Puget Sound Region Benthic Macroinvertebrate Field Collection and Laboratory Sorting Methods for a Side by Side Comparison of 3 ft^2 versus 8 ft^2

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King County

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Introduction and Background

In fall 2010, the King County Department of Natural Resources and Parks (DNRP) was awarded a 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." One of the tasks outlined in the grant proposal was a side-by-side comparison of 3 ft^2 versus 8 ft^2 sampling areas. This document describes the proposed sampling methodologies for the 2011 field sampling seasons. The purpose of this sampling effort is to collect data sufficient to compare impacts of field sample collection methods on benthic macroinvertebrate metrics that are typically used to calculate a benthic index of biotic integrity (B-IBI).

The Washington Department of Ecology (Ecology)[1] (Plotnikoff and Wiseman 2001, Cusimano et al. 2006), The Pacific Northwest Aquatic Monitoring Partnership (PNAMP) (Hayslip 2007) and the EPA (Klemm et al. 2006) utilize and recommend collecting macroinvertebrates from at least 8 ft^2. However, despite these recommendations, many local entities are reluctant to shift sampling protocols due to the risk of orphaning their existing long-term data sets from numerous site-visits collected from 3 ft^2. Sample collection from a larger surface area generally results in collection of a greater variety of taxa and an increase in species richness index values, regardless of the analytical method used (Cazier 1993, Vinson and Hawkins 1996). Thus, there is a need to establish a cross-walk between 3 ft^2 and 8 ft^2 methods to ensure that results reported from each method can be compared and reported interchangeably.

The goal of this sampling effort is to collect sufficient data to 1) determine if data collected from 8 ft^2 and 3 ft^2 are comparable, and 2) if they are not comparable develop a conversion algorithm or 'cross-walk' so that data (and associated B-IBI metrics) collected from both 8 ft^2 and 3 ft^2 at a given site can be readily compared. The algorithm will be developed from data associated with collection of side by side samples of 3 ft^2 and 8 ft^2 areas from the same stream. This will allow jurisdictions within the Puget Sound region to transition to collection of 8 ft^2 samples without losing the ability to track long term trends based on historical data collected from 3 ft^2 areas. In addition, the cross-walk will enable direct comparison of a larger pool of regional data and in doing so will promote data integration to evaluate ecosystem conditions across jurisdictional boundaries, a Puget Sound Partnership goal.

Site Selection

Side by side samples will be collected from approximately 50 locations in August and September 2011 and will be confirmed by additional sampling in 2012 if necessary[2]. Sites will primarily be selected from

[1] Ecology conducts both ambient (Plotnikoff and Wiseman 2001) and status and trends macroinvertebrate monitoring (Cusimano et al. 2006), in addition to special study monitoring but all rely on sampling 8 ft^2 of surface area.

[2] The cross-walk algorithm will be developed from a group of 'development' sites in 2011 (approximately 40) and will be confirmed by a group of independent 'test' sites if deemed necessary.

over 1,100 existing sample locations from the Puget Sound Stream Benthos database to limit property access issues and minimize the need for additional site reconnaissance. Where possible, we will join personnel from other local jurisdictions and tribes in the field during sample collection to discover opportunities to standardize data collection across the region.

Sites will be selected to represent a range of human disturbance (e.g., ranging from close to pristine to highly impacted). Analysis of existing macroinvertebrate data and natural features indicates that elevation, channel slope (gradient), and watershed area (up to at least 50 km^2 [19 mi^2]) do not have a consistent influence on B-IBI values across a range of urbanization, and therefore the sampling design will not address these factors (Fore 2011). Streams chosen for this effort will be representative of typical sites in the Puget Sound Stream Benthos (PSSB) database (2-30 mi^2 watershed area). Sites will also be selected based on the availability of (1) regional groups interested in partnering, or (2) suitable riffle habitat or other non-depositional, flowing aquatic habitat. Finally, where possible, sites with a history of sufficient organism counts (> 350) will be selected. Other complementary data such as fish, habitat, water quality, or flow data are desirable, but not required.

Field Methods

Field operations will be completed by a minimum of two people to gather macroinvertebrate samples and record basic site information. At each sampling station, macroinvertebrate samples will be collected from a total surface area of 8 ft^2 sampled across multiple riffles or fast-moving, non-depositional aquatic habitats using a Surber sampler or D-frame kick net with 500 µm mesh. These samples will be collected 1 ft^2 at a time divided into two sample containers for each site: one collected from 3 ft^2 and one from 5 ft^2. Sampling methods will generally follow Ecology's sampling protocol for regulatory purposes (Adams 2010) with some modifications. More details are outlined in each of the subsections below.

Type of Sampler

The most commonly used benthic macroinvertebrate collection devices in the Pacific Northwest are the D-frame kick net and Surber sampler (Figure 1). Both types of gear will work for the methods described in this document, and direct comparisons have found that samples with the same number of individuals are highly and consistently comparable between sampling devices (Barton and Metcalfe-Smith 1992, Cazier 1993, Cao et al. 2005). For the purposes of this study, either D-frame kick nets or Surber samplers can be used for sample collection; however, all samples at a single site will be collected with the same type of gear (i.e., either a D-frame kick net or a Surber sampler).

Figure 1. Sampling gear: D-frame kick net (left) and Surber sampler (right).

The D-frame kick net (Figure 1) has a D-shaped frame that is 1 ft. wide (along the spine) and 1 ft. 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 ft^2 for this study.

The Surber sampler (Figure 1) frame is 1ft tall and 1 ft. wide with a delineated sampling area in front of the net that is 1 ft^2. A vertical section of the frame has the net attached and captures the dislodged organisms from the sampling area. The use of the Surber is generally restricted to water depths of less than 1 ft.

Mesh Size

The size of the openings in the sampler net or in the sieves used for processing a sample determines the lower size limit of the organisms collected. 500 µm mesh size 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 bioassessment regardless of the type of sampler (D-frame kick net or Surber) (Hayslip 2007).

Habitats Sampled and Reach Length

Field methods will be standardized by concentrating macroinvertebrate sampling in a single, readily identifiable habitat: riffles or fast moving, non-depositional aquatic habitats (henceforth this document will use the term riffle for simplicity). Riffles have relatively fast currents, moderate to shallow depth, and cobble or gravel substrates and they are common features of wadeable streams throughout the Puget Sound region. No samples will be collected from areas where there is not sufficient current to extend the net in a downstream direction.

The sample reach will generally consist of sampling four distinct riffle habitats, with two 1 ft^2 collections from each riffle for a total sampled surface area of 8 ft^2 (Figure 2). However, at sites where four distinct

riffle habitats are not present, samples will be collected from fewer riffles. For example, if only two riffle habitats are present, then four 1 ft^2 samples will be collected from each of the two riffle habitats. If three riffle habitats are present, then the samples will be distributed so that there are two to three 1 ft^2 samples collected in each riffle for a total sampled surface area of 8 ft^2. Within each riffle, two 2 ft^2 samples can be collected side-by-side simultaneously or in cases of narrow streams, they can be collected up- and downstream stream at different times.

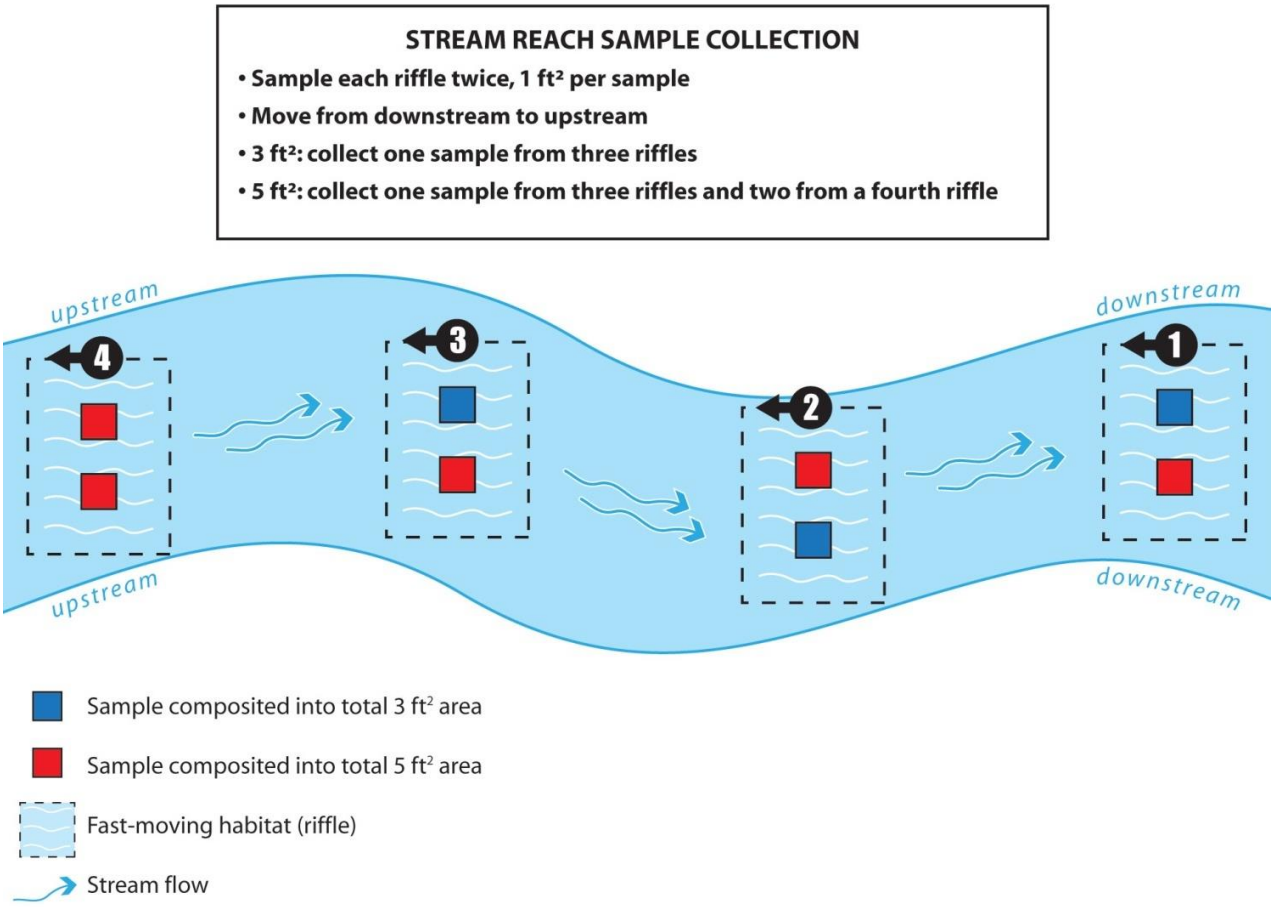

Figure 2. Schematic of stream reach sample collection procedures. Eight 1 ft^2 samples are collected from up to four riffles for a total sample area of 8 ft^2.

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. To allow for side by side comparison, collections for this study will be composited into two sample jars per site representing: (1) a 3 ft^2 sample from three riffles and (2) a 5 ft^2 sample from four

riffles (Figure 2). Overall, two separate 1 ft^2 fixed-area samples will be taken from four different riffle habitats for a total of 8 ft^2 of stream bottom sampled.

Placement of Sampling Device

Once the riffle habitat is identified for sample collection, the location within each riffle where the sampler is placed will be determined using best professional judgment following these guidelines:

- Sample within the stream's main flow,
- Avoid bedrock areas or substrates dominated by rocks larger than 12 inches (30.5 cm),
- Avoid the transition zone from the riffle to a downstream pool or other habitat (Karr and Chu 1999),
- Position the sampling device so that at least two 1 ft^2 collection areas can be sampled, if possible.

Sampling device placement should be done quickly and should not require lengthy discussion or analysis.

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, there is not sufficient water volume or flow to wash organisms over the lip of the sampling device into the net, year round perennial flow is unlikely, or no riffle habitats are present.

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

Once the sampling station is located, sampling will begin at the first riffle habitat encountered and will continue upstream with the next 3 fast-water habitat units. The stream reach is defined by the distance from the downstream riffle to the upstream most riffle sampled and ideally the entire length will have

similar gradient, valley shape, and riparian land cover throughout without any major tributaries joining the stream. Any exceptions to these conditions will be noted.

For each 1 ft^2 sample collection, the Surber sampler or D-net opening will be placed in the riffle 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 ft^2 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 ft^2 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 ft^2 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, screwdriver, piece of rebar, or garden tool to stir up the substrate in the 1 ft^2 area directly in front of the net. Front to back agitation is preferred as compared to side to side to ensure most organisms are washed into the net rather than downstream.

The net is then moved to the next upstream collection location (i.e. riffle), and this process will be repeated until the appropriate numbers of individual 1 ft^2 samples (3 or 5) are cumulatively sampled into one net (Figure 3). Once the desired sample area (3 or 5 ft^2) has 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 that might introduce new organisms. 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. This material will be carefully transferred to the sample container using spoons or spatulas, trying to minimize the amount of water washed into the sample container. A close visual inspection of the net, sieve, and collection cup (if applicable) will be performed for any remaining organisms, and forceps will be used to transfer these to the sample container.

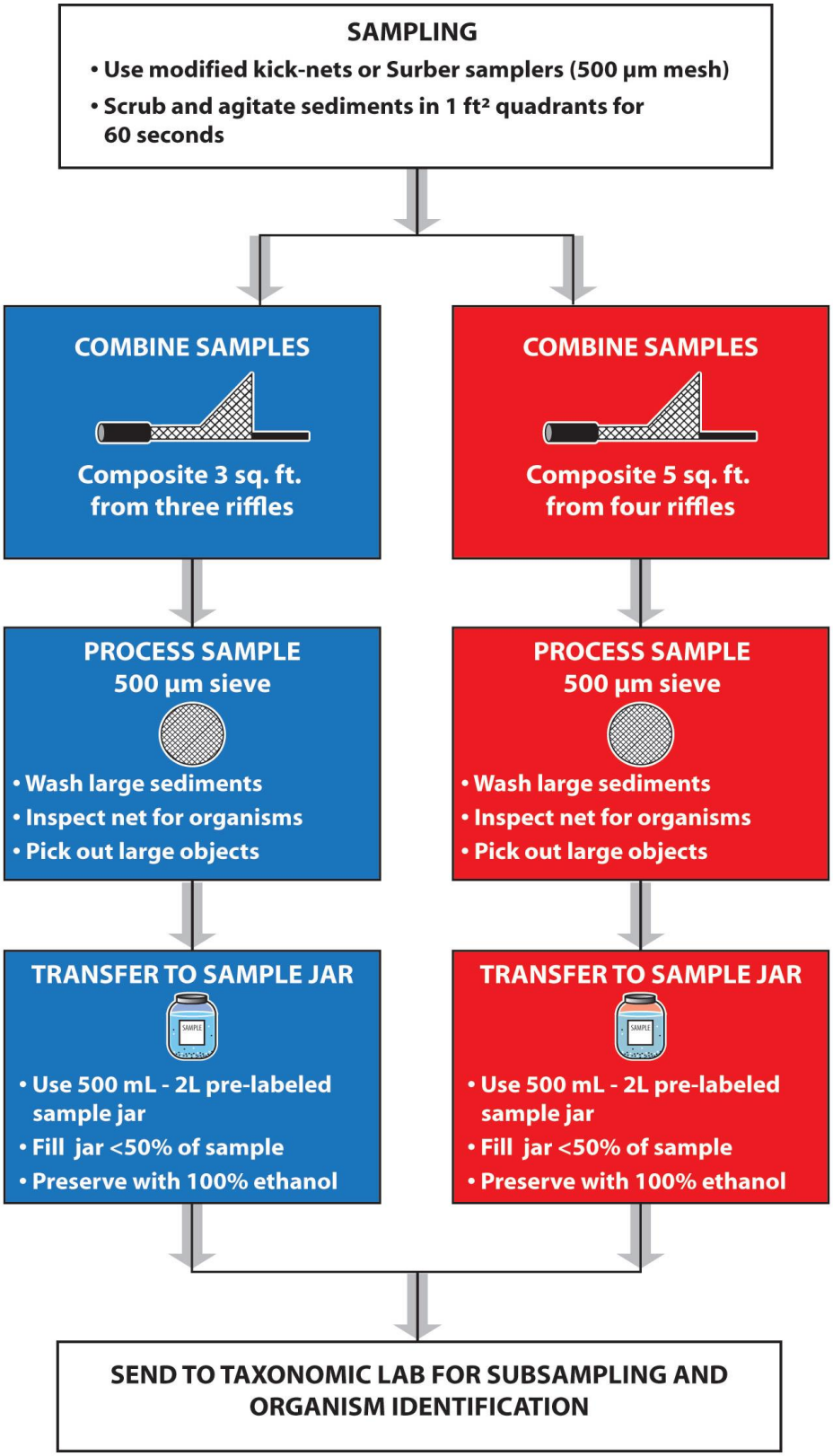

Figure 3. Schematic of stream compositing and sampling procedures.

Sample containers will be labeled both inside with pencil on waterproof 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 (3 or 5 ft^2), partner agency, and sampling personnel. The outside label will be covered with clear tape. 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.

The net will be rinsed thoroughly after each site to avoid cross-contamination. If sites with known New Zealand mud snail populations are sampled, secondary decontamination precautions will be taken following one of the methods outlined by WDFW (2011). The sample contents will be preserved in the field with 95-100% denatured ethanol[3], adding a minimum of 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 picked up by a taxonomic laboratory that specializes in the identification of macroinvertebrates and employs staff certified as competent in identifying taxa from the Pacific Northwest. 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 site on the 3 ft^2 sample first. Once these steps are completed, the 3 ft^2 and the 5 ft^2 samples will be combined (including those specimen picked out and identified in the 3 ft^2 sample) and the same steps will be conducted on this composited 8 ft^2 sample (Figure 3).

Subsampling

The taxonomic laboratory will process the two samples from each site into two fixed-count 500 minimum subsamples: one from 3 ft^2 and one from 8 ft^2 (composited from the 3 and 5 ft^2 samples). 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

[3] 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 at least two parts alcohol for each one part sample.

substrate and placed in 95-100% ethanol for subsequent identification. Grid selection, examination, and sorting will continue until at least 500 organisms are sorted. When 500 organisms are reached, the sorting continues until the current grid cell has been completely searched. When samples contain less than 500 organisms, the entire sample will be sorted.

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.

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 level[4] 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 95-100% ethanol in labeled vials, and archived at the taxonomic laboratory for a minimum of 1 year.

[4] Taxonomic identification in 2011 will match the resolution used for Ecology samples in 2010 (lowest practical for all organisms including Chironomidae, Acari, and Oligochaetes).

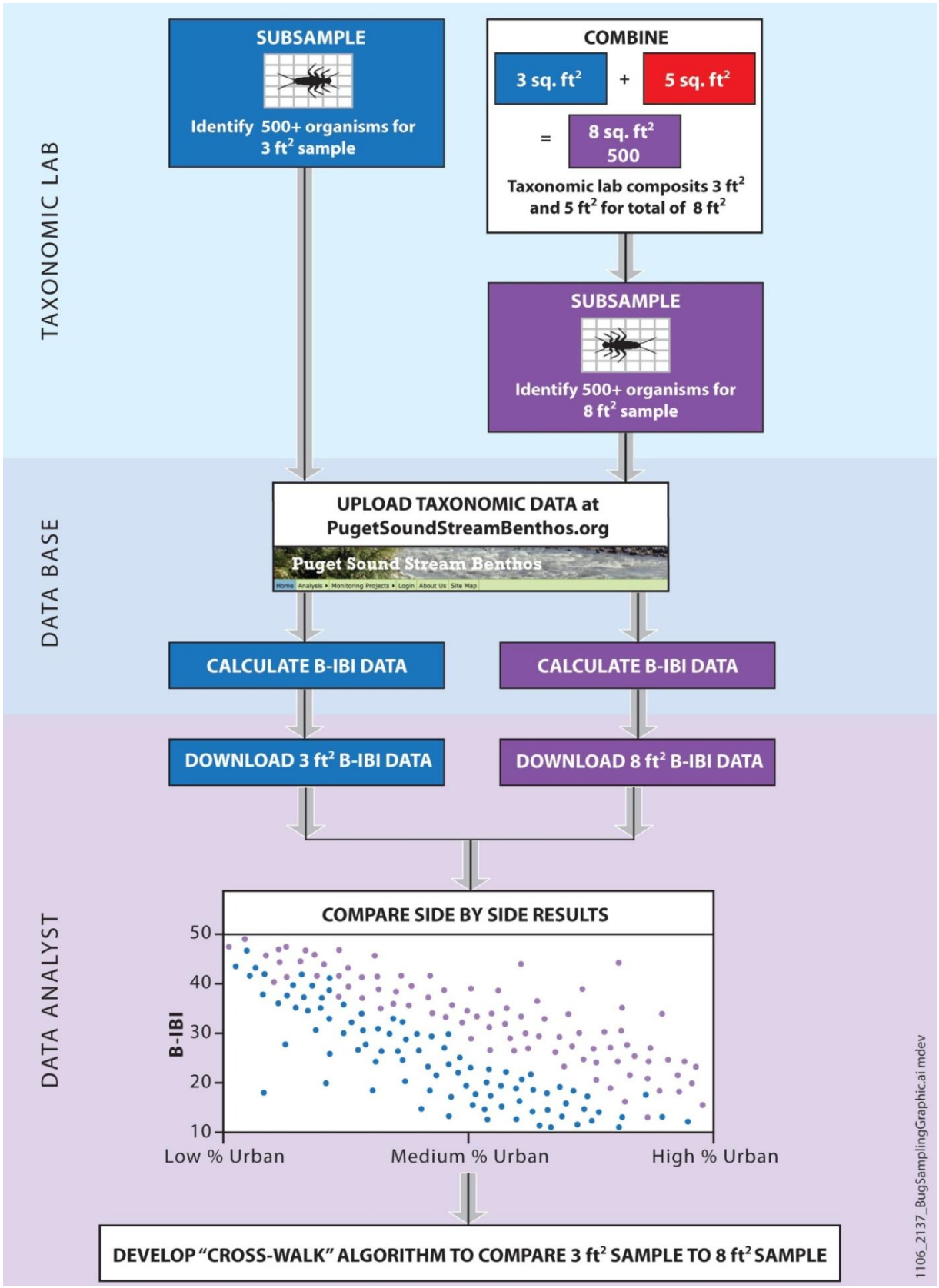

Figure 4. Schematic of laboratory procedures and subsequent data management and analysis.

Data Uploading, Analysis, and Reporting

Taxonomic data and counts will be uploaded into the 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 for both 3 ft^2 and 8 ft^2 surface areas. These data will be available for download as (1) a 500-count 3 ft^2 sample and (2) a 500-count 8 ft^2 sample to allow direct comparison of individual metrics and total B-IBI scores across a range of urban development. Based on the relationship of B-IBI scores from 3 ft^2 versus 8 ft^2 from approximately 40 sites, a cross-walk algorithm or model will be developed if necessary. If an algorithm is required, it will be confirmed against additional sites collected in 2012. The goal is to be able to compare total B-IBI scores regardless of sample area collected and encourage organizations to move towards the 8ft^2 sample area recommended by federal and state agencies.

Field data collected during the 2011 sampling effort and if needed a 2012 sampling effort will be analyzed and presented in a technical memorandum.

References

Adams, K. 2010. Standard operating procedures and minimum requirements for the collection of freshwater benthic macroinvertebrate data in wadeable streams and rivers. Prepared by Environmental Assessment Program, Washington Department of Ecology, Olympia, Washington.

Barbour, M. T. and J. Gerritsen. 1996. Subsampling of Benthic Samples: A Defense of the Fixed-Count Method. Journal of the North American Benthological Society **15**:386-391.

Barbour, M. T., J. Gerritsen, B. D. Snyder, and J. B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates and fish, second edition. EPA 841-B-99-002, U.S. Environmental Protection Agency, Office of Water, Washington, D.C.

Barton, D. R. and J. L. Metcalfe-Smith. 1992. A comparison of sampling techniques and summary indices for assessment of water quality in the Yamaska River, Québec, based on benthic macroinvertebrates. Environmental Monitoring and Assessment **21**:225-244.

Cao, Y., C. P. Hawkins, and A. W. Storey. 2005. A method for measuring the comparability of different sampling methods used in biological surveys: implications for data integration and synthesis. Freshwater Biology **50**:1105-1115.

Carter, J. L. and V. H. Resh. 2001. After Site Selection and before Data Analysis: Sampling, Sorting, and Laboratory Procedures Used in Stream Benthic Macroinvertebrate Monitoring Programs by USA State Agencies. Journal of the North American Benthological Society **20**:658-682.

Caton, L. W. 1991. Improving subsampling methods for the EPA's "Rapid Bioassessment" benthic protocols. Bulletin of the North American Benthological Society **8**:317-319.

Cazier, L. D. 1993. Bioassessment of small agricultural streams: the Palouse-Camas Prairie Region of Northern Idaho. University of Idaho, Moscow, Idaho.

Cusimano, R., G. Merritt, R. Plotnikoff, C. Wiseman, C. Smith, and Washington State Department of Fish and Wildlife. 2006. Status and Trends Monitoring for Watershed Health and Salmon Recovery: Quality Assurance Monitoring Plan. Prepared by Environmental Assessment Program, Washington State Department of Ecology, Washington State Conservation Commission, and Washington Department of Fish and Wildlife, Olympia, Washington. Ecology Publication No. 06-03-203.

Fore, L. 2011. Memorandum to Deb Lester and Jo Wilhelm, King County: Effect of natural features on B-IBI and recommendations for the 2011 sampling design. Statistical Design, Seattle, Washington.

Hayslip, G., editor. 2007. Methods for the collection and analysis of benthic macroinvertebrate assemblages in wadeable streams of the Pacific Northwest. Pacific Northwest Aquatic Monitoring Partnership (PNAP), Cook, Washington.

Karr, J. R. and E. W. Chu. 1999. Restoring life in running waters: better biological monitoring. Island Press, Washington, DC.

King County. 2002. Sampling and analysis plan: Ambient streams and rivers routine monitoring, ambient streams and rivers wet weather monitoring, and SWAMP streams and rivers trace metals and organics monitoring., Prepared by Bob Kruger, King County Environmental Laboratory, King County Water and Land Resources Division, Seattle, Washington.

Klemm, D. J., J. M. Lazorchak, and D. V. Peck. 2006. Section 10 Benthic Macroinvertebrates.*in* D. V. Peck, A. T. Herlihy, B. H. Hill, R. M. Hughes, P. R. Kaufmann, D. J. Klemm, J. M. Lazorchak, F. H. McCormick, S. A. Peterson, P. L. Ringold, T. Magee, and M. R. Cappaert, editors. Western Pilot Study: Field operations manual for wadeable streams. Office of Research and Development, U.S. Environmental Protection Agency, Washington D.C.

Plotnikoff, R. W. and C. Wiseman. 2001. Benthic Macroinvertebrate Biological Monitoring Protocols for Rivers and Streams. Washington Publication No. 01-03-028, Environmental Assessment Program, Department of Ecology, Washington Publication No. 01-03-028, Olympia, Washington.

Vinson, M. R. and C. P. Hawkins. 1996. Effects of Sampling Area and Subsampling Procedure on Comparisons of Taxa Richness among Streams. Journal of the North American Benthological Society **15**:392-399.

WDFW. 2011. Invasive species management protocols. Prepared by the Washington Department of Fish and Wildlife, Invasive Species Management Committee: Bill Tweit, Allen Pleus, Dave Heimer, Marc Hayes, Carl Klein, John Kerwin, Jesse Schultz, Larry Phillips, Bill Hebner, Annette Hoffmann, Mike Schmuck, Stacie Kelsey, and Rachel McDaniel, Olympia, Washington.

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
- Two 500 µm mesh sampling devices: either 1ft^2 Surber samplers or 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
- 95-100% denatured ethanol (add at least 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

Appendix B: Example Data Sheet

A two page data sheet is shown on the following pages.

Site Name/Number _____	Personnel: _____
Partner Organization _____	Date & Time: _____
Location Description _____	PSSB Data Entry: Check when complete ☐
_____	Date: _____ Initials: _____

Weather & Water Conditions	Current Weather: ☐ rain ☐ mostly cloudy (>50%) ☐ partly cloudy (10-50%) ☐ sunny	> 0.5" rain in last 24 hours? **Y** **N** Air Temperature (°C): _____ Water Temperature (°C): _____ Water Clarity: ☐ Clear ☐ Turbid/Opaque

Sampling Metadata	Reach length _____ ft Sampling Device: ☐ D-frame ☐ Surber # riffles sampled: **1** **2** **3** **4** Sample collected? **Y** **N**

Riffle Data

Sample Unit:
FT = Fast Turbulent (riffle, cascade, waterfall);
FN = Fast Non-Turbulent (sheet, run, glide)

Dominant Substrate:
(Snd) Fines/Sand
(Grvl) Gravel
(Crs) Coarse
(Oth) Other

Sample #	3 or 5 sq ft		Sample Unit		Substrate				Riffle Depth	Riffle #
1	3	5	FT	FN	Snd	Grvl	Crs	Oth	_____	_____
2	3	5	FT	FN	Snd	Grvl	Crs	Oth	_____	_____
3	3	5	FT	FN	Snd	Grvl	Crs	Oth	_____	_____
4	3	5	FT	FN	Snd	Grvl	Crs	Oth	_____	_____
5	3	5	FT	FN	Snd	Grvl	Crs	Oth	_____	_____
6	3	5	FT	FN	Snd	Grvl	Crs	Oth	_____	_____
7	3	5	FT	FN	Snd	Grvl	Crs	Oth	_____	_____
8	3	5	FT	FN	Snd	Grvl	Crs	Oth	_____	_____

Notes (regarding riparian condition, landuse, buffer width, sampling details, etc.)	

Schematic of Sampling (Hand draw approximate location of samples collected and location of riffles)