Enhancement and Standardization of Benthic Macroinvertebrate Monitoring and Analysis Tools for the Puget Sound Region

Quality Assurance Project Plan

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1. Distribution List

This Quality Assurance Project Plan (QAPP) will be distributed to the people listed in Table 1.

Table 1. List of QAPP recipients and associated contact information.

Name	Title/Affiliation	Phone Number
Karen Adams	Washington Department of Ecology	360-407-6530
Gretchen Hayslip	US Environmental Protection Agency	206-553-1685
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Ginna Grepo-Grove (USEPA R10 QA Manager)	US Environmental Protection Agency	206-553-1632

2. Project/Task Organization

The project involves staff from the King County Department of Natural Resources and Parks (DNRP) with collaborators from the US Environmental Protection Agency (EPA) and the Washington Department of Ecology (Ecology). Project staff also includes Leska Fore (Statistical Design) and Bob Wisseman (Aquatic Biology Associates). Roles and responsibilities for each staff member are listed in Table 2.

Table 2. List of project participants and associated roles and responsibilities.

Name	Project Title/Responsibility
Deb Lester	Project Manager
Jo Wilhelm	Field Sampling/GIS Lead
Doug Henderson	Data Management Lead
Leska Fore	Data/Statistical Analysis Lead
Ginna Grepo-Grove	EPA R10 QA Manager
Karen Adams	Ecology Liaison
Bob Wisseman	Contractor for Taxa Attributes Update
Gretchen Hayslip	EPA Technical Liaison

3. Problem Definition/Background

A. Problem Statement

The sampling effort described in this QAPP, specifically a side-by-side comparison of 3 ft^2 versus 8 ft^2 sampling areas, is intended 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).

Ecology[1] (Plotnikoff & 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 index values, regardless of the analytical method used (Cazier 1993, Vinson & 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.

B. Project Goals/Objectives

The goal of this sampling effort is to collect sufficient data to 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 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.

C. Intended Usage of Data

The data will be used to develop an algorithm or conversion factor so that benthic macroinvertebrate data collected from both 3 ft^2 and 8 ft^2 areas can be compared and used for long-term trend analysis across the Puget Sound region.

4. Project/Task Description

A. General Overview of Project

- Side by side samples will be collected from approximately 50 locations in August and September 2011 and 2012.

[1] Ecology conducts both ambient (Plotnikoff & 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.

- Sampling locations will be selected to ensure sites encompass a range of human disturbance gradients (e.g., close to pristine to highly impacted divided into minimal, moderate, and high disturbance). Other selection criteria may be considered if pilot data analysis necessitates it and if resources allow.
- Samples will be collected with either a D-net or a Surber sampler with 500 micron mesh.
- For both the 3 ft^2 and 8 ft^2 protocols, samples will be collected from riffle or fast flowing, non-depositional habitat following methods outlined in the King County Sampling and Analysis Plan for ambient monitoring (King County 2002) for 3 ft^2 and in the Ecology SOP for regulatory sampling (Adams 2010) for 8 ft^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 (approximately 10 in 2011 and 50 in 2012).
- Training will be conducted in July 2011 by Ecology staff to establish consistent sampling procedures across all staff collecting samples.

B. Project Timetable

The milestones associated with the sampling and analysis efforts are listed in Table 3.

Table 3. Sampling and Analysis Tasks Timetable.

Activity	Projected Start Date	Anticipated Completion Date
Preliminary Data Analysis	February 2011	July 2011
Sampling Design	April 2011	June 2011
Sample Location Selection & Site Reconnaissance	June 2011	August 2011, 2012
Sample Collection	August 1, 2011	September 30, 2012
Taxonomic Identification	October 2011	March 2012, 2013

5. Measurement Quality Objectives

A. Data Precision and Accuracy

Precision is the agreement of a set of results among themselves and is a measure of the ability to reproduce a result. Accuracy is an estimate of the difference between the true value and the determined mean value. The accuracy of a result is affected by both systematic and random errors.

Replicate samples have been collected at approximately 10% of the sampling sites for the annual King County ambient monitoring since 2002 for a total of 117 quality control replicates. These existing replicate data will be analyzed to quantify field sampling precision (i.e., operator consistency and bias) and microhabitat variability (i.e., variability due to small-scale spatial variability within a site reach). If results of that analysis suggest that field replicates yield consistent results (less than 10% difference in total B-IBI scores from at least 80% of the sites), no additional field replicates will be collected for this project. If the analysis suggests greater variability from the existing data, then replicate samples will be collected at 5% of the sampling sites.

Data quality objectives for the laboratory part of this effort emphasize accuracy and precision of benthic macroinvertebrate identification, which will be supported by following appropriate laboratory procedures. Sample processing and Quality Assurance/Quality Control (QA/QC) laboratory procedures are outlined below in Sections 10 and 11.

B. Data Representativeness

Representativeness of benthic community conditions is determined by the sample program design (King County 2002; Adams 2010). Representativeness expresses the degree to which sample data accurately and precisely represent a characteristic of a population, parameter variations at the sampling point or an environmental condition. Representativeness of samples is ensured by adherence to standard field sampling and measurement and laboratory protocols. These sampling protocols have been widely used and are designed to produce consistent and repeatable results in each stream reach. Targeting riffle or fast-moving, non-depositional habitats will minimize physical variability between samples. Benthic macroinvertebrate sampling using 3 ft^2 methods have been widely used and tested throughout King County and other Western Washington counties and cities; similarly 8 ft^2 methods have been used widely throughout Washington State.

Analysis tools are also selected to provide a reliable and consistent indicator of biological condition. For example, because population size varies too much under natural and impacted conditions to be a reliable indicator of biological condition (Karr and Chu 1999), analysis will focus on more robust metrics such as taxa richness and overall B-IBI scores. The B-IBI method has been widely used throughout the United States and has been subject to extensive regional evaluation in the Puget Sound lowlands (e.g, Booth et al. 2004, DeGasperi et al. 2009, Fore 1999, Kleindl 1995, Morley and Karr 2002). Samples will be subsampled to 500 organisms to standardize abundance within and between sites.

C. Data Comparability

Comparability is a qualitative parameter expressing the confidence with which one data set can be compared with another over time and space. Comparability will be maintained through using the same or very similar equipment and following established protocols, along with standardized data validation and reporting procedures. The sampling protocols used in this study follow those adopted by Ecology (Adams 2010) and King County (King County 2002). These protocols are designed to produce consistent and repeatable results in each stream reach ensuring data comparability by targeting riffle or non-depositional habitat, limiting the collection window to August and September during summer low flows, disturbing the substrate for a standard amount of time (60 seconds), and using the same net mesh size (500 µm). Training in field data collection protocols for all field staff will occur prior to sample collection to ensure consistency across sampling locations. Sample collection at most of the sites (>75%) will be led the same field staff, further enforcing site-to-site consistency by limiting variation that arises from using multiple personnel. All samples will be sent to the same taxonomic laboratory (Rhithron Associates, Inc., henceforth referred to only as Rhithron) to ensure taxonomic identification consistency and comparability.

D. Data Completeness

Completeness is defined as the total number of samples analyzed for which acceptable data are generated, compared to the total number of samples submitted for analysis. Sampling at stations with known position coordinates in favorable weather and stream flow conditions, along with adherence to standardized protocols will aid in providing a complete set of data for this project.

Sample loss will be minimized by thoroughly washing nets between site visits, carefully transferring the entire sample from the net to sturdy sample storage containers, adequately labeling each container, and properly preserving the samples with sufficient solution in a timely manner to prevent desiccation. Sample container type and labeling information are described below under Section 8. If the validity of the information from the sample is in question, the sample will be excluded from analysis. The goal for completeness of benthic macroinvertebrate data sets is 100% of the total samples collected. Completeness is defined as the total number of samples that we are confident in using for further data analysis following field collection.

6. Documentation and Data Management

All field data and associated observations will be recorded on standardized field sheets. All project data and macroinvertebrate taxonomic identifications will be stored in the Puget Sound Stream Benthos database (www.pugetsoundstreambenthos.org).

The Project Manager will provide supervision of all data acquisition and management activities.

7. Sampling Process Design

A. Rationale for Selection of Sampling Sites

Sites will primarily be selected from over 1,100 existing sample locations from the Puget Sound Stream Benthos database to avoid issues with access and minimize time needed for reconnaissance. Utilizing existing sites will allow participating entities to see the cross-walk applied at stream reaches they are familiar with, giving them direct evidence and higher confidence in this new analysis tool. Where possible, we will partner with jurisdictions and tribes in the field to promote standardized data collection across the region.

Approximately 50 sites will be selected to represent a range of human disturbance (minimal, moderate and high). Analysis of pilot data indicates that elevation, channel slope (gradient), and watershed area do not have a consistent influence on B-IBI values across a range of urbanization. Therefore, the sampling design will not address these factors. Streams will have a watershed area representative of typical sites in the Puget Sound Stream Benthos database (2-30 mi^2).

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 habitat. Other complimentary data such as fish, habitat, water quality, or flow data are desirable, but not required.

B. Sample Design Logistics

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 habitats using a Surber sampler or D-frame kicknet 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 follow Ecology's sampling protocol for regulatory purposes (Adams 2010) and more details are outlined in section 8 below.

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 the combined 3 ft^2 and 5 ft^2 samples. These data will be uploaded into the Puget Sound Stream Benthos data management system. For each sampling station, data downloads will be available as (1) a 500-count 3ft^2 sample and (2) a 500-count 8ft^2 sample to allow direct comparison of individual metrics and total B-IBI scores.

8. Sampling Method Requirements

Once the sampling station and stream reach are located, the Surber sampler or D-net opening will be placed in the most downstream riffle or fast-moving, non-depositional habitat 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. 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. 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 (Adams 2010, King County 2002) 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, or garden tool to stir up the substrate in the 1 ft^2 area directly in front of the net.

The net is then moved to the next upstream collection location (i.e. riffle or fast-flowing, non-depositional habitat) 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. Once the desired sample area (3 or 5 ft^2) has been collected, the net will be removed from the water and processed. The contents of the net will be carefully placed in a 500 µm mesh sieve. Rocks and debris too large to fit into the sample jars will be visually examined and all observed organisms will be removed and placed in the pre-labeled sample containers (0.5 to 2-liter high-density polyethylene [HDPE] bottles with labels identifying the site ID, sampling date, surface area sampled [3 or 5 ft^2], partner organization, and sampling personnel). The remaining contents of the sieve will be placed in the sample container along with any remaining organisms found and removed during a close visual inspection of the net.

The sample contents will be preserved in the field with 95% denatured ethanol. An additional internal label will be prepared in pencil on rite-in-the-rain paper (site ID, sampling date, surface area sampled [3 or 5 ft^2], partner organization, and sampling personnel) and placed inside of the sample container. The label on the outside of the sample container will be covered with clear tape.

9. Sample Handling and Custody Procedures

As described above, all samples will be preserved with 95% ethanol. All samples will be held at the King Street Center (201 S. Jackson Street, Seattle, Washington) in a locked storage room until they can be transported to the taxonomic laboratory (Rhithron) in late September or

October. A chain of custody (COC) form will be filled out prior to transferring the samples to the taxonomic laboratory. In addition to the COC forms, each site visit will be recorded in the Puget Sound Stream Benthos data management system including the site identification code, sample date, surface area sampled, partner organization, and collection personnel. The list entered in the database will be cross checked against the COC before the samples are transferred to the taxonomic laboratory. Upon receipt at the taxonomic laboratory the COC record will be cross checked with each sample.

10. Taxonomic Analysis Methods Requirements

A. Sample processing

The goal for all taxonomic analysis is to match Department of Ecology methods as closely as possible. As previously discussed above, upon arrival at the taxonomy laboratory, the samples will be checked against the inventory sheet and COC information. This information, along with the internal laboratory identification numbers will be uploaded into the Rhithron database prior to sample processing. 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. 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.

B. Taxonomic identification

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 resolution[2] using appropriate published taxonomic references and keys. Identification, counts,

[2] Taxonomic identification in 2011 will match the resolution used for Ecology samples in 2010 (lowest practical for all organisms including Chironomidae, Acari, and Oligochaetes). The resolution of taxonomic identification in 2012 may be adjusted for certain taxa based on 2011 results and the influence of taxonomic resolution on B-IBI metrics and overall values.

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 current 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.

11. Field and Taxonomic QA/QC Requirements

A. Field QA/QC Checks

For benthic invertebrate collection, a series of measures will be used for QA/QC. First, all samplers will be trained in established sampling protocols. Second, a core project team member will accompany and assist in most sample collection to ensure consistent and common application of protocols. Third, to reduce the chance of organisms being lost during sampling, nets will be visually inspected for holes and all rocks and nets will be thoroughly examined before additional samples are collected or before being discarded or stored. Finally, only recognized taxonomic labs with certified taxonomists and established quality control procedures will be used for identification and census of collected taxa, and data will be entered into a single database, the Puget Sound Stream Benthos data management system.

B. Taxonomic Laboratory QC Checks

Quality control procedures for initial sample processing and subsampling involve checking sorting efficiency. These checks will be conducted on 100% of the samples by independent observers who microscopically re-examine at least 20% of sorted substrate from each sample. Quality control procedures for each sample will proceed as follows: the quality control technician will pour the sorted substrate from a processed sample out into a Caton tray, redistributing the substrate so that 20% of it can be accurately lifted out by removing entire grids in a random fashion. Grids will be selected, and re-examined until 20% of the substrate is re-sorted. All organisms that were missed will be counted and this number will be added to the total number obtained in the original sort. Sorting efficiency will be evaluated by applying the following calculation:

$SE = n_1/n_1+n_2 * 100$

- where: SE is the sorting efficiency, expressed as a percentage,
- n_1 is the total number of specimens in the first sort,

- n_2 is the total number of specimens expected in the second sort, based on the results of the re-sorted 20%.

If 95% sorting efficiency is not achieved for a given sample, a failure will be recorded on the benchsheet and in the database.

Quality control procedures for taxonomic determinations of invertebrates involve checking accuracy, precision, and enumeration. A minimum of 10% of the samples will be randomly selected and all organisms will be re-identified and counted by an independent taxonomist. Taxa lists and enumerations will be compared by calculating a Bray-Curtis similarity statistic (Bray and Curtis 1957) for each selected sample. Samples achieving less than 95% similarity will be regarded as QA/QC failures, and each failure will trigger another sample quality check. Discrepancies between the original identifications and the QC identifications will be routinely discussed among the taxonomists, and necessary rectifications to the data will be made. Discrepancies that cannot be rectified by discussions will be sent out to taxonomic specialists for identification.

C. Data Analysis QC

All taxonomic data will be uploaded to the Puget Sound Stream Benthos database. Following completion of the uploading process, all data will be reviewed to check for erroneous results. See section 15 below for a discussion of data review processes.

12. Data Management

Except where noted otherwise, all field data and associated observations will be recorded on standardized field sheets (physical or electronic). All taxonomic data will be stored in the Puget Sound Stream Benthos database.

13. Assessment and Response Actions

Review of field activities will be the responsibility of the Field Sampling Lead, in conjunction with the Project Manager. If errors in sampling techniques are identified, the Field Lead will work with field staff to rectify and correct any problems.

All field activities may be reviewed by the EPA Quality Assurance Officer as requested. Any identified procedural problems will be corrected based on the recommendations from this officer.

14. Reports

Data collected by the 2011 sampling effort will be analyzed and presented in a technical memorandum by fall 2012. The data for both 2011 and 2012 will also be presented in a comprehensive report that summarizes the larger project due by December 2013.

15. Data Review, Validation, and Verification

All data will be subject to verification before data analysis, distribution to an outside party (i.e., not part of the King County or USEPA project team) or posting to a publicly accessible database. Prior to such use, the Project Manager will contact the appropriate project staff and field technicians responsible for collecting data to verify procedures were followed and the data were checked for errors. To provide a third-party review, at least one project team member (not project manager or technician involved in data collection) will review the data and collection procedures before data are committed to use in analysis or disseminated outside of the project team.

16. Validation and Verification Methods

As previously discussed above in Section 11B the taxonomic lab will use defined QA/QC procedures in their analysis of all macroinvertebrate samples.

Once the taxonomic data are entered into the Puget Sound Stream Benthos database, the Data Management Lead will review the uploaded data to assure that there are no errors in data entry.

17. Reconciliation with Data Quality Objectives (DQO)

As soon as possible after each sampling event, determinations for precision, completeness, and accuracy will be made and corrective action implemented, if necessary. If data quality indicators do not meet project specifications, data may be deemed unusable. Due to the time lag between sample collection and taxonomic analysis it may not be possible to repeat the sampling effort.

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