
**Puget Sound Region Benthic Macroinvertebrate
Field Collection and Laboratory Sorting Methods:
River Sampling (2013)
Quality Assurance Project Plan Addendum**

**EPA Puget Sound Science and Technical Assistance Grant under the
2010 Puget Sound Initiative: “Enhancement and Standardization of Benthic
Macroinvertebrate Monitoring and Analysis Tools for the Puget Sound
Region”**

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King County

Department of Natural Resources and Parks
Water and Land Resources Division

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River Sampling (2013)**

Quality Assurance Project Plan Addendum

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Prepared for

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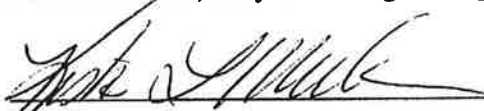
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Introduction and Background

In fall 2010, the King County Department of Natural Resources and Parks (DNRP) was awarded an U.S. Environmental Protection Agency (EPA) Puget Sound Science and Technical Assistance Grant under the 2010 Puget Sound Initiative titled: “Enhancement and Standardization of Benthic Macroinvertebrate Monitoring and Analysis Tools for the Puget Sound Region.” The lack of river sampling and river assessment analysis tools have been identified as a gap in the Puget Sound region by the Puget Sound Partnership Freshwater Workgroup. This document describes in detail the proposed sampling methodologies for a 2013 river field sampling pilot project. The intention of this sampling effort is to collect data to begin to assess the feasibility of sampling fluvial systems larger than those typically targeted in local agency, state, and Tribal bioassessment programs (e.g., watersheds >100 square miles, “non-wadeable” streams and rivers, Strahler stream order 4 or greater) and to start analyzing which biologic metrics (e.g., taxonomic richness, % predators, tolerant richness, etc.) might be the most effective in determining impairment level in these waterbodies in order to move towards a benthic index of biotic integrity (B-IBI) for Puget Sound rivers and large streams.

Site Selection

River samples will be collected from between 10 and 25 locations in August and September 2013. Sites will be selected from rivers draining to Puget Sound based on GIS and field reconnaissance. Where possible, we will join personnel from other local jurisdictions and tribes in the field to minimize reconnaissance time, maximize efficiency, and discover opportunities to standardize data collection across the region.

Sites will be selected to represent the extremes of human disturbance as much as possible (e.g., close to pristine or highly impacted divided into minimal and high urbanization). Streams chosen for this study will have a watershed area greater than typical sites in the Puget Sound Stream Benthos database (>50 mi², ideally > 100 mi²). Sites will also be selected based on the availability of regional groups interested in partnering. Sampling locations will be upstream of saltwater influence. Existing complimentary data such as fish, habitat, water quality, or flow data are desirable, but not required.

Puget Sound is home to over a dozen rivers, which represent the sample universe for this project. Table 1 lists possible river basins for 2013 sampling.

Table 1. Watershed area and discharge for Puget Sound rivers (from Czuba et al. 2011).

River Basin	Watershed Area (mi ²)	Mean Annual Discharge (ft ³ /s)
Skagit	3,200	18,000
Snohomish /Skykomish/ Snoqualmie /Tolt	1,800	10,000
Puyallup/White/Carbon/Greenwater	980	3,600
Nooksack	840	3,200
Nisqually	770	2,100
Stillaguamish	700	2,700
Duwamish/Green	500	1,400
Elwha	320	2,000
Skokomish	250	1,300
Dungeness	200	460
Deschutes	170	400
Samish	120	190
Dosewallips	120	670
Hamma Hamma	80	500
Duckabush	80	570
Big Quilcene	70	180

Field Methods

Field operations will be completed by a minimum of two people to gather macroinvertebrate samples and record basic site information. One kick sample (1 ft² each) will be collected at each of 8 transects for a total of 8 ft² per site. This method is based on Ecology's methods for wide streams and rivers (Merritt et al. 2010) which evolved from methods presented in Hayslip 2007, Peck et al 2006, Lazorchak et al. 2000). Sampling will be conducted while wading upstream if conditions permit or floating downstream if a boat is required for sampling.

More details are outlined in each of the subsections below.

Type of Sampler

For the purposes of this study, a D-frame kick net will be used for sample collection. The D-frame kick net (Figure 1) has a D-shaped frame that is 1 ft wide (along the spine) and 7-12" tall where the widest part of the "D" attaches to a long pole. The net is either cone or bag-shaped for the capture of organisms. The D-net must have a defined or delimited area that is sampled/kicked, which will be 1 ft² for this study.



Figure 1. Sampling gear: D-frame kick net.

Mesh Size

The size of the openings in the sampler net or in the sieves used for cleaning a sample determines the lower size limit of the organisms collected. 500 μm mesh will be used for all nets and sieves in this project. A 500 μm mesh size is consistently used across all states and federal biological assessment programs in the Pacific Northwest and is recommended for use in stream bioassessments regardless of the type of sampler (D-frame kick net or Surber) (Hayslip 2007).

Habitats Sampled and Reach Length

The sample reach will consist of 11 transects, with 1 ft^2 benthic macroinvertebrate collections from the littoral area of one bank for a total sampled surface area of 8 ft^2 .

Compositing

Compositing takes multiple macroinvertebrate collections from the study reach and combines them into a single sample that is sent to a taxonomic laboratory for enumeration and identification. Compositing collections is a commonly used practice in state bioassessment programs across the United States (Carter and Resh 2001) and has the advantage of being less expensive than processing many samples per location. The 8 kicks will be composited into one sample when habitats are relatively homogenous. To allow for comparison of stream flow (slack vs. moving water) and habitat (e.g., substrate type), kicks can be composited into similar-habitat composite samples (as determined by the best professional judgment of the collector) and these will be labeled accordingly.

Treatment of Large Taxa

Crayfish, snails, and mussels will likely be collected in some samples. Freshwater mussels are long-lived species that are on the decline throughout North America. Therefore, these organisms will be pulled out of the sample and returned to the stream. Their presence, identification, and abundance will be noted and photographs taken. In contrast, crayfish and snails will be included in the sample if collected. Crayfish are not known to be declining in the Pacific Northwest and there are concerns about the spread

of both invasive crayfish and snails. Collecting these organisms and getting identifications from qualified laboratories will help with early detection if invasive species are observed.

Determine Site Suitability

There may be some instances or conditions that make a site unsuitable for sampling. A site should not be sampled if any of the following conditions exist: it is unsafe to enter, access permission is denied by the land owner, the body of water is not a stream or river (e.g., a wetland or a lake), the water is not freshwater, or there is not sufficient water volume or flow to wash organisms over the lip of the sampling device into the net. If a site is visited, but cannot be sampled, the reason for not sampling will be noted along with the date and personnel.

Sample Collection: Step by Step Details

For each 1 ft² sample collection at the margin of 8 of the 11 transects, the D-net opening will be placed so that the net opening faces into the stream flow. The net will be secured on the stream bottom to eliminate any gaps under the frame. All large material (e.g., large gravel, cobble, boulders, and woody debris) within the 1 ft² sampling area that inhibit secure placement of the net will be scrubbed by hand so that the organisms are washed into the collection net. The 1 ft² sampling area can be delineated by a sampling frame or it can be visually imagined as a square plot in front of the net. After scrubbing and before being placed outside the sampling area, these large materials will be visually inspected for additional attached organisms and attached macroinvertebrates will be placed into the collection net. If a rock is lodged in the stream bottom, it will be rubbed a few times concentrating on any cracks or indentations. After removal and processing of any large stones or debris, the 1 ft² sampling area will be agitated to a depth of approximately 10 cm (3.9 in) for 60 seconds (King County 2002, Adams 2010) to suspend the substrate and any associated macroinvertebrates into the water column allowing the water flow to carry the macroinvertebrates into the net. This step can be accomplished by kicking with the feet or using a sturdy trowel, screw driver, piece of rebar, or garden tool to stir up the substrate in the 1 ft² area directly in front of the net.

The net is then moved to the next upstream collection location and this process will be repeated until all eight 1 ft² samples are cumulatively sampled into one net or composited as described above. Once the samples have been collected, the net will be removed from the water and processed. Sediments and organisms will be washed to the end of the net by immersing the net in the stream flow or by pouring water down the outside of the net taking care to avoid having any water or material enter the mouth of the net. The contents of the net and collection cup (if applicable) will be carefully placed in a 500 µm mesh sieve. Rocks and debris too large to fit into the sample jars will be rinsed with filtered stream water into the sieve. This large material will be visually examined and all observed organisms will be removed using forceps and placed in the plastic wide-mouth sample containers. The remaining contents of the sieve will be washed and concentrated to one side of the sieve using the spray bottles or by gently agitating the sieve in the water being careful not to lose any of the contents. Carefully transfer this material to the sample container using spoons or spatulas trying to minimize the amount of water washed into the sample container. Perform a close visual inspection of the net, sieve, and collection cup (if applicable) for any remaining organisms and use forceps to transfer these to the sample container.

Sample containers will be labeled both inside with pencil on rite in the rain paper and outside using permanent marker on pre-printed labels. Label information will contain at a minimum the site ID, sampling date, surface area sampled, flow description (flowing, slack water), habitat description, partner agency, and sampling personnel. If more than one sample container is required because of the amount of material collected, this will be indicated clearly on the labels. For example, if a single sample comprises 2 containers, two separate labels are required specifying 1 of 2 and 2 of 2 for each sample container in addition to the usual labeling information.

Rinse the net thoroughly after each site to avoid cross-contamination. The sample contents will be preserved in the field with 95% denatured ethanol¹ adding two parts by volume for each part sample. Samples will be transferred to a secure storage area and logged into a chain of custody data sheet.

Laboratory Methods

Once all project samples are collected, they will be either shipped to or picked up by a taxonomic laboratory that specializes in the identification of macroinvertebrates and is familiar with the taxa from the Pacific Northwest. 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.

Upon arrival at the taxonomy laboratory, the samples will be checked against the inventory sheet and chain of custody information. The procedures described below (subsampling, large and rare search, and taxonomic identification) will be conducted for each sample.

Subsampling

The taxonomic laboratory will process samples from each site into fixed-count 500 minimum subsamples. Subsampling is used to reduce the cost and time associated with processing benthic samples (Barbour et al. 1999) with the goal of providing an unbiased representation of a larger sample (Barbour and Gerritsen 1996).

Standard sorting protocols (Plotnikoff and Wiseman 2001) will be applied to achieve representative subsamples of a minimum of 500 organisms. Caton subsampling devices (Caton 1991), divided into 30 grids, each approximately 5 cm by 6 cm will be used. Each individual sample will be thoroughly mixed in its sample container(s), poured out and evenly spread into the Caton tray, and individual grids will be randomly selected. The contents of each grid will be examined under stereoscopic microscopes using 10x-30x magnification. All aquatic invertebrates from each selected grid will be sorted from the substrate, and placed in 95% ethanol for subsequent identification. Grid selection, examination, and sorting will continue until at least 500 organisms are sorted. When samples contain less than 500 organisms, the entire sample will be sorted.

¹ The ideal preservative is 95% denatured ethanol, however 70% or greater isopropyl alcohol or ethanol are acceptable as long as the sample is preserved with two parts alcohol for each one part sample.

Large and Rare Search

After the target number of organisms (500) is obtained in the subsample, the remainder of the sample material will be scanned in the Caton tray for a maximum of 15 minutes to find any large or rare taxa that may have been missed during the subsampling procedures. These organisms will be placed in a separate vial and labeled as “Large/Rare Organisms”, and they will be reported in the data uploaded to the PSSB database tagged as large/rare.

Taxonomic Identification and Resolution

Organisms will be individually examined by certified taxonomists, using 10x – 80x stereoscopic dissecting scopes (Leica S8E and S6E) and identified to the lowest practical taxonomic level² using appropriate published taxonomic references and keys. Identification, counts, life stages, and information about the condition of specimens will be recorded on bench sheets. Organisms that cannot be identified to the taxonomic targets because of immaturity, poor condition, or lack of complete regionally-applicable published keys will be left at appropriate taxonomic levels that are coarser than those specified. To obtain accuracy in richness measures, organisms will be designated as “not unique” if other specimens from the same group could be taken to target levels. Organisms designated as “unique” will be those that can be definitively distinguished from other organisms in the sample. Identified organisms will be preserved in 95% ethanol in labeled vials, and archived at the Rhithron laboratory for a minimum of 1 year.

Data Uploading, Analysis, and Reporting

Taxonomic data and counts will be uploaded into the Puget Sound Stream Benthos (PSSB) data management system (www.pugetsoundstreambenthos.org) by the taxonomic laboratory (Figure 4). The PSSB will calculate the B-IBI and individual metrics for each site and these will be compared for reference vs. disturbed sites to determine which metrics effectively distinguish impairment (see methods in Royer et al. 2001 for proposed analysis framework).

Data collected during the 2013 sampling effort will be analyzed and presented in a technical memorandum by summer 2014. The data for this sampling will also be presented in a comprehensive report that summarizes the larger EPA granted project due by October 2014.

Personnel Qualifications

Samples will be collected by those identified in the sub-permittees section of the Scientific Collection Permit. Sampling personnel have participated in invertebrate collection in previous years and have been trained on all sampling procedures and etiquette involved in minimizing impacts to the stream areas being sampled. Relevant publications from project personnel follow the qualifications table.

² Taxonomic identification in 2013 will match the resolution used for Ecology (lowest practical for all organisms including Chironomidae, Acari, and Oligochaetes).

Name	Title	Qualifications
Jo (Jennifer) Wilhelm	Environmental Scientist III, King County Water and Land Resources Division (WLRD)	Jo currently works in urban, agricultural, and forested watersheds to monitor and assess stream and wetland condition. She has over 16 years of experience working on ecology, restoration, and environmental education/outreach related projects. Macroinvertebrate sampling experience on small to medium streams in Michigan for three field seasons and in Washington for eight field seasons. Trained through aquatics coursework, research, and on the job. Studied with Dave Allan, Rich Merritt and Ken Cummins, world-wide experts on stream assessment, functional feeding group, and invertebrate taxonomy. Managed or co-managed numerous King County macroinvertebrate sampling protocols from 2005 onward including the ambient program (~ 140 sites annually), Urban Planned Development monitoring (~ 7 sites through 2010), Mercer Island (~ 5 sites through 2008), Regulatory Effectiveness (9 sites 2009-present). Subject matter expert for development of the Puget Sound Stream Benthos data management system (www.pugetsoundstreambenthos.org). Swiftwater training renewed in 2012. Education: M.S. in Resource Ecology with a focus on aquatics from the University of Michigan, B.A. in Biology from Macalester College. Relevant Publications - Wilhelm et al. 2005, Wessell et al. 2008, King County 2009b.
Deb Lester	Water Quality Planner III & Lead: Toxicology & Contaminant Assessment Group, King County WLRD	Deb manages WLRD's Toxicology and Contaminant Assessment Group and has over 28 years of experience working on various aspects of aquatic ecology and water quality related projects. She manages WLRD's Benthic Macroinvertebrate and Stream Monitoring programs. MS, Univ. of Vermont, School of Natural Resources. Relevant Publications - King County 2009a, 2009b.
Leska Fore	Statistician, Statistical Design	Leska has developed biological monitoring protocols and survey designs for fish, invertebrates and diatoms in rivers, streams, lakes, and coral reefs. Her company, Statistical Design, has worked with local, state and federal governments to bring science into the policy arena for 15 years. MS, Univ. of Washington, Quantitative Ecology and Resource Management. Relevant Publications – Fore et al. 1994, 1995, 1996, 2001.
Chris Gregersen	Environmental Scientist I, King County WLRD	Sampled benthic macroinvertebrates for the UPD, Regulatory Effectiveness, and King County ambient projects annually since 2008. B.S. in fisheries from Washington State University. Swiftwater training renewed in 2011.
Chris Knutson	Water Quality Planner I, King County WLRD	Sampled benthic macroinvertebrates for the UPD, Regulatory Effectiveness, and King County ambient projects annually since 2009. B.S. in environmental planning from Western Washington University. Swiftwater Training renewed in 2011.
Hans Berge	Environmental Scientist III, King County WLRD	Over 19 years experience sampling macroinvertebrates, fish, and habitat on small streams and large rivers including co-principal investigator on an EPA grant for WRIA 08 monitoring and assessment. Recognized by the American Fisheries Society as a Certified Fisheries Professional. M.S. in Fisheries and Aquatic Sciences from the University of Washington, and a B.S. from Utah State in Fisheries and Wildlife
Dan Lantz	Environmental Scientist II, King County WLRD	Worked for 11 years with US Fish and Wildlife Service where he led many types of fish and habitat surveys around the Northwest, including surveys in the Upper Sacramento River, Lake Washington, the Ship Canal, and the Cedar River. Worked for King County for two years on kokanee recovery in Lake Sammamish, the EPA WRIA 8 Status and Trends Monitoring grant, and conducting fish and habitat monitoring with Rivers and CIP staff. B.S. in Biology from Central Washington University.

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Appendix A: Equipment and Supplies Required for Sample Collection

The following are suggested lists of equipment needs for macroinvertebrate sample collection:

- Wide-mouth polyethylene jars (0.5 - 2 L) with screw caps
- One or Two 500 μm mesh D-frame kicknets
- Small rake, trowel, screw driver, piece of rebar, etc with marking tape at 10 cm for agitating the substrate
- Two sieves with 500 μm mesh
- Wash bottle, 1-L capacity
- Funnel lined with 500 μm mesh (for filling wash bottle or washing sieve)
- Plastic wash tub, dish pan or bucket
- Small spatula, scoops or spoons for transferring the sample
- Forceps
- Rubber gloves
- 95% denatured ethanol (add 2 parts by volume for each part sample)
- Interior rite-in-the rain labels
- Pre-printed exterior labels
- Soft-lead pencil
- Permanent markers (e.g., Sharpies)
- Clear tape
- Pocket knife
- Wading gear
- Field data forms on rite-in-the-rain paper
- Measuring tape (50-meter or longer)
- Stopwatch or timing device
- Flagging
- Camera to photograph site and surrounding environment
- Cooler for storing samples and ethanol
- Clipboard
- Large gear bag or bin
- Thermometer
- Coast guard approved personal flotation device
- Boat (may be a raft, inflatable kayak, or other suitable boat)