilities.

Jonathon Frodge	Program manager, SWAMP
Jim Simmonds	Program manager, Green Water Quality Assessment
Lorin Reinelt	Senior Technical Advisor
Kari Osterhaug	Project manager, Benthic Macroinvertebrate Study
	Greater Cedar River/Lake Washington Watershed
John Brooker	Project manager, Benthic Macroinvertebrate Study
	Greater Green/Duwamish Watershed
Jean Power	Laboratory Technical Coordinator, King County Environmental Laboratory
Jessica Kuchan	Project Technical Coordinator and Administrative Support
Rhithron Associates, Inc.	Consultant, Taxonomic Identification Lab
Leska Fore	Consultant, Statistical Design

3.2 Project Timeline

The following timeline details the project tasks for 2002 through 2004.

Table 3. Project timeline.

Spring/Summer 2002	Initial project planning
August-September 2002	Sample collection
Fall/Winter/Spring 2002-2003	Sample analysis (taxonomic ID lab)
	Review and evaluation of historic BMEP and METRO sites

	Create macroinvertebrate database
Summer 2003	Complete 2002 Benthic Macroinvertebrate Annual Report
August-September 2003	Sample collection
Fall/Winter/Spring 2003-2004	Sample analysis (taxonomic ID lab)
Summer 2004	Complete 2002-2003 Benthic Macroinvertebrate Summary Report

3.3 Project Deliverables

The data generated by this study will likely be used by King County and other jurisdictions both for local, WRIA and basin planning purposes. The following are the anticipated project deliverables:

- Benthic Macroinvertebrate SAP (December 2002)

- Benthic Macroinvertebrate SAP revisions (Spring/Summer 2003)

- 2002 Benthic Macroinvertebrate Monitoring Report (Summer 2003)

- Benthic Macroinvertebrate Monitoring Summary Report (Summer 2004)

- King County benthic macroinvertebrate website where the B-IBI scores, spatial data and links to the aforementioned reports will be available.

4 SAMPLE COLLECTION

PROCEDURES

The following is a description of the sampling methods, techniques and equipment necessary
for conducting this study.

4.1 Benthic Invertebrate Sample Collection

4.1.1   Sampling Equipment

The following collection equipment is required at each sampling site:
- Large canvas bag
- Surber sampler, (1 ft^2 frame with 500 μm mesh net, and removable plankton bucket with
  500 μm mesh openings)
- Dishpan, white or light-colored
- 2 - 500 μm sieves
- Weed pulling tool, marked 10 cm from end of tool
- 2 L Ethyl alcohol, 95%, denatured
- 1 - 2 L sample container with lid
- 1 – 1 L sample container with lid
- Clipboard
- 2 - Pencils
- 2 - Permanent marking pens
- 2 - Water indelible pens
- Waterproof paper
- Labeling tape
- Internal bottle labels
- Clear packaging tape
- Spray bottle
- Spatula, plastic spoon, plastic knife
- Metric ruler
- Measuring tape
- Thermometer
- 2 Pair of fine tip forceps (e.g., entomological forceps)
- Stopwatch
- Field datasheet

4.1.2   Riffle Selection

The location of the riffles to be sampled will be determined prior to entering the stream. To accomplish this, the stream reach at the site must be defined. Ideally, the reach should be representative of overall conditions in the area, and should be 20 times the average wetted channel width. Three riffles within the reach are selected and should be representative of varying riffle habitats (e.g., riffle depths, flow rates, and/or substrate characteristics). In the absence of well-defined riffles, choose the fastest flowing, most turbulent, non-depositional location possible. In the absence of three distinct riffles it may be necessary to sample at different locations within the existing one or two riffles. It is important to avoid walking in the stream or causing any disturbance upstream of any location yet to be sampled.

4.1.3   Surber Sampler Placement

Enter the stream below the furthest downstream sampling location. Collect the sample at the lateral center of each selected riffle. The Surber sampler should be placed on the substrate surface by approaching from downstream, with the team member averting their eyes from the stream bottom to avoid bias in setting the net. The sampler is placed firmly down onto the substrate with the net opening facing upstream. Press the net frame down into the substrate. The Surber frame must be securely "sealed" against the substrate to prevent organisms from washing under the frame. If any large cobble lying under the edge of the frame prevents a good "seal," it should be immediately pulled within the perimeter of the frame. Even if part of the cobble lies outside the frame area, it should be pulled into the frame area and included as part of the sample. In areas with high stream velocity, it may be necessary for one team member to hold the net down (typically from a downstream position) while another collects the sample. Care must be taken not to disturb the upstream substrate during this process. Once the net has been placed, the sample collection must be done quickly to minimize the movement of organisms into or out of the sampling area.

4.1.4   Sediment Agitation and Sample Collection

All large objects (e.g., large gravel and woody debris) within the sampling area will be picked up and scrubbed by hand inside the collection net. Examine the objects and remove any organisms that were not removed by the scrubbing process and discard the object downstream after inspection.

The weed tool is used to vigorously agitate the substrate within the perimeter of the frame to a depth of approximately 10-cm, for 60 seconds. The frame must stay securely anchored to the substrate during this process.

Large gravel or cobble particles that have washed into the net during agitation of the sediment should be picked up but not removed from the net. Physically scrub the object with your hand inside the net, and then inspect it to make sure all organisms were removed. Ensure that your hands are well rinsed by the stream water inside the net before removing them.

The Surber sampler should be removed from the stream by pulling the sampler in an upward and upstream motion. While holding the net vertically, use stream water and a squirt-bottle to rinse organisms and debris on the inside of the net into the plankton bucket at the bottom

of the net. When using stream water, the water will be splashed from the outside of the net or poured through a 500 μm sieve to ensure that no organisms are inadvertently added to the sample.

Three samples will be collected from three separate riffles at each site and combined to make one composite sample.

4.1.5   Sample Processing

One team member will be responsible for transferring the sample from the net to the sieve or dishpan while the other team member checks the inside of the net for any organisms that were not transferred. To remove the organisms from the net, detach the plankton bucket from the net and pour it into the sieve or dishpan. Rinse the plankton bucket carefully and inspect it to make sure all organisms are transferred into the sieve or dishpan. For mussels that were collected, a note of their number and presence will be made on the field data sheet and a penciled note representing the organism will be placed into the sample jar. The mussel should be gently returned to the stream. Also, note and return any fish to the stream.

The collected sample material is then gently sieved and rinsed with water from the spray bottle to remove any excess fine material (i.e., < 500 μm). Use the spatula, knife, spoon and/or spray bottle to concentrate the sample material on one side of the sieve and then transfer the material into the sample container.

4.1.6   Sample Preservation and Documentation

After the sample is transferred into the sample container, it will be preserved by filling the sample container with sieved water and 95% denatured ethanol to an approximate concentration of 70-80% alcohol.

Label the sample jars on the outside with a permanent marking pen and labeling tape and on the inside with pencil and a paper tag. A piece of clear packing tape will be used to cover the outside label once it has been completely filled out. Each label will consist of the sample identification number, date, the word "duplicate" (if necessary) and collectors' initials. The sample identification number will adhere to the following format:

> WRIA number – 3 digit sub-basin Identification Code – 4 digit site number

> Example: **09NEW2151**; was collected within the Green River Watershed (WRIA 9), in Newaukum Creek at site 2151.

After the sample jar is labeled (internally and externally), tightly secure the lid.

4.2 Habitat Evaluation

The following habitat and physical stream parameters will be measured at each site:
* Water and air temperature
* Riffle width, length, depth and flow velocity
* Water clarity

- Riparian bank vegetation parameters including; vegetation type, density, and size class
- Woody debris presence
- Wolman pebble counts (Wolman, 1954): 35 particles are counted just upstream of each Surber sample location to be combined for a total count of >100 particles.
- Distance to nearest known road crossing noted as either upstream or downstream and within set distance categories (from <10ft to crossing to >200ft).

For units and definitions of these parameters see Appendix B which includes the data sheet used for the 2002 sampling season. After the 2002-collecting season the habitat data will be analyzed and certain habitat parameters may be added, eliminated, or modified for the 2003 sampling season.

5 LABORATORY ANALYSIS

King County currently employs Rhithron Biological Associates from Missoula, Montana to process benthic macroinvertebrate samples including taxonomic identification of macroinvertebrates, QA/QC procedures, and analysis of data to provide metric and B-IBI scores. Future analysis will be done by Rhithron or another qualified taxonomic laboratory.

5.1 Taxonomic Laboratory Procedures

Rhithron uses Caton subsampling devices, divided into 30 grids, each approximately 5 cm by 6 cm, for all sample handling. To obtain subsamples of a minimum of 500 organisms, samples are poured into the device, grids are randomly chosen, and substrate materials lifted out into petri dishes. Using 10x-30x magnification under dissecting microscopes, technicians remove all organisms from the contents of each grid until 500 organisms are collected. The technician will then continue to remove all remaining organisms from the final grid, resulting in a subsample of somewhat more than 500 organisms. Quality assurance procedures (Section 7) are carried out for each sample. Sorted substrate and unsorted remainders are retained and stored until completion of the project. For a detailed account of Rhithron's laboratory procedures see Appendix C.

5.2 Taxonomic Resolution

The contract laboratory (Rhithron, Missoula, MT; or other qualified taxonomic laboratory) will provide taxonomic identification (at minimum) to the levels outlined in Table 4. The only exception is that early instars of some macroinvertebrates will only be able to be identified to higher taxonomic levels (e.g., genus) because diagnostic features have not fully developed.

Table 4. Level of taxonomic resolution for various aquatic invertebrate groups.

Group	Taxonomic Resolution
Ephemeroptera	Species (Genus only for Leptophelibidae and some species of Heptageniidae)
Odonata	Genus / Species when possible
Megaloptera	Genus / Species when possible
Plecoptera	Species (Genus only for Capniidae, Taeniopterygidae, Chloroperlidae)
Neuroptera	Genus
Hemiptera	Genus / Species when possible

Trichoptera	Genus / Species when possible
Lepidoptera	Genus
Collembola	Genus
Coleoptera	Genus
Diptera	Genus (including Chironomidae)
Amphipoda	Genus
Copepoda	Order
Isopoda	Genus
Ostracoda	Order
Cladocera	Family
Decapoda	Genus / Species when possible
Pelecypoda	Family / Genus when possible
Gastropoda	Genus
Tricladida	Class / Family/ Genus when possible
Hirudinea	Genus / Species when possible
Nematoda	Order
Nematomorpha	Order
Nemertea	Genus
Oligochaeta	Genus / Species for mature specimens
Hydrachnidia	Order

5.3 Reporting Methods

Once laboratory analysis of benthic samples is completed, final data and QC results will be submitted by the contract laboratory in both electronic and hard copy formats. The electronic format will be compatible with Microsoft Excel 97. The reporting timeline usually takes five to six months from submittal of samples to the lab.

6 DATA MANAGEMENT

The data will be maintained in a relational database, which is currently being developed by
King County WLRD. The database will contain (at minimum) the following types of sample
data and habitat information:

- Site name
- Sample date
- Watershed
- Sub-basin
- Geographic location, including GPS coordinates
- Macroinvertebrate identification to lowest practical level
- Metric Scores
- B-IBI Scores
- Quantitative and qualitative habitat data

7 QUALITY ASSURANCE AND QUALITY CONTROL

7.1 Field Sampling

To reduce the chance of sample organisms being lost during field collection, the following QA steps will be implemented.

7.1.1 Discarding Material

Before any rocks are discarded back into the stream, they will be visually inspected to ensure no organisms are still attached or trapped on the surface. Any organisms discovered will be transferred to the sample bottle.

7.1.2 Sampling Equipment

To assure that no organisms remain entrapped on the sampling equipment the following steps will be taken. One team member will turn the Surber sampler net inside out for visual inspection and remove any macroinvertebrates or organic material (e.g., macrophytes, detritus). The plankton bucket and sorting pan will also be inspected for entrapped sample material. All entrapped material will be removed with entomological forceps and placed into the sample bottle.

7.1.3 Duplicate Samples

To provide an indication of intra-site variability, a minimum of 5% of all samples will have a corresponding replicate sample collected. These duplicate samples will be collected in an identical manner as the normal samples and labeled as a "duplicate" on both the external and internal sample bottle labels. It will also be noted on the field data sheet that a duplicate sample was collected.

7.2 Laboratory Analysis

7.2.1 Sample Counts

To provide accurate sample organism counts, the technician at the contract laboratory will pour out the picked organisms into a petri dish and recount the organisms. If a shortage is apparent, the technician will continue the subsampling procedure to obtain the required number of organisms.

7.2.2   Subsampling

The subsampling quality assurance procedure will be performed by a technician who did not perform the original subsampling. The first technician will deliver all petri dishes of sorted substrate to the QA technician, who randomly chooses 10% of the dishes for examination. If fewer than 10 petri dishes (representing 10 of the 30 total Caton grids) are present, the technician will choose at least two dishes. The QA technician will examine the selected dishes under 10-30X magnification via dissecting microscope, and remove organisms missed by the subsampling technician. If more than four organisms are found in any single petri dish, all petri dishes of sorted substrate will be re-examined by the QA technician, and the organisms found in the procedure will be added to the organisms in the sample vial.

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APPENDIX A: B-IBI METRICS

The following are descriptions of the ten metrics used to calculate B-IBI for Puget Sound lowland streams.

Total taxa richness. The biodiversity of a stream declines as flow regimes are altered, habitat is lost, chemicals are introduced, energy cycles are disrupted, and alien taxa invade. Total taxa richness includes all the different invertebrates collected from a stream site: mayflies, caddisflies, stoneflies, true flies, midges, clams, snails, and worms.

Mayfly (Ephemeroptera) taxa richness. The diversity of mayflies declines in response to most types of human influence. Many mayflies graze on algae and are particularly sensitive to chemical pollution (e.g., from mine tailings) that interferes with their food source. Mayflies may disappear when heavy metal concentrations are high while caddisflies and stoneflies are unaffected. In nutrient-poor streams, livestock feces and fertilizers from agriculture can increase the numbers and types of mayflies present. If many different taxa of mayflies are found while the variety of stoneflies and caddisflies is low, enrichment may be the cause.

Stonefly (Plecoptera) taxa richness. Stoneflies are the first to disappear from a stream as human disturbance increases. Many stoneflies are predators that stalk their prey and hide around and between rocks. Hiding places between rocks are lost as sediment washes into a stream. Many stoneflies are shredders and feed on leaf litter that drops from an overhanging tree canopy. Most stoneflies, like salmonids, require cool water temperatures and high oxygen to complete their life cycles.

Caddisfly (Trichoptera) taxa richness. Different caddisfly species (or taxa) feed in a variety of ways: some spin nets to trap food, others collect or scrape food on top of exposed rocks. Many caddisflies build gravel or wood cases to protect them from predators; others are predators themselves. Even though they are very diverse in habit, taxa richness of caddisflies declines steadily as humans eliminate the variety and complexity of their stream habitat.

Intolerant taxa richness. Animals identified as intolerant are the most sensitive taxa; they represent approximately 5-10% of the taxa present in the region. These animals are the first to disappear as human disturbance increases.

Clinger taxa richness. Taxa defined as clingers have physical adaptations that allow them to hold onto smooth substrates in fast water. These animals typically occupy the open area between rocks and cobble along the bottom of the stream. Thus they are particularly sensitive to fine sediments that fill these spaces and eliminate the variety and complexity of these small habitats. Clingers may use these areas to forage, escape from predators, or lay their eggs. Sediment also prevents clingers from moving down deeper into the stream bed, or hyporheos, of the channel.

Long-lived (semi-voltine) taxa richness. These invertebrates require more than one year to complete their life cycles; thus, they are exposed to all the human activities that influence the stream throughout one or more years. If the stream is dry part of the year or subject to flooding, these animals may disappear. Loss of long-lived taxa may also indicate an on-going problem that repeatedly interrupts their life cycles.

Percent tolerant. Tolerant animals are present at most stream sites, but as disturbance increases, they represent an increasingly large percentage of the assemblage. Invertebrates designated as tolerant represent the 5-10% most tolerant taxa in a region. In a sense, they occupy the opposite end of the spectrum from intolerant taxa.

Percent predator. Predator taxa represent the peak of the food web and depend on a reliable source of other invertebrates that they can eat. Predators may have adaptations such as large eyes and long legs for hunting and catching other animals. The percentage of animals that are obligate predators provides a measure of the trophic complexity supported by a site. Less disturbed sites support a greater diversity of prey items and a variety of habitats in which to find them.

Percent dominance (3 taxa). As diversity declines, a few taxa come to dominate the assemblage. Opportunistic species that are less particular about where they live replace species that require special foods or particular types of physical habitat. Dominance is calculated by adding the number of individuals in the three most abundant taxa and dividing by the total number of individuals collected in the sample.

APPENDIX B: HABITAT DATA SHEET

Appendix B provides a copy of the macroinvertebrate habitat data sheet and assists with
interpretation of field measurements.

- **Site Name/Number:** Record the site name (see Section 3.1.6).
- **Location:** Enter nearest road, landmark or access point.
- **Nearest Road X-ing:** Indicate location and distance of nearest road crossing.
- **Personnel:** Name or initials of sampling team members.
- **Date:** Sampling date.
- **Weather:** Current weather (e.g., sunny, cloudy, mostly sunny, etc.).
- **Air Temperature:** Current air temperature (°C).
- **Water Temperature:** Current water temperature (°C).
- **Water Clarity:** Indicate if water is clear or turbid.
- **Stream Width:** Wetted channel width at the location of the riffle.
- **Riffle Length:** Length of the riffle where the sample is being collected.
- **Riffle Depth:** Average depth of the riffle where the sample is being collected.
- **Riffle Flow: 1)** Record the distance between the starting and stopping points.
  2) Record the time it takes the float to travel the measured distance.
- **Riparian Vegetation Type:** "Check" only one box for each bank (left and right).
- **Riparian Vegetation Density:** "Check" only one box for each bank (left and right).
- **Riparian Vegetation Size:** "Check" only one box for each bank (left and right).
- **Woody Debris:** Indicate if present and detail in the notes section.
- **Pebble Count:** Measure 35 pebbles from a transect across the stream at each riffle
  location, for a total of 105 measurements (Wolman 1954).

(Insert Field Habitat Data Sheet – page 1)

(Insert Field Habitat Data Sheet – page 2)

APPENDIX C: LAB PROTOCOLS

The following details Rhithron Biological Associates Subsampling and Quality Assurance Procedures:

When samples require subsampling, we (Rhithron) use the following protocol: Substrate from a single sample is poured out into a subsampling device. At Rhithron, we use Caton subsamplers, which are rectangular stainless steel sieves with a mesh size of 0.367μm. The sieve rests inside an acrylic tray, and is scored into 30 grids each measuring 5cm x 6cm. Use of the Caton subsampler allows the substrate to be floated very slightly above the surface of the sieve, and facilitates removal of grid contents for subsampling.

In either case, the subsampling technician randomly chooses a grid from the 30, and the contents of the grid, substrate and organisms, is lifted out of the subsampling device using a scoop designed for the purpose and a paintbrush, and placed in a petri dish. The dish of substrate is examined under a dissecting microscope with magnification of 10x – 30x, and organisms are sorted from the substrate and counted. Organisms are placed into vials of 70% ethanol; vials are labeled.

To proceed with the subsampling task, the technician stores the petri dish of sorted substrate for subsequent quality assurance procedures, randomly chooses another grid from the subsampling device, and continues sorting and counting organisms until the required number are removed. For the King County samples, we remove a minimum of 500 organisms, when possible, for subsequent identification. All grids selected are thoroughly picked, resulting in sample sizes exceeding 500 organisms in some cases, but assuring a better estimate of the proportion of the total sample used in the subsample. Subsampling records document the number of grids picked, the total number of organisms sorted, and the results of the quality assurance procedure applied to the sample.

A sample count quality assurance procedure is performed by the subsorting technician, and proceeds as follows: The subsampling technician pours out the picked organisms into a clean petri dish and picks and recounts the organisms, returning them to the vial. If a shortage is apparent, the subsampling technician continues the subsampling procedure to obtain the required number of organisms. This count QA not only results in more accurate subsample counts, but also delivers a cleaner collection of organisms to the taxonomist. More experienced subsampling technicians sort the organisms by order or family into spot plates. If after completion of the subsampling, substrate remains in the subsampling device, this material is placed back into the original sample jar. This material is not discarded until the taxonomist has identified and recounted the organisms from that sample. Retention of this unsorted material assures a source of additional organisms should part of the subsample prove to be unidentifiable due to damage.

The subsampling quality assurance procedure is performed by a technician who did not perform the original subsampling. The first technician delivers all petri dishes of sorted substrate to the QA technician, who randomly chooses 10% of the dishes for examination. If fewer than 10 petri dishes (representing 10 of the 30 total Caton grids) are present, the technician chooses at least two dishes. The QA technician examines the selected dishes under 10-30X magnification via dissecting microscope, and removes organisms missed by the

subsampling technician. If more than four organisms are found in any single petri dish, all petri dishes of sorted substrate are re-examined by the QA technician, and the organisms found in the procedure are added to the organisms in the sample vial.