

FINAL Quality Assurance Project Plan
for
Bioassessment Monitoring and Analysis to Support
Stormwater TMDL Development

EPA National Watershed Contract
EP-C-08-004
Task Order 88

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Quality Assurance Project Plan (QAPP)

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Background

The 1998 TMDL Consent Decree required U.S. EPA Region 10, and by delegated authority, required Washington Department of Ecology (Ecology) to develop and implement TMDLs based on the 1998 303(d) water quality impairment listings. After 13 years of work in developing more than 600 TMDLs, the Litigants responsible for bringing about this Consent Decree reviewed Ecology's progress. The Litigants agreed that Ecology had made good progress toward the original goal, and wanted to ensure that current issues involving stormwater impacts and biological impairments were addressed under continuing requirements of the settlement agreement.

Under the National Watershed Contract, Tetra Tech was asked by U.S. EPA Region 10 and Ecology to develop a Technical Approach for use of biological information in evaluating and determining progress in abating impacts from stormwater. Biological information is used in conjunction with regulation of stormwater through TMDL development. Two watersheds (Squalicum and Soos Creek watersheds) have been identified where existing information will be used along with more recent biological assessments in order to relate physical and chemical factors altered by stormwater events with predictable biological responses. Major components that will be developed for integrating biological assessments along with the water quality TMDL in each drainage are as follows:

- STEP 1** Identify biological evaluation tools and methods for analysis;
- STEP 2** Biological information and water quality information that needs to be combined as part of an integrated assessment of stormwater impacts; and
- STEP 3** How to interpret possible outcomes for biological conditions and water quality conditions following assessment.

Some of the reaches in the watersheds of interest have been the focus of extensive environmental data collection effort. For example, Squalicum Creek at Cornwall Park is a Category 2 listing (waters of concern) based on a River Invertebrate Prediction and Classifications System (RIVPACS) assessment score below acceptable threshold. This listing has been included on both the 2004 and 2008 303(d) list of impaired waterbodies in the State of Washington.

Biological impairments included on the 303(d) list are often included in Category 2 or Category 4C. These categories acknowledge that direct measurements of aquatic life conditions are not meeting expectations. In the absence of companion environmental information that could identify specific pollutant(s) responsible for the impairment, a stepwise process for identifying and systematically eliminating potential causes for impaired biological condition has been developed and used by Ecology (Adams 2010). The CADDIS approach (Causal Analysis Diagnosis/Decision Information System) for identification of specific pollutants likely the cause for impairment had been originally developed by U.S. Environmental Protection Agency and adopted by Ecology.

The identification of stressors and stressor groups (chemicals or physical elements in the aquatic environment that have the same effect on biological response) is the next step following 303(d) listing based on biological impairment of a stream segment. Specific parameters that will be measured in this



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project are identified from several sources: the 303(d) listings, local monitoring effort, existing monitoring data that detected high concentrations of toxics, and specific physical or chemical characteristics known to impair habitat and biota from nearby, similar streams. A simple description for the process is provided in Figure 1. The intent for using this process and following steps in this diagram is to accurately identify pollutant(s) causing biological impairment, and through a series of management actions, use strategies to abate pollution problems and restore healthy biological conditions.

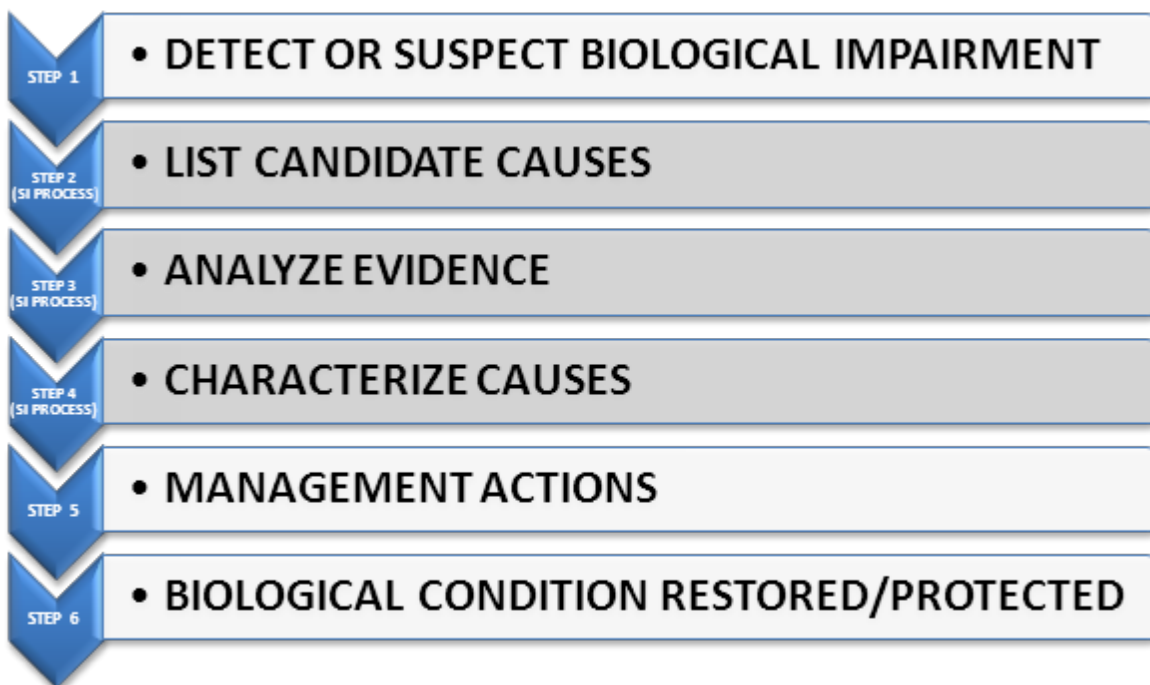


Figure 1. Steps in the Stressor Identification process that identify probable cause(s) for biological impairment.

The intent of this work will lead to development of implementation-ready TMDLs for the Squalicum Creek and Soos Creek drainages that use biological endpoints. Biological endpoints integrate one or more stressors in a particular stream setting and are considered to be one of several parameters used to evaluate compliance with TMDL expectations. Use of bioassessment data in the TMDL development process may result in greater stakeholder understanding of effects from stressors and support for management actions in these watersheds. Additionally, as response-stressor relationships are documented, biological assessments in concert with stressor data can be used to help predict and track environmental outcomes of management actions.

Project Description

Candidate sites in the Squalicum Creek and Soos Creek watersheds will be identified that have measurable stormwater impacts and are under further consideration as demonstration locations for determining utility of using multiple indicators. The use of multiple indicators is advantageous for the following reasons:

- To identify specific pollutants from stormwater input,
- To identify impacts to stream temperature and dissolved oxygen from stormwater input, and
- To identify habitat where toxics are transported and increase exposure potential of aquatic life (benthic macroinvertebrates (BMI), periphyton).

The selection of sites in several types of stream reaches should have a range of characteristics beginning with those considered to be like high quality Western Washington streams (assessed using the RIVPACS predictor variables). Additional, related variables that are degraded by stormwater input should also be reflected at sites within the same drainage so that direct comparison between high quality and stormwater impaired sites can be examined for specific differences (or combination of differences) that are attributable to this impact. For watersheds where physical habitat and stream dynamics have not been well-described additional data collection will be necessary for determining principal factors that explain why BMI communities change at any time of year following exposure from stormwater input.

There are three objectives for developing a bioassessment monitoring and analysis strategy to support TMDL development in the Squalicum Creek and Soos Creek watersheds. The following primary objectives for developing this assessment strategy are:

OBJECTIVE 1 Identify biological evaluation tools and methods for analysis;

- Identify sites for biomonitoring in summer 2012 using the following criteria:
 - Use information from existing biomonitoring sites
 - Partition drainage into areas with dominant land use types
 - Identify major gradient breaks on the mainstem
 - Identify likely locations for “TMDL Compliance Points”
 - Final site locations integrate the above elements (as much as possible)
- Use Ecology Biomonitoring protocols for field collection of BMI, periphyton, and companion physical habitat and water quality data,
- Identify sites in the Squalicum Creek and Soos Creek drainages as reference according to the RIVPACs model scores,
 - These sites will be further examined for physical setting characteristics as a guide for determining prescriptions on managing stormwater impacts

OBJECTIVE 2 Combine biological information with water and sediment quality information as part of an integrated assessment for identifying impairment from stormwater input;

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- Develop relationships between physicochemical parameters influenced by stormwater or stream sediments and biological response scores (i.e. RIVPACs)
- Analyze BMI data using the Western Washington Model for generation of RIVPACs scores for each site,
- Use the CADDIS (Stressor Identification) process as guidance to develop these relationships between BMI assessments and companion physical habitat and water and sediment quality conditions
- Analyze periphyton data using assessment methods developed by (Bahls, Montana DEQ) to determine impacts from stormwater inputs
- Identify how biological conditions respond to stressor groups and couple with setting characteristics

OBJECTIVE 3 How to interpret possible outcomes for biological conditions and water and sediment quality conditions following assessment.

- Identify stressors where response is stronger in either the biological indicators or water quality indicators (sensitivity to the pollutant stressor)
- Determine if there is a biological response to pollutants used in the TMDL
- Develop a list of water quality and biological indicators where response is consistent to stressor groups or a specific pollutant.

Development of biological endpoints (or “triggers”) used to identify pollutants for which a TMDL is developed are unique to a drainage. Just as TMDLs are drainage-specific, biological endpoints related to stressor(s) are unique to a drainage setting.

Several important concepts for detecting stormwater impacts and developing a management strategy are addressed in this Technical Approach. These following concepts should be the focus in development of technical tools and in development of the stormwater TMDLs:

- 1) TMDL Compliance Point Limits
 - a. Couple biological assessments with TMDL expectations
- 2) Relationship between BMI condition (indicators) and TMDL Loading Capacity and Allocations
 - a. Inverse/direct relationship between treatment/response variables
- 3) Biological thresholds description
 - a. Endpoints for biological response (physical habitat, water quality indicators)
- 4) Interpreting biological response to stormwater quality
 - a. Describing biological condition improvements
 - b. Using predictions to determine location and type of impairment
- 5) Use of Multiple Indicators to detect/diagnose stormwater impacts
 - a. Management decisions informed by an integrated monitoring approach
 - b. Sensitivity of BMI to specific stormwater stressors
 - i. Sporadic water quality/physical impacts
 - ii. Continuous effects; stressor influence on BMI condition

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Specific physical and chemical conditions are altered by stormwater input and respond in either a negative or positive direction. The development of technical tools to integrate biological information into the TMDL process can be informed by reviewing elements from the following two tables.

Table 1 lists several factors that are associated with stream dynamics and physical habitat that will influence stream community health. This table serves as a checklist for determining the stressor groups contributed by stormwater input and what combinations of these occur at a site. A healthy aquatic life community in a stream is measured against expectations from similar settings. The Department of Ecology uses the RIVPACS assessment tool (for Western Washington streams) to determine quality of a stream, in part, based on health of the benthic macroinvertebrate (BMI) community. The RIVPACS tool requires that several predictor variables be used to calculate expected BMI species at a site. The predictor variables reflect reference stream conditions including region in the state, climate, and physical site characteristics. Those variables are the following:

- Slope of the streambed
- Elevation of the site
- Julian day
- Ecoregion (Level III)

The candidate stream segment and corresponding biological condition will be compared against stormwater-related impacts for patterns that emerge among the set of biomonitoring sites. The stormwater-related factors are listed in Table 1. Existing data may have companion habitat and chemical information similar to that listed in Table 1 and will be used to describe how site-specific conditions are related to biological response.

Table 1. Stormwater influence on stream dynamics and habitat that are directly related to changes in aquatic communities.

Stream Reach	PHYSICAL IMPACT			CHEMICAL IMPACT				VULNERABLE HABITAT		
	Flow Variation			Factors Influencing Toxics Exposure Potential				Point of Toxics Exposure		
	Substrate Movement Is substrate size subject to transport and at what intensity of stormwater input?	Substrate Size Are substrate size changes a result of stormwater input?	Water Velocity Do changes in water velocity patterns following stormwater input affect BMI communities?	Sediment / Water Column In which media do toxics aggregate and present exposure potential?	Water Chemistry Are toxics impacting stream temperature and dissolved oxygen concentrations.	Residence Time Are toxics resident in media for extended periods of time?	Habitat Association Are there characteristic habitats in a candidate watershed where toxics aggregate in harmful levels?	Mixing Zone Exposure Is there a mixing zone and does toxics exposure present a greater impact to BMI?	Suspended Contaminated Sediment Is suspended material carrying a high toxics load?	Substrate Deposition Are contaminated toxics redistributed on a routine basis?
Site 1										
Site 2										
Site 3										
Site 4										
Site 5										
Site 6										

Existing Monitoring Data from Squalicum Creek/Soos Creek

Squalicum Creek

The Squalicum Creek basin is approximately 15,800 acres. The majority of the creek is within the City of Bellingham. The upper extent of the creek and headwaters are in Whatcom County. SMA (Shoreline Master Agreement) jurisdiction associated with this creek is approximately 423 acres. Land use is dominated by urban residential and industrial development as well as large segments of undeveloped parcels. Public access is available via Cornwall Park and Sunset Pond.

Existing biological information has been collected and described by the City of Bellingham. The City of Bellingham uses Ecology protocols for collection benthic macroinvertebrates. The same consultant providing taxonomic services is being used for analysis of biological samples (Rhithron Associates, Inc.). The biomonitoring sites are distributed across a broad set of locations throughout the drainage and represent influence from several different land uses and the impact of stormwater in these stream locations. A detailed analysis of this information was conducted by Western Washington University (*Vandersypen et al., 2006*). Although the uppermost Squalicum Creek site had slightly better macroinvertebrate indices, all sites contained low numbers of sensitive organisms and were dominated by pollution tolerant taxa, including amphipods, chironomids, and worms. Pollution tolerant mayflies (*Baetis tricaudatus*) were also observed in higher numbers than normally expected.

All reaches, except the segment between James Street and Hannegan Road, indicated some level of pollution for dissolved oxygen, temperature, fecal coliform, zinc and/or pentachlorophenol (Ecology 2009; Anderson and Roose, 2004). Habitat is generally impaired throughout creek. Due to the amount of undeveloped property in the creek valley and floodplain, good habitat, or habitat potential through restoration, remains along most of the creek. The potential for habitat connectivity along the entire length of the creek still exists to a high degree despite transportation corridor barriers. Undeveloped floodplain also provides opportunities to improve stream habitat (meanders and in-stream structures).

Ecological functions are an important element to examine for determining presence and extent of stormwater impacts to aquatic life. Characteristic ecological functions of the creek and adjacent buffers decline in downstream areas beginning from Interstate-5. Increased development over the past 20 years has resulted in the loss of habitat for aquatic life and secondary functions contributed by riparian buffers. Moderate to high functioning condition remain upstream of Interstate-5. These are areas where buffer widths are greater and native vegetation still remains. The large wetland complex populated by a wide range of native vegetation remains in upper reaches of Squalicum Creek (Reaches 6 through 9; City of Bellingham Draft Shoreline Characterizations 2011). The quality of ecological function in this area is reduced by a single factor; transportation barriers that bisect the stream reach and by decline in condition of native vegetation allowing non-native and invasive species to dominate. Buffer widths are 50 feet or greater along the entire creek and 200 feet or great in other areas.

Anadromous fish populations that use Squalicum Creek include: Sea-run cutthroat, Chinook, Coho, and Steelhead salmon. Bull trout have been identified in Squalicum Creek up to the first long culvert in Reach 2 and could likely be present in the remaining reaches. Spawning redds will be avoided when identified within BMI sampling reaches. Chum salmon are identified from Reaches 1 through 5 and likely found in



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remaining reaches. Chinook salmon and Bull trout are listed as Federal Threatened species and listed by the State of Washington as a species of concern (WADNR Natural Heritage Program). Sea-run cutthroat and Coho salmon are listed as a Federal species of concern and do not appear under any State listing status.

Salmon and steelhead spawning and rearing occur at select locations throughout the drainage and potentially at different times of the index period identified for assessing biological communities in Squalicum Creek and Soos Creek. The following are web site addresses that report up-to-date life cycle activity for each of the protected species in the project area:

Salmon & Steelhead

<http://www.nwr.noaa.gov/salmon.cfm>

Chinook (see Table 1, pages 41-45)

<http://www.nwr.noaa.gov/Publications/Biological-Status-Reviews/loader.cfm?csModule=security/getfile&pageid=21664>

Steelhead (see Table 3, pages 16-22)

<http://www.nwr.noaa.gov/Publications/Biological-Status-Reviews/loader.cfm?csModule=security/getfile&pageid=21828>

Bull trout

<http://www.nwcouncil.org/fw/subbasinplanning/methow/plan/e-Appendix%20F%20Independ%20Populations%20&%20Limiting%20Factors/FocalSpeciesStatCP.pdf>

Range maps for select species can be found at the following:

<http://www.ecy.wa.gov/services/gis/maps/wria/sasi/sasi.htm>

Soos Creek

Land use in the Soos Creek basin consists of rural residential, agriculture, and highly urban commercial and residential areas (Herrera, 2005). The western area in particular has been subject to heavy urbanization. Increased impervious surface area has contributed to decreases in summertime low flows and increases in winter storm water flows (King County, 1990). Some areas of the Soos Creek basin are expected to have winter peak stream flows increase 3.5 times the 1985 levels due to the shift from highly permeable soils being converted to urban areas with impervious surfaces (King County, 1989). Increased groundwater withdrawal also contributes to the decline in in-stream flows. The City of Kent, the Covington Water District, and King County Water District #111 are the largest consumers of water in the basin (King County, 2011).

Available benthic data was previously identified in the Stormwater Pilot-Candidate Watersheds Assessment (Technical Memorandum to Ecology 2012). One USGS gage on Soos Creek had co-located benthic data collection during one sampling event in 2007 and a total of 4 sampling events in 1996-1998. In addition, King County has also conducted benthic sampling as part of King County's Benthic Invertebrate Program. In Soos Creek, two sampling events occurred where one station was sampled in



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2002 and two stations were sampled in 2010.

Collection protocols for characterizing benthic macroinvertebrate communities differ between King County and Ecology (bottom area collection of 3 ft² versus 8 ft², respectively). Current work is underway to identify how the collection area affects results when quantifying density and calculating biometric expressions and will be referenced in the final report for this project.

Organization and Schedule

The organizational aspects of a program provide the framework for conducting tasks. The organizational structure can also facilitate project performance and adherence to quality control (QC) procedures and quality assurance (QA) requirements. Key project roles are filled by those persons responsible for ensuring the collection of valid data and the routine assessment of the data for precision and accuracy, as well as the data users and the person(s) responsible for approving and accepting final products and deliverables. The project organization chart, presented in Table 2, includes titles and responsibilities among participants and data users. The responsibilities of these persons are described below. Table 3 reports project Task timelines to ensure that deliverables are completed on time.

Table 2. Project Organization and responsibilities for each of the team members.

STAFF	TITLE	RESPONSIBILITIES
Jayne Carlin	Task Order Manager	U.S. EPA Region 10 Task Order Manager (TOM). She will provide coordination of the technical and QA resources of the Agency and its contractors in executing this project.
Gina Grepo-Grove	EPA Region 10 QA Manager	U.S. EPA Region 10 Quality Assurance Manager (QAM), or her designee, will be responsible for reviewing and approving the QAPP and any other deliverables, as requested by the TOM.
Dave Ragsdale	EPA Technical Lead	U.S. EPA Region 10 TMDL Technical Lead who serves as an on-site TMDL expert and a resource to Department of Ecology staff.
Brandi Lubliner	Squalicum Technical Lead	Ecology's principal contact and technical lead for Squalicum Creek bioassessment monitoring and TMDL project. Field lead and laboratory coordination for sediment sampling on Squalicum Creek.
Stephanie Brock	Soos Technical Lead	Ecology's primary contact for Soos Creek TMDL development and monitoring.
Amy King	Task Order Leader	Tetra Tech primary contact for project management and liaison for communication between EPA/Ecology and the Tetra Tech team.
Robert Plotnikoff	Technical Lead	Tetra Tech senior aquatic ecologist and primary contact for development of the monitoring and analysis approach in determining stormwater impacts to stream biota.
Harry Gibbons	QA Officer	Tetra Tech senior environmental scientist that reviews and approves content of the reports.
John O'Donnell	QA Officer	Tetra Tech senior quality assurance officer that reviews and approves content of the Quality Assurance Project Plan.

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Table 3. Project components, timeline, and lead staff assigned to complete technical products.

FIELD AND LABORATORY WORK	DUE DATE	LEAD STAFF
Task 1. QAPP/Monitoring Plans		
Draft QAPP and Monitoring Plan	April 20, 2012	Robert Plotnikoff
Final QAPP and Monitoring Plan including response to comments	May 17, 2012	Robert Plotnikoff
Task 2. Biological Monitoring		
Field Sample Collection	July 1 – July 31, 2012	Robert Plotnikoff Brandi Lubliner
Biological Sample Collection	August 2012	Jennifer Lawson Colin Spence Jessica Blizard
Monitoring Results Summary		Robert Plotnikoff
Copies of Field and Laboratory Data Sheets		Jennifer Lawson
Task 3. Bioassessment Analysis		
Draft Memorandum on Biological Assessment	Early September 2012	Robert Plotnikoff Jennifer Lawson
Stressor Identification		Robert Plotnikoff
Recommended Targets		Robert Plotnikoff
Task 4. Recommended Targets and Reports		
Final Memorandum on Biological Assessment, including:	Within 20 days from receipt of comments on Draft Memo	Robert Plotnikoff Jennifer Lawson
Stressor Identification		Robert Plotnikoff
Recommended Targets		Robert Plotnikoff
Response to comments		Robert Plotnikoff

Task 1. QAPP/Monitoring Plans

This draft Quality Assurance Project Plan (QAPP) using Ecology monitoring protocols for Squalicum and Soos Creeks was developed prior to site visits to each watershed. The plan includes estimated costs for monitoring and plans for data analysis and interpretation including potential use of CADDIS.

After receipt of comments from the agency technical review Team (June 18, 2012), the QAPP for Squalicum and Soos Creeks was revised in consultation with the technical review Team and responses to major issues will be prepared.

Task 2. Biological Monitoring and Sediment Quality Monitoring

Benthic macroinvertebrate communities will be characterized using a combination of existing information collected by local agencies from the past 20 years. More recent community composition will be characterized with benthic macroinvertebrate and periphyton collections at 6 targeted sites in each of the Squalicum Creek and Soos Creek watersheds and combined with past data collections where



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comparability of data quality objectives are acceptable.

Sediment quality monitoring for metals, total organic carbon, pesticides and polychlorinated biphenyls (PEST1PCB list can be found in Appendix B), will be conducted at only the Squalicum benthic monitoring sites by Ecology. This concurrent sampling will be used in the Stressor Identification process to evaluate candidate stressors to the observed benthic results.

Task 3. Bioassessment Analysis

BMI community data will be evaluated in conjunction with sediment, periphyton, physical and water quality data to determine the relationship between cause-and-effect biological responses. The tools used for this analysis will include RIVPACS, B-IBI, in addition to other relevant assessment tools that identify casual factors and biological response.

An abbreviated CADDIS stressor identification model will be constructed to identify the predominant stressors in the Soos Creek and Squalicum Creek basins and the response by the BMI and periphyton community. The stressor identification and biological impairment analysis will be quantified.

Task 4. Recommended Targets and Reports

Restoration targets for the BMI community will be proposed based on reasonable assumptions made for restoration to the habitat, water quality and other implicated stressors. Methods, assumptions, and the process used for the biological assessment, stressor identification, and recommended allocations or restoration targets to support beneficial uses will be included in a technical memorandum. Agency review team will provide comments and these will be incorporated into the memorandum. A written response will be compiled in response to each of the review comments. Following technical review and response, the information will be incorporated into the draft TMDL sections for each of the drainages.

Quality Objectives

Field and Laboratory Quality Assurance

The integrity of the data collected by this project is upheld by maintaining a high level of quality addressing the five objectives below. The quality of the sampling protocol is checked by analyzing the degree of sampling and visit precision, attempting to maintain less than 20% variation among reference stream data for taxa richness in benthos and periphyton samples. The aim is to collect samples that are representative of community and ecological conditions for each stream. Data are collected with common protocols used by other regional biological monitoring programs. This improves data comparability and usefulness among colleagues in biomonitoring.

Sampling and Visit Precision

Sampling precision measures the extent of random variability among replicate measurements of the same property and is a data quality indicator (USGS, 1998). Sampling precision will be estimated by collection of a second set of biological samples from within the same reach each of the days. The Squalicum Creek sites will be visited by the Tetra Tech team and the Ecology team who will each collect a separate set of data for within-site comparison (precision estimate). Sampling precision is calculated using the relative standard deviation (RSD) among the replicate samples and should be < 20% in reference streams when using the taxa richness metric (Plotnikoff, 1992). Collections of BMI and periphyton samples from multiple locations should have similar community structure in reference streams.

Visit precision measures variability in the sampling method and is related to the variability of collecting a composite sample in a reach. Visit precision is estimated by collecting duplicate composite samples of the invertebrate and periphyton communities within the same reach during the same day at 10% of the reaches sampled. Visit precision is calculated using the relative standard deviation (RSD) from two replicate composite samples and should be < 20% in reference streams when using the taxa richness metric.

Precision in the sediment samples will be evaluated using laboratory duplicates and matrix spike and matrix spike duplicate.

Bias

Sampling bias is the difference between the population mean and the true value. Bias usually describes a systematic difference reproducible over time and is characteristic of both the measurement system, and the analyte(s) being measured (Kammin, 2010; Ecology, 2004). Bias may be caused by the same field investigator conducting the same task at each site. It may also occur due to consistent misinterpretation of protocols by a group of field investigators. Bias will be evaluated in the sediment samples through the use of laboratory and field duplicates and laboratory control standards.



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Representativeness

Representativeness is a data quality indicator that measures the degree to which a sample reflects the population from which it came (USGS, 1998). For ambient monitoring, sites should be representative of minimally or least disturbed conditions in the sampled stream. For targeted monitoring, the sites should be representative of the range of conditions in the sample area. The sampling protocols in the appendices are designed to produce consistent and repeatable results in each stream reach. Physical variability within reaches is accounted for through reach-wide sampling of the various depths, substrates, and flow conditions throughout the stream.

Completeness

Completeness is defined as the amount of valid data obtained from a data collection project compared to the planned amount and is usually expressed as a percentage (USEPA, 1997). Sample loss is minimized with sturdy sample storage vessels and adequate labeling of each vessel. Sediment samples will be shipped in glass jars and plastic bags and using a generous amount of packing material to prevent breakage. Sample vessel type and labeling information are described under "Sampling Stream Macroinvertebrates", and "Sampling Periphyton" in the section "Sampling Procedures". Sample contamination occurs when containers are improperly sealed or stored. Loss of material or desiccation diminishes the integrity of the sample. If the validity of the information from the sample is in question, the sample will be flagged and excluded from analysis. Completeness is determined by four criteria:

- Number of samples collected compared to the sampling plan.
- Number of samples shipped and received in good condition by Manchester Environmental Laboratory (MEL) and the taxonomy contractor.
- Laboratory's ability to produce usable results for each sampling event.
- Sample results accepted by the project manager.

Comparability

Comparability describes the degree to which different methods, data sets, and decisions agree or can be represented as similar (USEPA, 1997). Comparable data sets make sharing data with other organizations that adhere to the same protocols, such as field sampling and analytical methods, possible.

Biological monitoring efforts within Ecology use the applicable protocols followed by Washington's Status and Trends Monitoring Program. These protocols are similar to those of others in the region, including the Oregon Department of Environmental Quality's bioassessment program, and the U.S. Environmental Protection Agency's "Regional Environmental Monitoring and Assessment Program" (R-EMAP). Following these commonly accepted protocols will result in data that is comparable to other regional programs when methods and other critical data quality objectives are similar to those used in this project (Table 4).

Sediment collection will follow Ecology's guidance materials for collecting sediment samples.

- EAP040 - Standard Operating Procedure for Obtaining Freshwater Sediment Samples (Ecology, 2008)



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Table 4. Measurement quality objectives.

Analysis	Equipment Type and Method	Duplicate Samples Relative Standard Deviation (RSD)	Method Reporting Limits and/or Resolution
Field Analysis			
Periphyton	Adams (2010), Barbour et al. (1999)	<20% RSD	NA
BMI	Plotnikoff and Wiseman (2001)	<20% RSD	NA
Water Quality Field Parameters			
Stream Discharge	Marsh-McBirney Flow-Mate Flowmeter	+/- 0.1 ft/s	0 cfs
Temperature	Hydrolab MiniSonde®	+/- 0.1° C ¹	0.01° C
Dissolved Oxygen	Hydrolab MiniSonde®	10% RSD	0.1 mg/L
Specific Conductivity	Hydrolab MiniSonde®	+/- 0.5%	0.1 µS/cm 0.2 @ 25° C
pH	Hydrolab MiniSonde®	0.05 SU	1 to 14 SU
Sediment Parameters*			
Total Organic Carbon (TOC)	PSEP (1986, with 1997 update) MEL (2008) page 120	<20%	0.1%
Grain Size	PSEP 1986	<20%	0.1%
Base Neutral Acids	GC/MS Method 8270 (EPA 1996) ^a MEL (2008) page 144	<40%	25- 250 µg/Kg, dry
Pesticides and PCB Aroclors (PEST1PCB) ¹	GC/ECD SW-846 Method 8081/8082 MEL(2008) pages 25, 155 and 164	<40%	0.25- 2.5 ug/Kg, dry
Arsenic (As)	ICP Method 200.8 (EPA 1983) ^a MEL (2008) page 134	<35%	0.1 mg/Kg, dry
Copper (Cu)	ICP Method 200.8 (EPA 1983) ^a MEL (2008) page 134	<20%	0.1 mg/Kg, dry
Lead (Pb)	ICP Method 200.8 (EPA 1983) ^a MEL (2008) page 134	<25%	0.1 mg/Kg, dry
Zinc (Zn)	ICP Method 200.8 (EPA 1983) ^a MEL (2008) page 134	<20%	5 mg/Kg, dry
Physical Habitat			
Riffle Pebble Count and Embeddedness	Ruler	10% RSD	10 to 300 mm



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Bankfull Width	Tape	10% RSD	0-100%
Bank Stability	Tape; Adams (2010)	10% RSD	Categorical
Wetted Width	Tape	10% RSD	0-100%
Slope	Clinometer	10% RSD	0-100%
Canopy Cover	Densiometer	10% RSD	0-100%
Current Velocity	Marsh-McBirney	10% RSD	Not known
Stream Discharge	Marsh-McBirney	20% RSD	Not known
Laboratory Analysis			
Chlorophyll <i>a</i>	SM 10200H(3)	<10% RSD	0.1 ug/L

*Described in Adams (2010); Appendix B-5

^a Find method quality objectives in Meredith and Furl (2008), Table 2.

¹ Pest/PCB analyte list is combined list analyzed at MEL. Analytes are shown in Appendix B.

Sampling Process Design (Experimental Design)

Candidate sites in the Squalicum Creek and Soos Creek watersheds are identified that will serve in measuring the intensity and effect of stormwater impacts and will be used as demonstration locations for determining utility of using multiple biological indicators. The use of multiple indicators is advantageous for the following reasons:

- To identify specific pollutants from stormwater input,
- To identify impacts on stream temperature and dissolved oxygen from stormwater input, and
- To identify habitat where toxics are transported and increase exposure potential of aquatic life (BMI, periphyton).

Examination of existing data for identification of stream reaches where additional biomonitoring will be collected recognizes the relationships between stream setting and potential for human influence. Potential stormwater stressors like flow characteristics that are characterized by indicators like $T_{Q_{Mean}}$ and Richards-Baker Flashiness Index will be related to biological responses. The relationship between indicators (e.g., physical habitat or water quality) and landscape setting will also be used to develop the sequence for implementing improvements in order to achieve TMDL Management Plan goals.

The selection of sites in several types of stream reaches should have a range of characteristics beginning with those considered to be like high quality Western Washington streams (assessed using the RIVPACS predictor variables). Additional, related variables that are degraded by stormwater input should also be reflected at sites within the same drainage so that direct comparison between high quality and stormwater impaired sites can be examined for specific differences (or combination of differences) that are attributable to this impact. For watersheds where physical habitat and stream dynamics have not been well-described additional data collection will be necessary for determining principal factors that explain why BMI communities change at any time of year following exposure from stormwater input.

A more detailed explanation for how data collected in this project may be used was provided to EPA in a Technical Approaches Memo (Tetra Tech, Inc. March 2nd, 2012), which is included as Appendix A.

Sampling Design and Rationale

A combination of existing site information and proposed biological sampling is identified in Table 5 for Squalicum Creek and in Table 6 for Soos Creek. Comparability is possible between existing data and the proposed monitoring effort because similar sampling protocols will enable both sets of data to be combined and analyzed.



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Table 5. Existing biomonitoring sites (City of Bellingham) and proposed biomonitoring sites (Tetra Tech) in the Squalicum Creek drainage used for identifying response to stormwater impacts.

Drainage	Project	Site Code	Latitude	Longitude
Squalicum Creek	City of Bellingham Macroinvertebrate Monitoring Program	SqualBghamAbBaker	48.775666	-122.486996
Squalicum Creek	City of Bellingham Macroinvertebrate Monitoring Program	SqualBghamBaker	48.775010	-122.491541
Squalicum Creek	City of Bellingham Macroinvertebrate Monitoring Program	SqualBghamBelBaker	48.762061	-122.508344
Squalicum Creek	City of Bellingham Macroinvertebrate Monitoring Program	SqualBghamWestern	48.789217	-122.438570
Squalicum Creek	EPA Benthos Grant (King County)	Squalicum Irongate	48.777734	-122.453729
Squalicum Creek	Tetra Tech (Tt)	Below SR542	48.800451	-122.408164
Squalicum Creek	Tt	Upper Squalicum	48.801360	-122.390144
Squalicum Creek	Tt	Above Hannegan Rd	48.784126	-122.439607
Squalicum Creek	Tt	Below Sunset Pond	48.775324	-122.465137
Squalicum Creek	Tt	At West Street	48.765875	-122.500094
Squalicum Creek	Tt	Baker Creek	48.776980	-122.490842

Table 6. Existing biomonitoring sites (USGS) and proposed biomonitoring sites (Tetra Tech) in the Soos Creek drainage used for identifying response to stormwater impacts.

Drainage	Project	Site Code	Latitude	Longitude
Soos Creek	USGS*	NEWAUKUM CREEK NEAR BLACK DIAMOND, WA	47.275656	-122.059559
Soos Creek	USGS*	SPRINGBROOK CREEK AT TUKWILA, WA	47.465655	-122.232622
Soos Creek	USGS	BIG SOOS CREEK ABOVE HATCHERY NEAR AUBURN, WA	47.312323	-122.165396
Soos Creek	USGS*	DUWAMISH RIVER AT GOLF COURSE AT TUKWILA, WA	47.478988	-122.258734
Soos Creek	USGS*	GREEN RIVER ABOVE TWIN CAMP CREEK NEAR LESTER, WA	47.181779	-121.388704
Soos Creek	USGS*	INTAKE CREEK NEAR LESTER, WA	47.205668	-121.405926
Soos Creek	Ecology	Big Soos Cr at 208th St	47.416500	-122.157100
Soos Creek	Tt	At 148th Ave SE	47.386341	-122.144080
Soos Creek	Tt	Near Brandt Road	47.341697	-122.135568
Soos Creek	Tt	Near SR 58	47.317578	-122.147453
Soos Creek	Tt	At 168 th Way SE	47.3193	-122.1193
Soos Creek	Tt	SR 58 Crossing nr Kent-Black Diamond Road SE	47.3122	-122.0965
Soos Creek	Tt	At 164th Ave SE	47.393689	-122.117106

*Not located within the Soos Creek and Covington Creek drainage.



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Location of proposed bioassessment monitoring sites in the Squalicum Creek drainage is reported in Figure 2. Not all of the existing biomonitoring sites reported in Table 6 are located in the Soos Creek drainage, but have been included to acknowledge past work completed in the area. Location of proposed bioassessment monitoring sites in the Soos Creek drainage and Covington Creek drainage is reported in Figure 3. Each of the proposed sites was chosen to reflect stormwater influence from several land use types on biota in the adjacent stream channel. The sites will represent response reaches where impacts from stormwater are more readily detected through examination of the biological community.

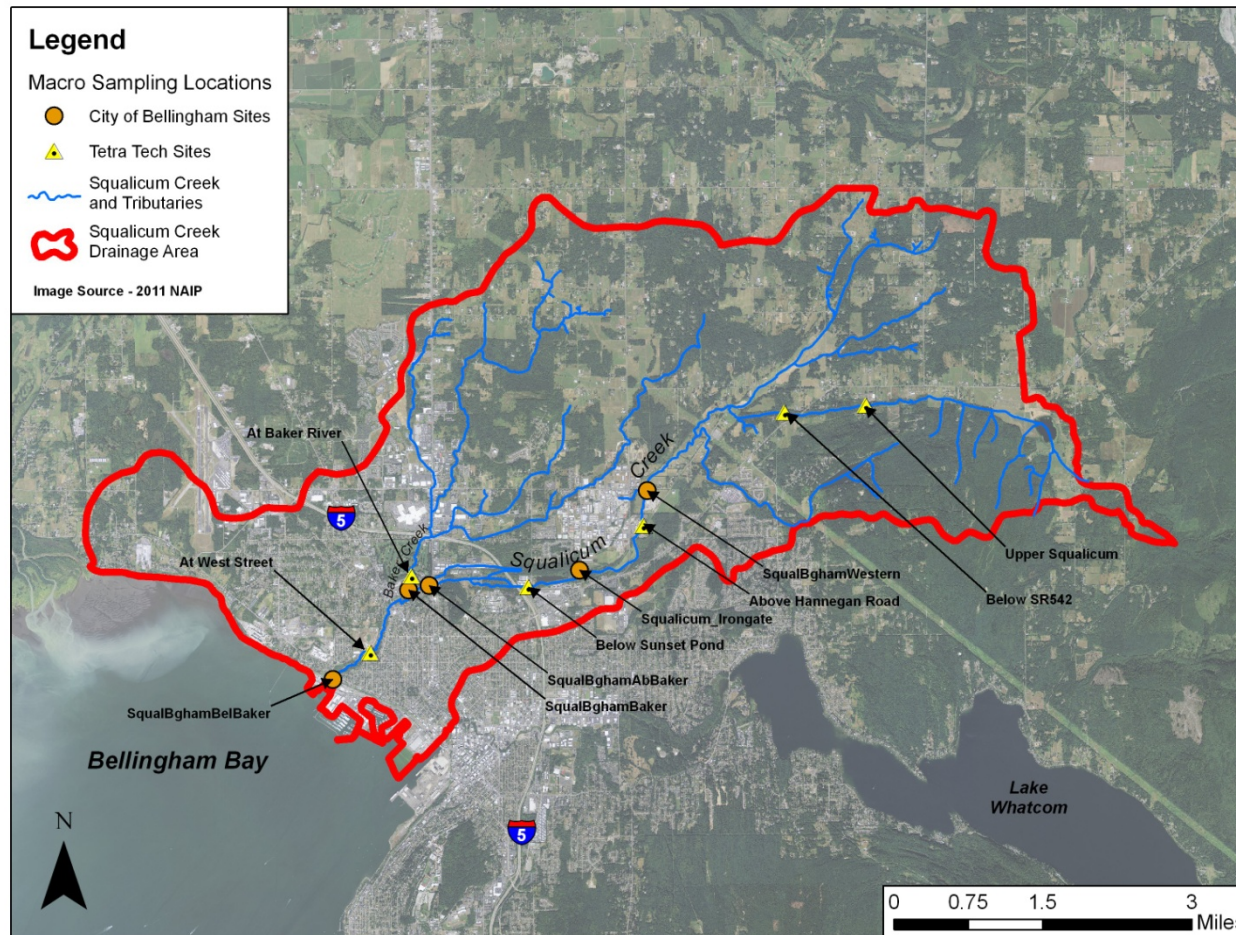


Figure 2. Existing biomonitoring sites (City of Bellingham) and 6 proposed biomonitoring sites (Tetra Tech) in the Squalicum Creek drainage used for identifying response to stormwater impacts.

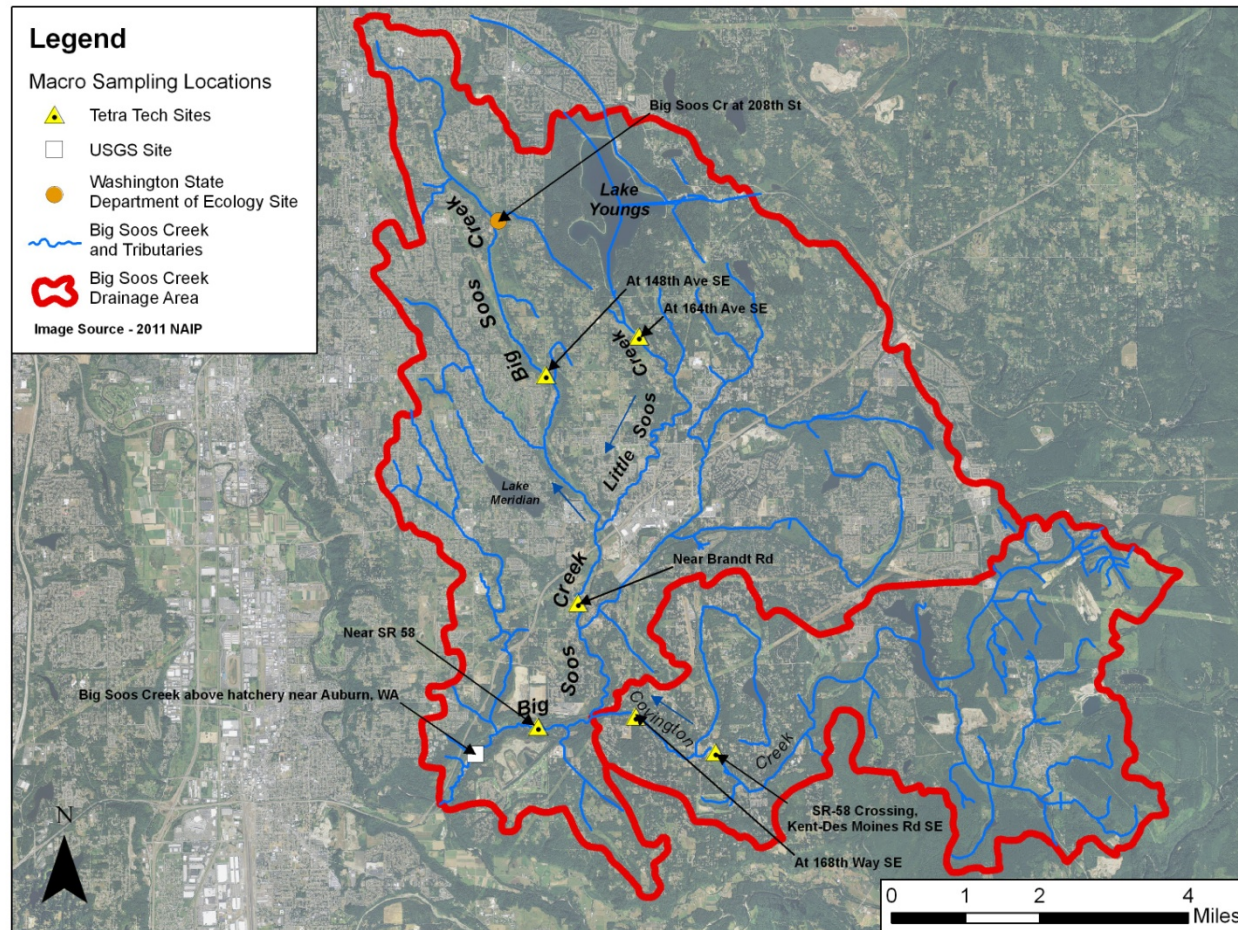


Figure 3. Existing biomonitoring sites (USGS) and 6 proposed biomonitoring sites (Tetra Tech) in the Soos Creek and Covington Creek drainages used for identifying response to stormwater impacts.

Sampling Procedures

Field and Laboratory Preservatives

Biological samples collected from streams must be preserved immediately following storage in containers. Inadequate preservation often results in (1) loss of prey organisms through consumption by predators, (2) eventual deterioration of the macroinvertebrate specimens, and (3) deformation of macroinvertebrate tissue and body structures, making taxonomic identification difficult or impossible.

The field preservative used in this program is 85% denatured ethanol. The preservative is prepared from a stock standard of 95% denatured ethanol. Flammability, health risks, and containment information are listed on warning labels supplied with the preservative container. Detailed information can be found with the Materials Safety Data Sheets (MSDS) online at: <http://hazard.com/msds/>. Minimal contact with the 95% denatured ethanol solution is recommended.

The preservative used in handling sorted laboratory samples is 95% ethanol (non-denatured). Seventy percent non-denatured ethanol is used for preservation of voucher specimens in two dram vials (8 mL). Hazard Communication Training is recommended for all personnel who come into contact with hazardous materials while conducting projects that require use of preservatives for biological samples.

Miscellaneous

Field activities should be conducted by at least two persons, especially when in remote streams. A contact person should be designated at the Lead Project Team's main office for field personnel daily check-in at pre-designated times.

Careful planning of field activities is essential and permission to access private land must be obtained. Access to private land is usually obtained through verbal agreement with the land owner while at the proposed sampling site.

Sampling

Index Period

The index period is a time span during the year in which samples are collected. The index period used in this study (July 1 - August 15) was chosen for the following reasons:



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- Adequate time is available for the instream environment to stabilize following natural disturbances (e.g., spring floods).
- Many macroinvertebrates reach body sizes that can be readily identified.
- Representation of BMI species reaches a maximum, particularly during periods of pre-emergence (typically mid-spring to late-summer).
- To avoid collecting samples during bull trout spawning and immediately afterwards to minimize redd disturbance. If sampling must take place during or immediately after spawning, field personnel will use extreme caution in affected areas so as not to disturb redds.

Biological assessments can yield different interpretations depending on the index period chosen. This is because natural seasonal disturbances and physical stream conditions strongly affect the diversity, abundance, and life-stage progression of aquatic insects (Hynes, 1970; Vannote et al., 1980).

Macroinvertebrate Sampling

At each site, stream reach length is determined by identifying the lower end of the study unit and estimating an upstream distance of 20 times the bankfull width or a minimum of 1000 feet. The lower end of a study unit is located at the point of access to the stream and is always below the first upstream riffle encountered. This reach length ensures that characteristic riffle sequences are represented and potentially sampled.

The sampling routine used at each site includes collection of surface water information and determination of discharge at the furthest downstream portion of the sample reach. Collection of BMI samples follows the initial surface water chemical and physical measurements. The last component of a site visit is habitat characterization. Thus stream disturbance is minimized before the biological information is collected.

Eight biological samples are collected from riffle habitat in a reach. Two samples are collected from each of four riffle habitats. A variety of riffle habitats are chosen within the reach to ensure representativeness of the biological community. Sampling among several riffles in a stream increases representation of physical differences in this habitat. Also, this sampling design maximizes the chance of collecting a larger number of benthic macroinvertebrate taxa from a reach than from fewer riffles. Variations in physical condition of the riffle habitat provide an opportunity to collect both common and rare taxa.

Macroinvertebrate samples are collected from riffle habitats with a D-Frame kicknet (500-micrometer net mesh). A device fastened to the base of the D-Frame kicknet encloses a one-foot by one-foot area in front of the sampler (sampling area= 1 square foot). Larger cobble and gravels within the sampler will be scraped by hand and soft brush, visually examined to ensure removal of all organisms, then discarded outside and downstream of the sampler. Remove all algae and periphyton attached to substrate since BMI reside on these materials. Thoroughly agitate the remaining substrate within the sampler, if possible, to a depth of no more than two to three inches (5 to 8 cm). Visually examine two to three hands full of substrate to confirm that all organisms have been removed.



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Excess sediment and detritus (e.g., algae, leaves, plant material) retained in the sampler serve as a visual warning of the potential for net clogging. Empty the D-frame sampler into a tub between sample locations before signs of net clogging (backwash out the front of the sampler). The eight D-frame samples may be collected and composited in the net without emptying the sampler if net clogging is not suspected.

If the net becomes full and there is danger of backwash or loss of material from around the opening of the net, then the net must be emptied. Hold the net upright, splash water on the outside of the D-frame sampler netting to wash organisms and detritus to the bottom of the net. Holding the net over a tub, invert the net and gently pull the net inside out. Using stream water previously filtered through a U.S. Standard No. 35 (500 µm) sieve, rinse and then examine the net to ensure that all organisms are removed. Remove cobbles and large gravels from the tub after close examination. Pour tub contents into a U.S. Standard No. 35 sieve. Rinse the tub and examine it to be sure all organisms are removed. Repeat the procedure at the remaining randomly selected locations until eight samples have been collected.

Place all of the sieve contents in the sample bottles. Fill each sample container not more than 2/3 full to allow room for the sample preservative. Add ethanol.

Wipe the bottle threads (and the cap if necessary) to remove any sand or dirt so that the cap will tighten properly, and tighten the screw cap (500 and 1000 mL bottle caps require 40-60 inch pounds of torque to be leakproof). Then gently invert the container three to four times so the preservative will penetrate into all of the organisms. Any liquid leaking from the bottle cap with the bottle inverted indicates an incomplete seal, most likely due to dirt or debris in the bottle or cap threads. Label the bottles and place them in a box, wooden container, or cooler for transport to the laboratory.

Periphyton and Chlorophyll *a* Sampling

Periphyton are important primary producers and chemical modulators in stream ecosystems. As such, periphyton can be more sensitive to certain stressors such as nutrients, salts, sediment, and temperature compared to other aquatic organisms. Measures of periphyton structure, diversity, and density are useful in the assessment of biological condition for surface waters. For more information on periphyton and their use in bioassessments, refer to Barbour et al. (1999) and Stevenson et al. (1996).

Eight biological samples are randomly collected from riffle habitat in a reach. Two samples are collected from each of four riffle habitats. Samples will be collected in close proximity to (but not within) the randomly selected D-frame sample locations. See *Macroinvertebrate Sampling* above for description of selecting sample locations.

Carefully remove one or two rocks from each of the eight randomly selected sample locations while retaining the rock's orientation as it occurred in the stream to avoid loss of periphyton. Rocks should be relatively flat and range in size from about 4 cm (coarse gravel) to 10 cm (small cobble) in diameter. Collect only one rock per randomly selected sample location if the diameter of the first rock selected is equal to or exceeds 7.5 cm. If the diameter of the first rock selected is less than 7.5 cm, select a second



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rock. If possible, select rocks that are similar with respect to size, depth, and exposure to sunlight. A total of eight to 16 rocks are collected at each sampling site. Gently place the rocks (as they were oriented in the stream) in a plastic tray; do not stack rocks upon one another. Transport the tray to a convenient sample-processing area. Where possible, process the sample out of direct sunlight to minimize degradation of chlorophyll.

Scrub only the upper surface of each rock with a firm-bristled toothbrush using a circular motion. In circumstances where rocks are much greater than 10 cm (medium to large cobbles), firmly brush only a portion of the upper rock surface around 10 cm in diameter. Do not brush the sides or bottom of rocks. If needed, remove any filamentous algae and mosses by scraping with a knife and place in a separate plastic tray. Use a knife or scissor to cut algal filaments or moss into roughly 2 to 3 mm segments. Gently brush other larger plant material that may be attached to the rocks, but do not collect the plants.

Rinse the sampled rock surface, attached plants, and toothbrush bristles with a rinse bottle containing deionized or distilled water. Use rinse water sparingly, but be thorough. Collect rinsate in the plastic tray containing any filamentous algae or mosses. Repeat for the remaining rocks. Keep the sample volume less than 500 mL. After sample processing is complete, measure and record the total rinsate volume (now considered the composite sample volume) on the datasheet and pour the rinsate through a funnel into a 500 mL Nalgene® sample bottle.

For each rock processed, cover the surface with a sheet of aluminum foil. Either trim the foil with a knife or fold the foil to match the area sampled. Place the trimmed/folded foil templates into a labeled collection envelope and attach to the field data sheets

Process the composite sample following steps described in *Subsample Processing Procedures* to extract subsamples for chlorophyll *a* analysis and taxonomic identification.

Sub-sample Processing Procedures

Each composite periphyton sample processed in the field is used to extract subsamples for chlorophyll *a* analysis and taxonomic identification. Successful execution of subsample processing procedures described here is dependent on measuring and tracking the various volumes as the composite sample is processed. One subsample is extracted from each composite sample for the purpose of determining chlorophyll *a* in the laboratory. The remaining volume of the composite sample is considered the ID subsample and is preserved for taxonomic identification.

Subsampling processing procedures for periphyton composite samples are as follows:

- 1) In an area out of direct sunlight, assemble the filtration apparatus by attaching the filter base with rubber stopper to the filtration flask. Join the flask and a hand-operated vacuum pump (with pressure gage) using a section of tubing.
- 2) Place a 47 mm, 0.7 micron glass microfiber filter (for example, Whatman® GF/F) on the filter base and wet with deionized or distilled water. *Note:* Wetting the filter will help it adhere to the base in windy conditions. Attach the filter funnel to the filter base.



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- 3) Prior to subsample extraction; homogenize the composite sample by vigorously shaking or using a battery-powered stirrer for 30 seconds.
- 4) Extract one 10 mL aliquot of homogenized composite sample using a disposable serological volumetric glass pipette, and dispense onto the middle of the wetted glass microfiber filter.
- 5) Filter the aliquot with the vacuum pump using 7 to 10 psi.
 - a. Examine the filter. An adequate amount of periphytic biomass for analysis is indicated by the green or brown color of material retained on the filter. If needed, extract additional 5 mL aliquots and filter until a green or brown color on the filter is apparent. *Note: For composite samples with abundant organic material and/or fine sediment, filtration of a 10 mL aliquot may not be possible. In these circumstances, filter one 5 mL aliquot. If no difficulties were apparent when filtering the first 5 mL aliquot, proceed with filtering a second 5 mL aliquot.*
 - b. The filtered aliquot(s) represent the chlorophyll *a* subsample. Determine the number of aliquots filtered and record the chlorophyll *a* subsample volume on the datasheet. For example, 2 aliquots x 5 mL/aliquot = 10 mL subsample volume.
 - c. Rinse the sides of the filter funnel with deionized or distilled water; allow the water to be vacuumed completely before releasing the vacuum from the filtering apparatus.
 - d. Using forceps, fold the filter into quarters with the filtered biomass inside. Remove the filter from the funnel base with forceps and wrap in a small piece of aluminum foil. Place the aluminum foil wrapped filter in a separate 47 mm Petri dish.
 - e. Seal the sides of the Petri dish with plastic tape and label the Petri dish with the following required information:
 - i. Site name
 - ii. Sample ID
 - iii. Collection date (mm-dd-yyyy)
 - iv. Collection time (24 hr.)
 - v. Composite sample volume (mL)
 - vi. Subsample volume (mL)
 - f. Repeat the aliquot extraction and filtration processes if necessary for quality control duplicates.
 - g. Insert the labeled Petri dish(s) in a re-sealable plastic bag and place in a cooler containing dry ice. About 4.5 kg (10 pounds) of dry ice is needed for a small cooler (< 2 gal). Insulate the cooler with newspaper to minimize sublimation of dry ice. *Note: Wet ice can be used if dry ice is not available. Make a note on the data sheet when wet ice is used. Alternately, pre-dosed vials with acetone can be used to store the folded filter (folded into quarters) for transport to the laboratory.*
 - h. Coolers should be shipped within a few days after the subsamples have been prepared because of a 25-day holding time limit. Subsamples can be temporarily stored in a freezer (at -20°C) at the field office over weekends. Contact laboratory personnel to notify them of plans to ship (via

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overnight shipping service) coolers containing dry ice and frozen subsamples. Be sure to disclose to the carrier the amount of dry ice in the cooler prior to shipping.

- 6) Measure the volume of the remaining composite sample (which represents the ID subsample volume) and record on the datasheet.
- 7) Preserve the ID subsample with 5 to 10% Lugol's solution (*see Sample Preservative-Lugol's Solution* for preparation; also commercially available). Five percent should be sufficient for most samples, although up to 10% can be used for samples rich in organic matter. Record the preservative volume on the datasheet. The quantities of Lugol's solution required for selected sample volumes are:
 - 500 mL ID subsample, add 25 mL Lugol's solution.
 - 400 mL ID subsample, add 20 mL Lugol's solution.
 - 250 mL ID subsample, add 12 mL Lugol's solution.
- 8) Label the ID sub-sample with the following required information:
 - a. Site name
 - b. Sample ID
 - c. Collection date (mm-dd-yyyy)
 - d. Collection time (24 hr.)
 - e. ID subsample volume (mL) [ID subsample + preservative]

Periphyton Sample Preservative-Lugol's Solution

Periphyton samples will be preserved using a ready-made Lugol's solution. Store Lugol's solution in an opaque plastic bottle.

Riffle Pebble Count and Embeddedness Measurements

The embeddedness measurement procedure presented herein is a modified version of the procedure described by MacDonald, Smart, and Wissmar (1991). It is most applicable to channels with gravel- or cobble-dominated beds. It may have limited, if any, use in high-energy, steep-gradient channels where fine sediment deposition is unlikely. It may not be as appropriate in basins where the sediment load is mostly comprised of silts and clays, and in low-gradient reaches that lack the coarse particles needed to measure embeddedness.

Embeddedness and riffle pebble count is evaluated at the same time when, and in the same riffle/run habitat where, the macroinvertebrate D-frame samples are collected (*See Macroinvertebrate Sampling*). Measurements are made after rocks are scrubbed in the D-frame. The channel bed upstream and within the riffle/run habitat should not be disturbed prior to making measurements (description of method in Appendix B-7, Adams 2010).

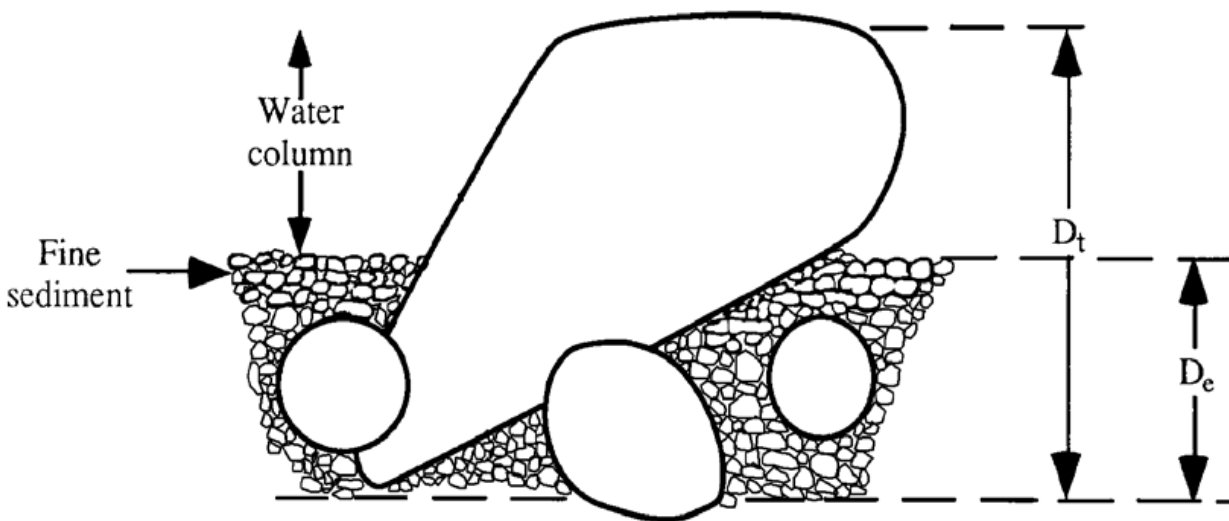
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- 1) Each of the four riffles are divided into three equidistance transects. A total of 11 particles are measured across each transect as follows:
 - a. At the left bankfull stage.
 - b. 10% distance across the channel.
 - c. 20% distance across the channel.
 - d. 30% distance across the channel.
 - e. 40% distance across the channel.
 - f. Half way across the channel.
 - g. 60% distance across the channel.
 - h. 70% distance across the channel.
 - i. 80% distance across the channel.
 - j. 90% distance across the channel.
 - k. At the right bankfull stage.

- 2) Data are collected in the size range of ≥ 10 mm to ≤ 300 mm median diameter. Areas, regions, or “pockets” of homogenous fine sediment that cover gravels and cobbles are defined as 100% embedded. Hardpan and bedrock are by definition 0% embedded (consider the applicability of embeddedness measures for these bed materials).

- 3) Individual particles are selected from the streambed in front of the predetermined random locations where D-frame samples were collected. Particles are selected from the “wetted” or “active” bed of the channel. The particles are “blindly” selected by looking away from the selection site and extending an index finger to the first particle touched on the streambed. Before the particle is removed from the bed, its top and sides are closely examined to determine if it is covered or embedded by fine sediment. A piece of plexiglass may be used to break the water surface and provide a clearer view of the particle. This is done to verify that stain lines on the particle are not the result of past sedimentation or periphyton growth on the upper surface.

- 4) Remove the particle from the streambed while retaining its spatial orientation to measure and record both its total vertical height (D_t) and embedded height (D_e) perpendicular to the bed surface. A stain line may be noticeable to differentiate the embedded portion from the portion that is above the plane of embeddedness. The particle’s median or intermediate diameter (D_m) is measured and recorded after D_t and D_e are measured.



- 6) The number of particles to be collected in front (upstream) of each D-frame collection location may require some pre-planning, depending on the size of the riffle and the relative proximity of each randomly determined D-frame location. The individual D_t and D_e values for all 100 particles are summed, and a percent embeddedness value is calculated for the riffle/run habitat from the formula:

$$\text{Percent Mean Embeddedness} = 100 (\sum D_e / \sum D_t)$$

Bankfull Width

Determining bankfull width is a qualitative evaluation and a distance/elevation measurement, followed by a calculation of the entrenchment ratio. Bankfull stage is defined as the point where stream water just begins to overflow into the active flood plain (approximately the 1.5 to 2.0 year flood). Bankfull stage must be determined at each wadeable monitoring sample location.

Observe bankfull stage indicators such as (1) the flat, depositional surface adjacent to the channel (best indicator, but may be absent in certain stream types); (2) top of point bars; (3) a change in vegetation (especially the lower limit of perennial species); (4) a slope or topographic break along the bank; (5) a change in particle size of bank material; (6) undercuts in the bank, which usually reach an interior elevation slightly below bankfull stage; and (7) stain lines or the lower extent of lichens on boulders.

Stretch a tape across the stream, perpendicular to the flow at the bankfull stage elevation. The tape should be level. If the tape is sloped, the bankfull indicators need to be re-evaluated.

Determine and record bankfull width by measuring the distance from bank to bank.

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Bank Stability

For wadeable streams, evaluate how much of a 10-m length of each bank (centered on the primary transect) is unstable. Limit your observations of bank stability to the portion of the bank at and below the bankfull stage. A bank is unstable if it has eroding or collapsing banks. It may have the following characteristics:

- Sparse vegetation on a steep surface.
- Tension cracks.
- Sloughing.

Wetted Width

Determining wetted width is a qualitative evaluation and a distance measurement. Wetted stage is defined as the point where stream water is present in the channel during time of the site visit. Wetted stage must be determined at each wadeable monitoring sample location.

Stretch a tape across the stream, perpendicular to the flow at the wetted stage elevation. The tape should be level. If the tape is sloped, the wetted width indicator needs to be re-evaluated.

Determine and record wetted width by measuring the distance from one side of the stream channel to the opposite side.

Water Surface Slope

This method describes how to measure stream slope and bearing of the main channel at each site during a data collection event. It applies to waded streams. This method requires use of a hand level, measuring rod, and a compass to make incremental measurements across each sample riffle.

This is a quantitative measurement of the change in elevation over a measured distance. Riffle gradient refers to the percent slope of the monitoring site riffle over a distance of 100 feet OR the entire length of the riffle if it is less than 100 feet.

To measure stream gradient, place a staff or rod in a vertical position at the stream's "wetted edge" (edge of water) at the most downstream portion of the riffle. Stand next to the staff at the same elevation as the wetted edge, hold a clinometer to one eye, align the cross hairs with the zero, and record the reference point on the staff.

Measure 100 feet OR the entire length of the riffle if it is less than 100 feet upstream from the staff, and leave the tape in place. Record the actual distance if it is less than 100 feet. Do not enter the stream. Stand at the wetted edge, hold the clinometer to one eye, and align the cross hairs with the reference point on the staff. Record percent slope per 100 feet or for the length of the riffle.

Canopy Cover

Percent canopy cover is estimated with a convex/concave densiometer (Lemmon, 1957) that has been modified according to Mulvey et al. (1999). Canopy cover is estimated at each sampling riffle. Four readings are taken at the sample point (facing upstream, facing downstream, facing the right bank, and



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facing the left bank). In addition, one reading is taken facing the bank at the wetted right bank and left bank, respectively. Each measurement is taken one foot above the water surface. The composite value is the sum of the four readings taken from the macroinvertebrate sample location.

Current Velocity and Flow

Approximately 15-20 equally-spaced stations across the stream (possibly fewer for very small streams) will be determined before making measurements. To determine spacing between stations, the transect width will be divided by 20 and rounded up to a convenient number. Stations for making measurements across the transect should not be closer than 10 cm to each other, even if this results in less than 15 stations. The first station is located at the left wetted margin, and the last station is located at the right wetted margin.

A calibrated flow meter will be used that is equipped with a top-setting wading rod that has depth increments in tenths of feet. At each station across the transect, a record of the tape distance (cm) will be taken from left to right. Water depth will be recorded to the nearest 0.1 feet. The flow monitoring sensor will be placed at 60% of the distance down from the water surface. Additional measurements will include water velocity (nearest 0.01 f/s).

Bottom Sediments

Prior to sampling, all equipment will be thoroughly decontaminated in accordance with Puget Sound Estuary Program protocols (PSEP, 1997). Stainless steel equipment and utensils will be cleaned by washing with Liquinox detergent, followed by sequential rinses with tap water, 10% nitric acid, deionized water, and pesticide-grade acetone and hexane. The equipment will then be air-dried and wrapped in aluminum foil. All sampling and handling activities will be conducted by personnel wearing non-talc nitrile disposable gloves. Gloves will be changed often, as appropriate, to prevent contamination. The requirements for sample containers, preservations, and holding times (MEL, 2008) are shown in Table 7. Sediment sampling methods for this study are fully described in Adams (2010). All bottles will be ordered from MEL. The metals and organics containers will be shipped with the Certificate of Analysis which means they are contaminant free.

Bottom sediments will be collected as grabs using either a stainless steel scoops (Cubbage, 1994) or a petite ponar sampler (Wilson and Norton, 1996). To obtain sufficient mass for analyses and to enhance the representativeness of the material, a grab consisting of a minimum of 5 sub-grabs will be composited at each site. Solids will be homogenized in a stainless steel bowl using stainless steel utensils, and subsamples will be transferred to pre-cleaned glass jars and sent to MEL for storage and analyses. Field personnel will ensure that sufficient headspace remains in the sample jars to prevent breakage of sample jars.

A summary of sampling procedures and description of materials need to collect or measure environmental media is reported in Table 7.

Table 7. Sample Containers, Preservation and Holding Times

Parameter	Matrix	Minimum Quantity Required ¹	Container	Preservative	Holding Time
Field Analysis					
Periphyton	Substrate	NA	250 mL HDPE – taxonomic sample	Lugol's	Indefinite
BMI	Substrate	NA	1 gallon freezer bags	95% Non-denatured ethanol	Indefinite
Water Quality Field Parameters					
Temperature	Water	NA	NA	NA	NA
Dissolved Oxygen	Water	NA	NA	NA	NA
Conductivity	Water	NA	NA	NA	NA
pH	Water	NA	NA	NA	NA
Sediment Parameters					
Total Organic Carbon (TOC)	Sediment	2 oz	Glass ²	Cool to $\leq 6^{\circ}\text{C}$; may freeze at -18°C	14 days; 6 months if frozen at -18°C
Grain Size	Sediment	8 oz	HDPE Plastic	NA	NA
Base Neutral Acids (BNAs)	Sediment	8 oz	Glass ²	Cool to $\leq 6^{\circ}\text{C}$; may freeze at -18°C	14 days; 1 year if frozen at -18°C
Pesticides and PCBs ³ (PEST1PCB)	Sediment	8 oz	Glass ²	Cool to $\leq 6^{\circ}\text{C}$	14 days; 1 yr if frozen at -18°C
Arsenic (As)	Sediment	4 oz	Glass ^{2,3}	Cool to $\leq 6^{\circ}\text{C}$; may freeze at -18°C	6 months; 2 years if frozen at -18°C
Copper (Cu)					
Lead (Pb)					
Zinc (Zn)					
Physical Habitat					
Riffle Pebble Count and Embeddedness	Instream and Riparian	NA	NA	NA	NA
Bankfull Width	Instream and Riparian	NA	NA	NA	NA
Wetted Width	Instream and Riparian	NA	NA	NA	NA
Slope	Instream and Riparian	NA	NA	NA	NA
Canopy Cover	Instream and Riparian	NA	NA	NA	NA

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Current Velocity	Instream and Riparian	NA	NA	NA	NA
Stream Discharge	Instream and Riparian	NA	NA	NA	NA
Laboratory Analysis					
Chlorophyll <i>a</i>	Substrate	5 aliquots	47mm Petri Dish – filter (Chl <i>a</i>)	NA	Indefinite

¹ Fill jars ¾ full to assure minimum sample size for collection, except TSS which should be filled completely.

² Teflon lined cap, certified clean per OSWER Cleaning Protocol #9240.0-05 (MEL, 2008).

³ Pest1PCB analyte list is combined list. Analytes are shown in Appendix B.

³ Metals are combined for analysis

Sample Labeling and Chain of Custody

Labeling

Labeling is used to identify each sample’s location and the analyte(s) in that sample to be analyzed. Laboratory-prepared bottles will be labeled to identify the cleanliness and/or preservative contents for each bottle. Labels will be premade. Bottles will be either numbered or pre-labeled to ensure proper handling. Labels will be filled out in pencil or permanent pen, placed on sample containers, and taped with packing tape to reduce water damage to the label. Sample labels will contain the following information:

- (1) Station name/identification
- (2) Analysis to be performed
- (3) Date and time of sampling
- (4) Sample ID or coding information
- (5) Sample numbers (1 of 3, 2 of 3, and so on)
- (6) Name/initials of field tech performing the sampling

This labeling information will be written in the chain of custody forms, which are discussed below.

Chain of Custody

Chain of custody (COC) can be defined as a systematic procedure for tracking a sample or datum from its origin to its final use. Chain of custody procedures are necessary to ensure thorough documentation of handling for each sample, from field collection to laboratory analysis. The purpose of this procedure is to minimize errors, maintain sample integrity, and protect the quality of data collected. A COC form will accompany each cooler of samples (Appendix C). Biological samples will be stored in coolers following collection, preservation and labeling. The chain of custody form provided by Rhithron Associates, Inc. will be shipped with samples back to the laboratory. Rhithron Associates, Inc. will pick up samples from Tetra Tech upon completion of field work for transport by automobile to Missoula, MT. After completion of the form and packaging of samples for shipping, the sampler should retain a copy of the form for their records. Individuals who manipulate or handle these samples are required to log their activities on the form. Definitions of custody from MEL’s *Laboratory’s Users Manual* (2008) are described below:



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A sample is considered to be under a person's custody if it is:

In the individual's physical possession

In the individual's sight

Secured in a tamper-proof way by that person, or

Secured by the person in an area that is restricted to authorized personnel

Elements of chain-of-custody include:

Sample identification

Security seals and locks

Security procedures

Chain-of-custody record

Field log book

When the laboratory receives the cooler, it will assume responsibility for samples and maintenance of the COC forms. The laboratory will then conduct its procedures for sample logins, storage, holding times, tracking, and submittal of final data to the responsible parties.

Measurement Procedures

The sequencing and type of measurements made at each sampling reach proceed in a deliberate order so as not to contaminate each of the sample types. The following types of measurements made and the order in which they proceed are described in detail.

Stream Discharge

Instantaneous discharge measurements will be taken at the base of each sampling reach according to field methods described by the American Fisheries Society (Gallagher and Stevenson, 1999) and according to methods in the meter manufacturer's operating manual. One duplicate discharge measurement will be recorded for every 1 of 5 sampling sites visited.

Temperature, Conductivity , pH, and Dissolved Oxygen

Temperature, conductivity, pH, and dissolved oxygen measurements will be collected at each sampling site using a Hydrolab MiniSonde®. Measurements will be collected according to field methods described in the *Standard Operations for Hydrolab® DataSonde® and MiniSonde® Multiprobes* (Swanson, 2007). Multi-probe, pre-and post-calibration procedures (Swanson, 2007) will be performed for each sampling run.

Sequence for Conducting Field Operations

Field procedures follow a sequence of measurements that ensure quality information is collected and a reasonable amount of time is spent at each site. The sequence and spatial arrangement of field operations is outlined in Figures 4 and 5, respectively.

- 1) Field staff members collect surface water and discharge information for water quality measurements at the furthest downstream portion of the sample reach.
- 2) Field crew lead selects biological sampling locations in four different riffles.
- 3) The lead identifies the biological sampling location with numbered flags along the bank.
- 4) Field crew collects macroinvertebrate samples from all four sampling locations.
- 5) The lead collects two substrates from the sides of the D-frame net and hands them to a field assistant for periphyton collection.
- 6) Field crew collects periphyton samples.
- 7) The lead collects particles across the channel at each of the three riffle transects and determines particle embeddedness and size; substrate size representation is estimated from the pebble count.
- 8) Field crew deposits collected BMI into a container and preserves the samples with 85% *isopropanol*.

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- 9) Field crew collects sediment samples for characterization of toxics immediately following collection of the BMI samples at each location. Sediment samples will be collected to the side of each BMI site and from the nearest finely deposited sediment.
- 10) Field crew evaluates slope and reach-wide bank stabilization.

With the above sampling sequence, stream disturbance is minimized before surface water and biological information is collected.

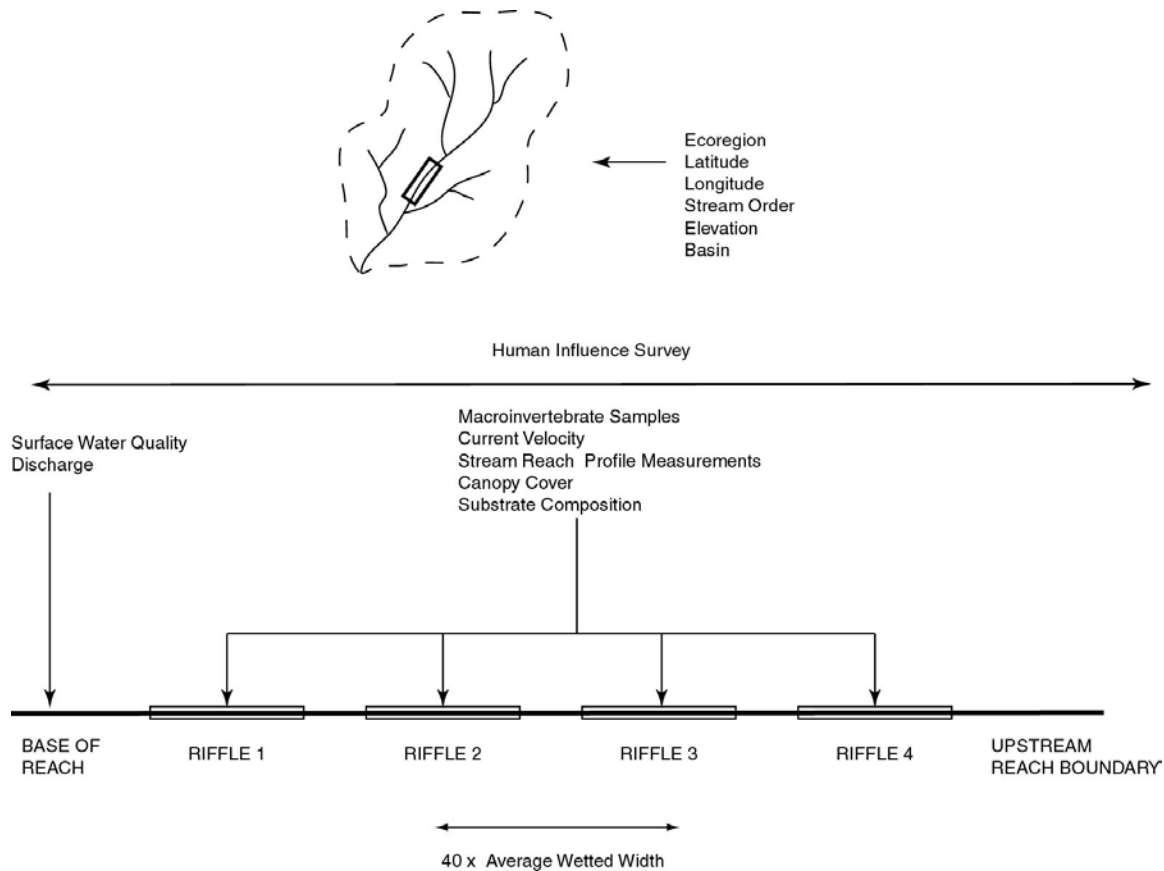


Figure 4. Sequence of field operations. (from Plotnikoff and Wiseman, 2001)

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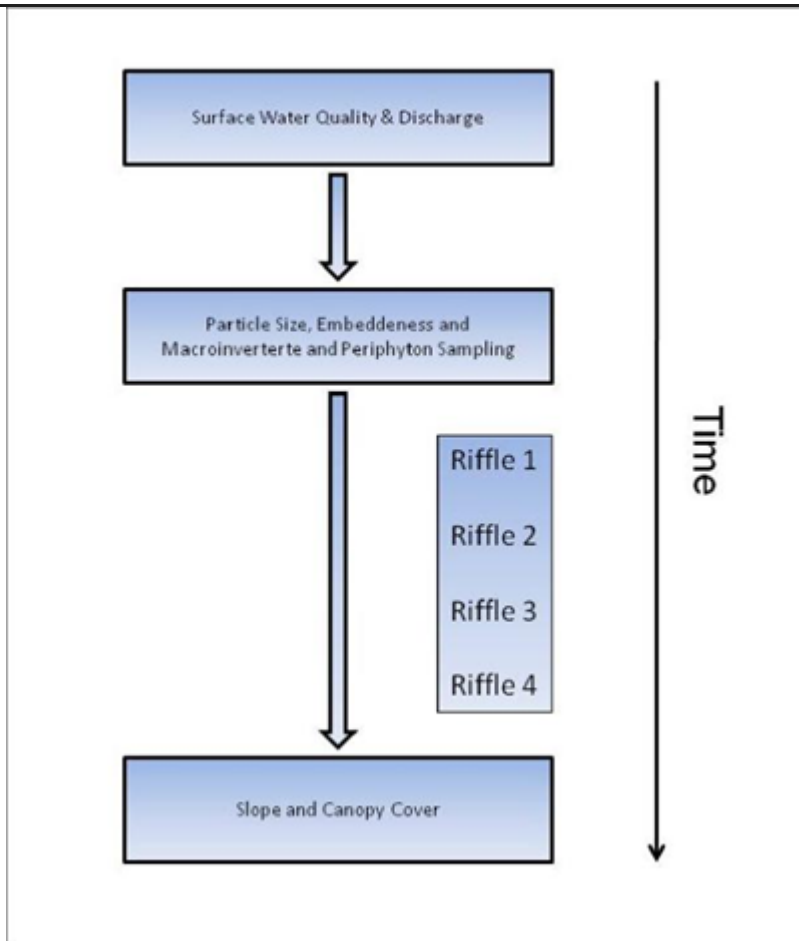


Figure 5. Spatial distribution of field operations. (Modified from Plotnikoff and Wiseman, 2001)

Measurement methods for characterization of physical, chemical, and biological conditions are described in Table 8. Information contained in this table provide a summary of additional detail for the field conditions expectations and the type and number of samples that should result from field collection effort.

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Table 8. Measurement methods (Field and Laboratory)

Analyte	Sample Matrix	Number of Samples (one sample per site)	Expected Range of Results	Reporting Limit	Sample Prep Method	Analytical (Instrumental) Method
Bioassessment Parameters						
a. Chlorophyll <i>a</i> analyzed by Aquatic Research, Inc.						
b. BMI/Periphyton analyzed by Rhithron Associates, Inc.						
Chlorophyll <i>a</i>	Substrate	≥5 aliquots at each site	NA	0.1 µg/L	SM 10200H(3)	Cell count & volumetric analysis
Periphyton	Substrate	6 samples (1 composite of 8 samples)	NA	NA	20% Lugol's Solution	Microscopic Identification
BMI	Substrate	6 samples (1 composite of 8-1sq.ft. samples)	NA	NA	95% Non-denatured Ethanol	Microscopic Identification
Water Quality Field Parameters						
Temperature	Water	2 measurements per site	0 - 25 °Celsius	0.01° C	NA	Hydrolab MiniSonde®
Dissolved Oxygen	Water	2 measurements per site	0 – 12 mg/L	0.1 mg/L	NA	Hydrolab MiniSonde®
Conductivity	Water	2 measurements per site	50 – 250 µmhos/cm	0.1 µS/cm 0.2 @ 25° C	NA	Hydrolab MiniSonde®
pH	Water	2 measurements per site	6 – 9 standard units	1 to 14 SU	NA	Hydrolab MiniSonde®
Sediment Parameters analyzed by MEL (except grain size by contract laboratory)						
Total Organic Carbon (TOC)	Sediment	6 samples (1 composite per site)	<1 to 20%	0.1 %	Acid digest and combustion @ 900°C	PSEP, 1997 SM5310B
Grain Size	Sediment	6 samples	-4 to 10 phi	0.1%	Sieve and pipette	PSEP, 1986
Base Neutral Acids (BNAs)	Sediment	6 samples	<10 to 50,000 µg/Kg	25-250 µg/Kg dry	GC/MS	EPA 8270
Pesticides ¹	Sediment	6 samples	<0.5 to 50,000 µg/Kg	10-100 µg/Kg dry	GC/ECD	SW-846 Method 8081
Polychlorinated Biphenyls (PCBs) ¹	Sediment	6 samples	<0.5 to 50,000 µg/Kg	<0.5 to 5,000 µg/Kg	GC/ECD	SW-846 Method 8082
Arsenic (As)	Sediment	6 samples	<1 to 50	0.1 mg/Kg dw	ICP/MS	EPA 200.8



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Copper (Cu)	Sediment	6 samples	<1 to 200	0.1 mg/Kg dw	ICP/MS	EPA 200.8
Lead (Pb)	Sediment	6 samples	<1 to 1500	0.1 mg/Kg dw	ICP/MS	EPA 200.8
Zinc (Zn)	Sediment	6 samples	<1 to 3000	5.0 mg/Kg dw	ICP/MS	EPA 200.8
Physical Habitat						
Riffle Pebble Count and Embeddedness	Instream and Riparian	4 locations corresponding with BMI collection	NA	NA	Adams, 2010	Visual, ruler
Bankfull Width	Instream and Riparian	4 locations corresponding with BMI collection	NA	NA	Adams, 2010	Tape
Bank Stability	Instream and Riparian	4 locations corresponding with BMI collection	NA	NA	Adams, 2010	Visual Observations
Wetted Width	Instream and Riparian	4 locations corresponding with BMI collection	NA	NA	Adams, 2010	Tape
Slope	Instream and Riparian	4 locations corresponding with BMI collection	NA	NA	Adams, 2010	Clinometer (%)
Canopy Cover	Instream and Riparian	4 locations corresponding with BMI collection	NA	NA	Adams, 2010	Densiometer
Current Velocity	Instream and Riparian	4 locations corresponding with BMI collection	NA	NA	Adams, 2010	Marsh-McBirney (or equivalent)
Stream Discharge	Instream and Riparian	1 measurement at base of sampling reach	NA	NA	Adams, 2010	Marsh-McBirney (or equivalent)

¹ Pesticides and PCBs (PEST1PCB) is a combined analyte list run at Manchester Environmental Laboratory. The list of analytes can be found in Appendix B.

Quality Control

Data quality is addressed, in part, by consistent performance of valid procedures documented in the SOPs found in Adams (2010) and Collyard (2009). It is enhanced by the training and experience of project staff and documentation of project activities. This QAPP, including its appendices, will be distributed to all sampling personnel. A QC Officer will ensure that samples are taken according to the established protocols and that all forms, checklists, and measurements are recorded and completed correctly during the sampling event.

Measurement performance criteria for data to be collected during this project are discussed in the following sections.

Field Quality Assurance

Precision

Precision is a measure of internal method consistency. It is demonstrated by the degree of mutual agreement between individual measurements or enumerated values of the same property of a sample, usually under demonstrated similar conditions. The usability assessment will include consideration of this condition in evaluating field measures from the entire measurement system. Although precision evaluation within 20 percent relative percent difference (RPD) are generally considered acceptable for water quality studies and analyses, no data validation or usability action will be taken for results in excess of the 20 percent limit. Instead, the results will be noted and compared with the balance of the parameters analyzed for a more comprehensive assessment before any negative assessment, disqualification, or exclusion of data.

This QC calculation also addresses uncertainty due to natural variation and sampling error. Precision is calculated from two duplicate samples by RPD as follows:

$$RPD = \frac{|C_1 - C_2|}{(C_1, C_2)} \times 100\%$$

Where C_1 = the first of the two values and C_2 = the second of the two if precision is to be calculated from three or more replicate samples (as is often the case in laboratory analytical work), the relative standard deviation (RSD) will be used and is calculated as:

$$RSD = \frac{s}{\bar{x}}$$

Where \bar{x} is the mean of the replicate samples, and s is the standard deviation and is determined by the following equation:

$$SD = \sqrt{\frac{\sum_{i=1}^n (\chi_i - \bar{\chi})^2}{n-1}}$$



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Where χ_i is the measured value of the replicate, $\bar{\chi}$ is the mean of the measured values, and n is the number of replicates.

For this project, replicate field samples (BMI, periphyton, or physical habitat) will be collected for all six Squalicum Creek sites. There will be one site in the Soos Creek drainage where a replicate will be collected to assess sampling precision. Replicate water quality measurements, where applicable, are made for one of five sample sites visited.

Accuracy

Accuracy is defined as the degree of agreement between an observed value and an accepted reference or true value. Accuracy is determined by using a combination of random error (precision) and systematic error (bias) due to sampling and analytical operations. Bias is the systematic distortion of a measurement process that causes errors in one direction so that the expected sample measurement is always greater or lesser to the same degree than the sample's true value. EPA now recommends that the term *accuracy* not be used and that *precision* and *bias* be used instead.

Because accuracy is the measurement of a parameter and comparison to a *truth*, and the true values of environmental physicochemical characteristics cannot be known, use of a surrogate is required. Accuracy of field measurements will be assumed to be determined through use of precision.

The accuracy of field equipment for the measurement of temperature, DO, conductivity, salinity, and pH will be determined at a minimum of two points that span the expected range of values for these parameters. Instruments used and procedures for determining accuracy include the following:

Temperature sensors:

The accuracy of temperature sensors used in this project will be checked using a standard thermometer.

DO sensors:

The accuracy of DO sensors and methods used in this project will be determined using the ambient air oxygen concentration to calibrate the multi-parameter probe before each day's instantaneous measurements. The actual concentration of DO at saturation is determined by measuring temperature and reading the corresponding concentration from a standard table and by making the required correction for nonstandard atmospheric pressure conditions (if the instrument is not set-up for automatic adjustment).

Conductivity sensors:

The accuracy of the salinity and conductivity sensor used in this project will be checked using certified calibration solutions appropriate for the range of expected results. Initial calibration standards should bracket most sample results, for example in low conductivity streams a calibration may consist of a 0 and 100 $\mu\text{S}/\text{cm}$ standard, or a larger river may require a 0 and a 1,000. Daily verification of calibration should be evaluated using a 100 $\mu\text{S}/\text{cm}$ solution.

pH sensors:

The accuracy of pH sensors used in this project will be checked using a certified pH 7 buffer solution. Initial calibration may include 3 points 4, 7, and 10 to cover all possibilities, or a



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calibration range appropriate for the pH in the study area may be more appropriate 4-7 for low pH streams and 7-10 for larger rivers and higher pH regimes.

Accuracy of data entry into the project database (or spreadsheets) will be controlled by double-checking all manual data entries.

Representativeness

Data representativeness is defined as the degree to which data accurately and precisely represents a characteristic of a population, parameter, and variations at a sampling point, a process condition, or an environmental condition. It therefore addresses the natural variability or the spatial and temporal heterogeneity of a population. The number of sampling points and their location within the study area were selected from a random draw to ensure that representative sample collection of each area of the watershed and each assessment characteristic occurs.

Completeness

Completeness is defined as the percentage of measurements made that are judged to be valid according to specific criteria and entered into the data management system. To achieve this objective, every effort is made to avoid accidental or inadvertent sample or data loss. Accidents during sample transport or lab activities that cause the loss of the original samples will result in irreparable loss of data. Lack of data entry into the database will reduce the ability to perform analyses, integrate results, and prepare reports. Samples will be stored and transported in unbreakable (plastic) containers wherever possible. All sample processing (subsampling, sorting, identification, and enumeration) will occur in a controlled environment within the laboratory. Field personnel will assign a set of continuous identifiers to a batch of samples.

Percent completeness (%C) for measurement parameters can be defined as follows:

$$\%C = \frac{V}{T} \times 100\%$$

Where V = the number of measurements judged valid and T = the total number of measurements planned.

For this project, sampling will be considered complete when no less than 90 percent of the samples collected during a particular sampling event are judged valid.

Comparability

Two data sets are considered to be comparable when there is confidence that the two sets can be considered equivalent with respect to the measurement of a specific variable or group of variables. Comparability is dependent on the proper design of the sampling program and on adherence to accepted sampling techniques, and QA guidelines.

Laboratory Quality Assurance

Lab Quality Assurance Samples - Macroinvertebrate Sorting (Standard Procedure for Commercial Services)



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Samples are either sorted whole, or in the case of large sediment volumes, sub-sampled so that only a fraction of the original is analyzed. Precision of the sub-sampling process is evaluated by re-sorting a new sub-sample of the original samples. Ten percent of the benthic macroinvertebrate samples (1 of 10 samples) are re-sorted by a second laboratory technician. Sorting results that are less than 95% similar would indicate the need for (1) more thorough distribution of sample materials across the sub-sampling grid, and (2) special attention given to easily missed taxa when sorting (for example, increased magnification).

Taxonomic Accuracy and Precision

Taxonomic misidentification results in inadequate biological characterization of a stream. Errors in identification should be less than 5% of the total taxa in the sample. Re-identification of samples is conducted for 10% of the total number of samples in each year. Secondary identification is conducted by experienced taxonomists to maintain confidence in the data set. Difficult taxa should be sent to museum curators whose specialty includes members of the order in question. A voucher collection has been maintained by Ecology for biomonitoring project samples and transferred to the Orma J. Smith Museum of Natural History in Caldwell, Idaho for curation. A voucher collection should be prepared from the set of samples for the year and shipped to the address below:

The Orma J. Smith Museum of Natural History
College of Idaho
2112 Cleveland Blvd
Caldwell, ID 83605-4432

Frequency of replicate collection for determining precision and checks on cross-contamination when transporting samples will be reviewed by using blanks, check standards and replicates for field and laboratory samples. Specific requirements for conducting these quality control procedures are provided for each of the parameters in Table 9.

Chemistry Accuracy, Precision and Bias

Laboratory QC samples to be used to assess accuracy, precision and bias of data obtained in this study are shown in Table 9. To limit QC costs, all sediment samples and duplicates will be collected in one sampling event, such that one batch represents the whole study. The QC procedures routinely followed by MEL or required of its contractors will be satisfactory for the purposes of this project. QC procedures include blanks, control samples, laboratory duplicates, matrix spikes, and surrogate spikes. Sufficient material will be sent for complete laboratory QC samples to be exercised.

Laboratory control samples contain known amounts of analytes and indicate bias due to matrix effects, calibration, and/or sample preparation. Results of duplicate samples provide estimates of analytical precision. Matrix spikes may reveal bias due to matrix interferences or provide an estimate of the precision of the results. Accuracy is assessed using standard reference materials. The organic compound analyses involve spiking each sample with labeled compounds or congeners (BNAs and PCBs, respectively). The concentration of the target compounds are corrected for recovery of the labeled compounds or congeners; the remaining compounds or congeners are determined by an internal quantitation technique.

Table 9. QC Samples, Types, and Frequency



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Parameter	Field		Laboratory			
	Blanks	Replicates	Check Standards	Method Blanks	Analytical Duplicates	Matrix Spikes
Water Quality Field Measurements						
Water Temperature	N/A	1/5 sample sites	N/A	N/A	N/A	N/A
Dissolved Oxygen	N/A	1/5 sample sites	N/A	N/A	N/A	N/A
Specific Conductivity	N/A	1/5 sample sites	1/run	N/A	N/A	N/A
pH	N/A	1/5 sample sites	1/5 sample sites	N/A	N/A	N/A
Stream Discharge	N/A	1/5 sample sites	N/A	N/A	N/A	N/A
Bioassessment Parameters						
a. Chlorophyll <i>a</i> analyzed by Aquatic Research, Inc.						
b. BMI/Periphyton analyzed by Rhithron Associates, Inc.						
Chlorophyll <i>a</i>	N/A	1/6 for each of the Creeks	N/A	N/A	N/A	N/A
BMI	N/A	6/6 for Squalicum Cr 1/6 for Soos Creek	N/A	N/A	N/A	N/A
Periphyton	N/A	1/6 for each of the Creeks	N/A	N/A	N/A	N/A
Sediment Parameters Analyzed by MEL						
Total Organic Carbon (TOC)	1/batch	1/batch	1/batch	1/batch	1/batch	NA
Grain Size	NA	NA	NA	NA	3/batch	NA
BNAs	N/A	1/batch	1/batch	1/batch	N/A	1/batch
PEST1PCB	N/A	1/batch	1/batch	1/batch	N/A	N/A
Arsenic (As)	N/A	1/batch	1/batch	1/batch	N/A	1/batch
Copper (Cu)	N/A	1/batch	1/batch	1/batch	N/A	1/batch
Lead (Pb)	N/A	1/batch	1/batch	1/batch	N/A	1/batch
Zinc (Zn)	N/A	1/batch	1/batch	1/batch	N/A	1/batch
Physical Habitat						
Riffle Pebble Count and Embeddedness	N/A	4-8	N/A	N/A	N/A	N/A
Bankfull Width	N/A	4-8	N/A	N/A	N/A	N/A
Bank Stability	N/A	4-8	N/A	N/A	N/A	N/A
Wetted Width	N/A	4-8	N/A	N/A	N/A	N/A
Slope	N/A	4-8	N/A	N/A	N/A	N/A
Canopy Cover	N/A	4-8	N/A	N/A	N/A	N/A
Current Velocity	N/A	4-8	N/A	N/A	N/A	N/A
Stream Discharge	N/A	4-8	N/A	N/A	N/A	N/A

Sediment sampling will be conducted simultaneously with physical habitat, benthic macroinvertebrate, and periphyton sampling. Ecology will collect and analyze samples for several parameters. Cost for analysis of these sediment parameters are in Table 10.



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Table 10. Cost of sediment sample analyses.

Analysis	Lab	Number of Samples	Field QA Samples	Lab QC Samples	Cost per Sample ³	Cost Subtotals ⁴
Grain Size	Sub-contract ¹	6	0	0	\$110	\$660
TOC	MEL	6	1	0	\$45	\$315
Metals ²	MEL	6	1	1	\$108	\$864
BNAs	MEL	6	1	1	\$298	\$2,384
PEST1PCB	MEL	6	1	1	\$255	\$2,040
Total Cost:						\$6,263

¹ Contracting for grain size analysis will be handled by the MEL, and a 25% surcharge is included.

² Metals analysis includes arsenic, copper, lead, and zinc.

³ With the exception of MS/MSDs, laboratory QC is included in unit costs.

⁴ Costs include a 50% discount for analyses conducted at MEL

Data Management Procedures

Samples will be documented and tracked on Field Data Record forms, Sample Identification labels, and Chain of Custody records. The Field Task Leader will be responsible for ensuring that these forms are completed and reviewed for correctness and completeness by the designated field QC Officer. Tetra Tech (Tt) will maintain copies of these forms in the project files. A sampling report will be prepared following each sampling event. Another person will manually check data entered into any spreadsheet or other format against the original source to ensure accurate data entry. If there is any indication that requirements for sample integrity or data quality have not been met (for samples or measurements collected by Tt), the Tt QAO will be notified immediately (with an accompanying explanation of the problems encountered).

Benthic macroinvertebrate, periphyton and water quality laboratory results will be provided by electronic and hard copy. Hard copy data packages will be paginated, raw data packages that include an analytical narrative with a signed certification of compliance with this QAPP and all method requirements; copies of Chain of Custody forms; sample inspection records; laboratory sample and QC results; calibration summaries; example calculations by parameter; and copies of all sample preparation, analysis, and standards logs adequate to reconstruct the entire analysis. The CD-ROM data will include a full copy of the paginated report scanned and stored in portable document format (PDF) for potential future submission to the client, if requested, and for long-term storage in the project files. Initially, the full raw data package will be submitted to the Tt QAO for assessment of compliance with the program goals and guidance.

All computer files associated with the project will be stored in a project subdirectory by Tt (subject to regular system backups) and will be copied to disk for archive for the 5 years subsequent to project completion (unless otherwise directed by the EPA TOM). The BMI and habitat data will be entered in to the Puget Sound Stream Benthos online database. Benthic count data and physical and chemical data will be submitted for entry to Ecology's EIM database. "Benthic data collected for Squalicum Creek will be available on Ecology's Environmental Information Management (EIM) website at www.ecy.wa.gov/eim/index.htm. Search User Study ID, BRWA0007."

Laboratory Data

Procedures for laboratory data reduction, review, and reporting are outlined in the *Lab User's Manual* (MEL, 2008). Laboratory staff will be responsible for the following functions:

- Data verification.
- Proper transfer of data to the Laboratory Information Management System (LIMS).
- Reporting data to the Ecology project manager.
 - Uploading BMI and habitat data to the Puget Sound Stream Benthos website

Chemistry and physical data collected will be formatted and electronically delivered to Ecology project managers for inclusion in the EIM database. Data will be entered after data verification and validation. The project manager will perform the following functions:

- Review data for errors
- Apply corrective measures to minimize errors and validate the quality of the data.



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The project manager may approve data that do not meet MQOs, but only after consultation with QA Project Plan signatories, and only with appropriate data qualification.

Laboratory Reports

The taxonomic consulting laboratory and water quality laboratory will report all laboratory results to the Tetra Tech project manager within 30 days of sample delivery (may be 45 days for the taxonomic consulting laboratory). The reports will include narratives, numerical results, data qualifiers, and costs. The taxonomic contractor will report all results to the project manager within two months of sample delivery.

MEL and contract laboratories will compile analytical results in electronic formats. The lab data packages will include chain of custody forms, case narratives discussing any problems with the analyses, corrective actions taken, changes to the referenced methods, and an explanation of data qualifiers. All laboratory QC results associated with the data will also be provided in the data packages, including results for blanks, control samples, duplicates, matrix spikes, and surrogate recoveries. This information will be used to evaluate data quality and to determine whether the MQOs were met.

Field Data

Field observations and measurement data will be recorded by pencil onto a notebook with waterproof pages. The Tt project manager will review the field data after each sampling run and calculate discharge from water velocity measurements. The Tt project manager will review calculated data for errors and make procedural adjustments as necessary. All field data will then be entered into Excel® spreadsheets templates that have been formatted for Ecology's EIM database. Data entry and verification will be performed by staff within Ecology's Environmental Assessment Program. All entered data will be validated by an internal, independent reviewer. Errors found will be identified, flagged, and corrected by the Ecology project manager.

Audits and Reports

The taxonomic contractor will submit laboratory reports, QA worksheets, and chain-of-custody records that will be examined by the Tetra Tech Technical Lead. Any problems and associated corrective actions will be reported by the laboratory to the Tetra Tech Technical Lead. The Tetra Tech Technical Lead is responsible for periodic audit updates to the team and client as well as for the final report.

The taxonomic contractor and water quality laboratory will submit laboratory reports and QA information to the Tetra Tech project manager according to the project timeline. Taxonomic reports will be delivered within 2 months from the date they were submitted and should include taxa lists, taxa counts, and standard and requested metrics for BMI and periphyton. The water quality laboratory will report all results for water chemistry, soil chemistry, and chlorophyll *a* to the Tetra Tech project manager within 30 days of sample delivery. The reports will include narratives, numerical results, data qualifiers, and costs.

The laboratory will report any problems and associated corrective actions to the Tetra Tech Technical Lead who will flag data. These data may be dropped from analysis if the problem can't be addressed. The project manager is responsible for periodic audit updates to the sampling team as well as for any reports upon request.

Data Verification and Validation

Data Verification

Data verification involves examining the data for errors, omissions, and compliance with quality control (QC) acceptance criteria. The water quality laboratory and taxonomic consulting laboratory is responsible for performing the following functions:

- Reviewing and reporting QC checks on instrument performance such as initial and continuing calibrations.
- Reviewing and reporting case narratives. This includes comparison of QC results with method acceptance criteria such as precision data, surrogate and spike recoveries, laboratory control sample analysis, and procedural blanks.
- Explaining flags or qualifiers assigned to sample results.
- Reviewing and assessing MEL's performance in meeting the conditions and requirements set forth in this QA Project Plan.
- Reporting the above information to the project manager or lead.

After field staff record measurement results, the results are verified by the project manager to ensure that:

- Data are consistent, correct, and complete, with no errors or omissions.
- Results of QC samples accompany the sample results.
- Established criteria for QC results were met.
- Data qualifiers are properly assigned where necessary.
- Data specified in the Sampling Process Design were obtained.
- Methods and protocols specified in this QA Project Plan were followed.

The Tt project manager is responsible for verifying all taxonomic results.

Field results will also be verified by field staff before leaving the site after measurements are made. Detailed field notes will be kept to meet the requirements for documentation of field measurements. The field lead is responsible for checking that field data entries are complete and error free. The field lead will check for consistency within an expected range of values, verify measurements, ensure measurements are made within the acceptable instrumentation error limits, and record anomalous observations.

Data validation and review services provide a method for determining the usability and limitations of data and provide a standardized data quality assessment. All Field Data forms will be reviewed by the Tt TOLs (assisted by the QAO, as needed) for completeness and correctness. Tt will be responsible for



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reviewing data entries and transmissions for completeness and adherence to QA requirements. Data quality will be assessed by comparing entered data to original data or by comparing results to the measurement performance criteria determine whether to accept, reject, or qualify the data. Results of the review and validation processes will be reported to the TOLs.

Data Quality (Usability) Assessment

The Tt project manager will examine the complete data package to determine compliance with procedures outlined in the QA Project Plan and Standard Operating Procedures. The project manager is also responsible for the data usability assessment by ensuring that the MQOs for precision, bias, and sensitivity are met.

Part of this process is an evaluation of precision. Precision will be assessed by calculating relative standard deviations (RSDs) for field and laboratory duplicates. Laboratory duplicates will yield estimates of precision performance at the laboratory only. Field replicates will indicate overall variability (environmental + sampling + laboratory). Acceptable precision performance is outlined in the MQOs (Table 4).

The project manager will assess completeness by examining the (1) number of samples collected compared to the sampling plan; (2) number of samples shipped and received at MEL and the taxonomic contractor in good condition; (3) lab's ability to produce usable results for each sample; and (4) sample results accepted by the project manager.

To analyze data for its usability, the project lead will consider precision, completeness, and documentation of adherence to protocols. Data will also be examined for extremes (i.e., against historical records and against the distributions of these project data). Extreme values will require logical explanations. Identified sources of bias will be described in the final project report.

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Acronyms and Abbreviations

BMI	Benthic macroinvertebrate
°C	degrees Celsius
cm	centimeters
DO	Dissolved oxygen
DQI	Data quality indicators
DQO	Data Quality Objectives
Ecology	Washington Department of Ecology
EIM	Environmental Information Management
EPA	Environmental Protection Agency
g	grams
GRTS	Generalized Random Tessellation Design
m	meter(s)
µS/cm	microSiemens per centimeter
mg/L	milligrams per liter
NPS	Nonpoint source
PDF	Portable Document Format
PNAMP	Pacific Northwest Ambient Monitoring Partnership
QA	Quality assurance
QAM	Quality Assurance Manager
QAO	Quality Assurance Officer
QAPP	Quality assurance project plan
QC	Quality control
QCO	Quality Control Officer
RBP	Rapid Bioassessment Protocol
RPD	Relative percent difference
RSD	Relative standard deviation
SOP	Standard Operating Procedure
TMDL	Total Maximum Daily Load
TOL	Task Order Leader
TOM	Task Order Manager
Tt	Tetra Tech, Inc.
WDFW	Washington Department of Fish and Wildlife
WWTP	Waste Water Treatment Plant

Appendices

Appendix A: Biomonitoring and Analysis of Data to Support Stormwater TMDL Development

The 1998 TMDL Consent Decree required U.S. EPA Region 10, and by delegated authority, required Washington Department of Ecology (Ecology) to develop and implement TMDLs based on the 1998 303(d) water quality impairment listings. After 13 years of work in developing more than 600 TMDLs, the Litigants responsible for bringing about this Consent Decree reviewed Ecology's progress. The Litigants agreed that Ecology had made good progress toward the original goal, but wanted to ensure that current issues involving stormwater impacts and biological impairments were addressed under continuing requirements of the settlement agreement.

Under the National Watershed Contract, Tetra Tech was asked by U.S. EPA Region 10 and Ecology to develop a Technical Approach for use of biological information in evaluating and determining progress in abating impacts from stormwater. Biological information is used in conjunction with regulation of stormwater through TMDL development. Two watersheds (Squalicum and Soos Creek watersheds) have been identified where existing information will be used along with more recent biological assessments in order to relate physical and chemical factors altered by stormwater events with predictable biological responses. Major components that will be developed for integrating biological assessments along with the water quality TMDL in each drainage are as follows:

- STEP 1** Identify biological evaluation tools and methods for analysis;
- STEP 2** Biological information and water quality information that needs to be combined as part of an integrated assessment of stormwater impacts; and
- STEP 3** How to interpret possible outcomes for biological conditions and water quality conditions following assessment.

The description of the Technical Approach has several elements organized in a series of logical steps, beginning with biological response to stormwater stressors. The orders in which the technical tools are developed and used are shown in Figure 1. These categories are more fully described in the "Biomonitoring Tools" section of this Technical Approach.



Step 1. Biological Response to Stormwater Stressors

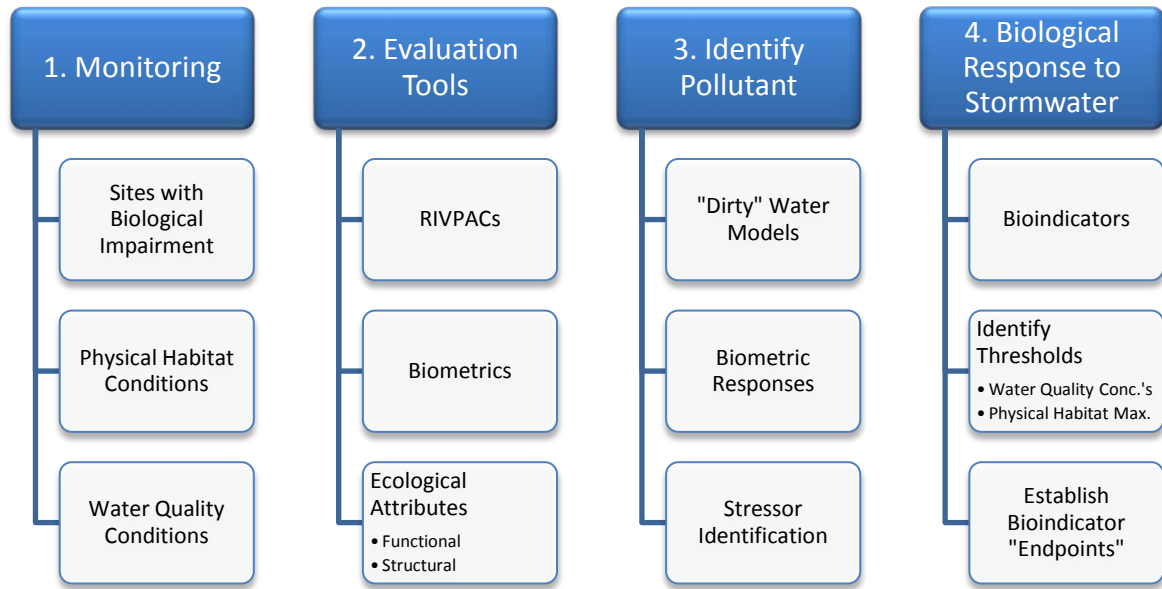


Figure 1. Biological information and analytical tools used to identify the biological response resulting from exposure to stormwater stressors.

Although several tools exist that serve as the “building blocks” for integrating biological information with water quality-based indicators, the combination of physicochemical and biological information is necessary (Figure 2).

Step 2. Initial Steps in Combining Biological and Water Quality Information

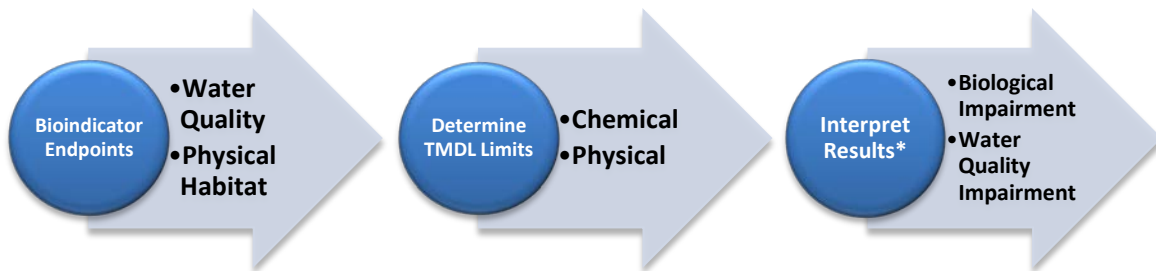


Figure 2. The process for combining biological and water quality information (*refer to Figure 3).

The sensitivity of monitoring information (e.g., water quality and biological) may differ in the presence of the same stressor and assessment of conditions could vary if an integrated assessment (e.g., biological, chemical, and physical) is not used. Figure 3 describes the possible outcome of individual assessments and resulting management implications.

Step 3. Interpreting possible outcomes for biological conditions and water quality conditions.

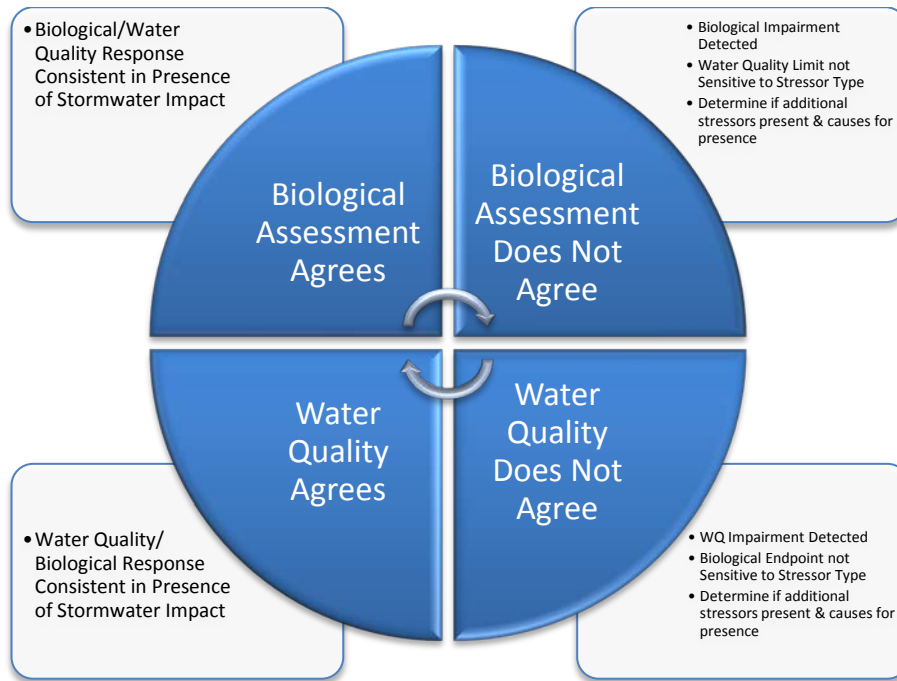


Figure 3. Combinations of assessment outcomes and using the integrated approach for determining combination of related stressors.

The use of an integrated monitoring approach will ensure that all stressors in the aquatic environment are detected by either the water quality assessment or by directly measuring biological condition. Failing to abate all stressors affecting biological communities through management practices will mask progress in improving water quality (including physical habitat condition) and fail to meet water quality goals.

Squalicum Creek and Soos Creek Biological Monitoring

Candidate sites in the Squalicum Creek and Soos Creek watersheds will be identified that have measurable stormwater impacts and are under further consideration as demonstration locations for determining utility of using multiple indicators. The use of multiple indicators is advantageous for the following reasons:

- To identify specific pollutants from stormwater input, and
- To identify habitat where toxics are transported and increase exposure potential of aquatic life (benthic macroinvertebrates).

Some of the reaches in the watersheds of interest have been the focus of extensive environmental data collection effort. For example, Squalicum Creek at Cornwall Park is a

Category 2 listing (waters of concern) based on a River Invertebrate Prediction and Classifications System (RIVPACS) assessment score below acceptable threshold. This listing has been included on both the 2004 and 2008 303(d) list of impaired waterbodies in the State of Washington. Examination of existing data for identification of stream reaches where additional biomonitoring will be collected recognizes the relationships between stream setting and potential for human influence. Potential stormwater stressors like flow characteristics that are characterized by indicators like $T_{Q_{Mean}}$ and Richards-Baker Flashiness Index will be related to biological responses. The relationship between indicators (e.g., physical habitat or water quality) and landscape setting will also be used to develop the sequence for implementing improvements in order to achieve TMDL Management Plan goals.

The selection of sites in several types of stream reaches should have a range of characteristics beginning with those considered to be like high quality Western Washington streams (assessed using the RIVPACS predictor variables). Additional, related variables that are degraded by stormwater input should also be reflected at sites within the same drainage so that direct comparison between high quality and stormwater impaired sites can be examined for specific differences (or combination of differences) that are attributable to this impact. For watersheds where physical habitat and stream dynamics have not been well-described additional data collection will be necessary for determining principal factors that explain why BMI communities change at any time of year following exposure from stormwater input.

Biomonitoring Tools

Several interpretative tools have been developed for use in interpreting benthic community conditions in streams of Western Washington and for diagnosing the potential for causes of impairment.

1) BMI Assessment Tools

The predictive model developed for assessing benthic macroinvertebrate communities in Western Washington streams has been used to determine presence of impairment, and to a limited extent, suggests the type of impairment assimilated in the biotic community.

- a. RIVPACS Scores (Charles P. Hawkins; Western Center for Monitoring and Assessment of Freshwater Ecosystems)

The primary assessment tool for use in evaluating health of the benthic macroinvertebrate (BMI) community is the RIVPACS predictive tool. This tool measures biological condition in any wadeable stream in Western Washington and is based on sampling of almost 300 reference sites in the following ecoregions: Coast Range, Cascades, and Puget Lowland. There is no requirement that a reference condition be available among the set of sites in a drainage. The variability in reference condition description for wadeable Western Washington streams is a component of the reference condition threshold (usually one standard deviation about the mean for the reference site distribution of scores).

- b. Species Attributes (Output from RIVPACS model)
 - i. Increases
 - ii. Decreases



Attributes associated with the RIVPACs score are derived from further examination of species presence (or absence) when they are predicted to be in a stream setting. The general definition for “increasers” is tolerance to stressors present at a site whereas “decreasers” are intolerant to stressors present. These properties of the RIVPACS predictive model can be calibrated with known stressors; the biological “endpoint” is determined for stressors associated with stormwater input. The development of a RIVPACS model is described by a series of steps in generating biological information from reference sites and then synthesizing that data using several statistical applications. A series of steps for developing the RIVPACs model is described in simple terms in Figure 4.

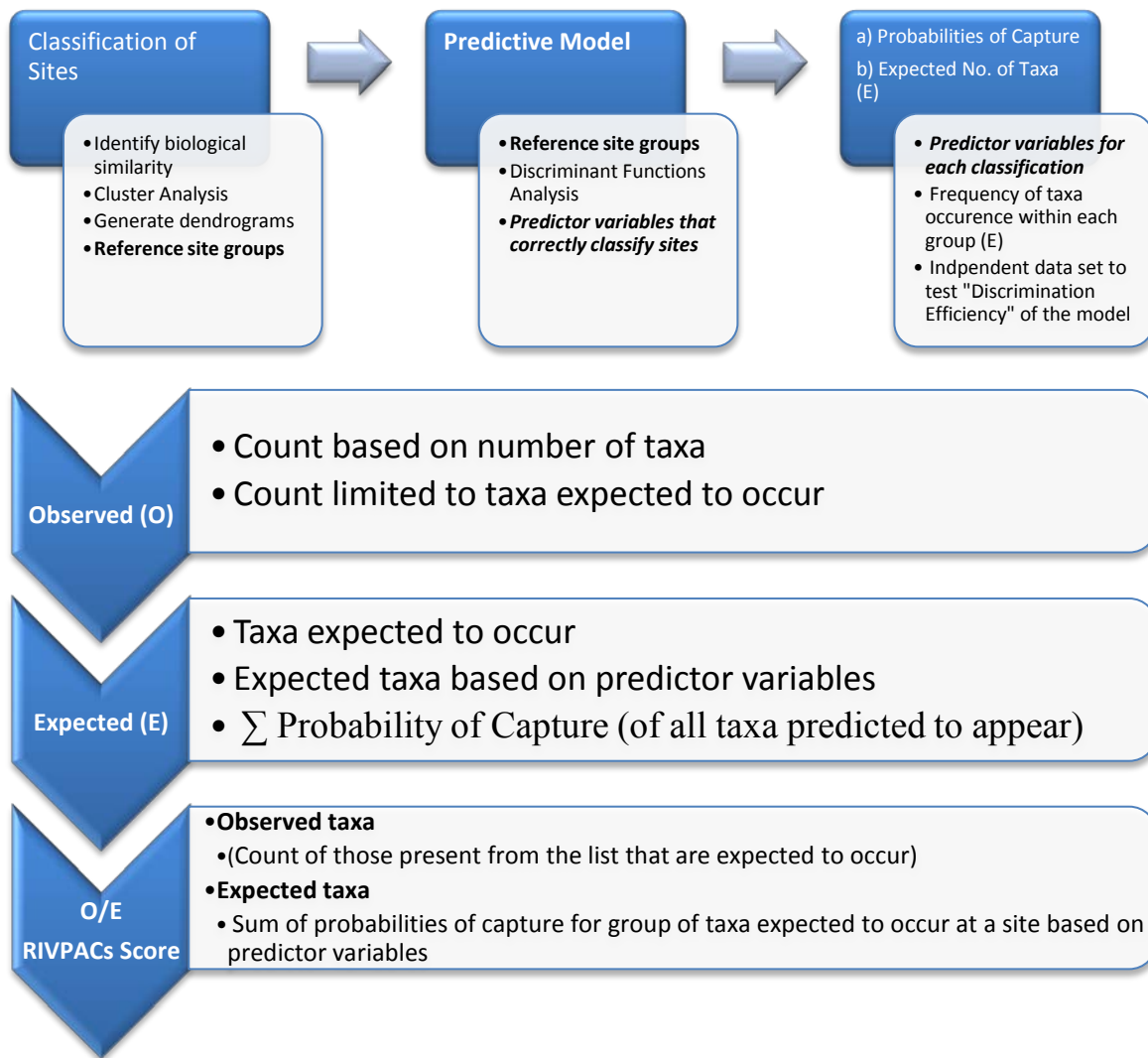


Figure 4. Steps used to build “Predictive” RIVPACs models in the flow chart above and description for how number of taxa expected to be present and the sum of the probabilities used to calculate O/E.

c. Biological Metrics



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- i. Functional Attributes
 - ii. Behavioral Attributes

Several biological metrics (or biometrics) are associated with behavioral and feeding attributes. Experienced benthic ecologists will often note how representation of certain functional feeding groups will shift in the presence of a stressor type (e.g., nutrient enrichment or metals toxicity). A shift may be in an increasing or decreasing direction (expressed as a percentage of community density, a numeric count, or the number of species aggregated within a higher taxonomic level) and is calibrated with a corresponding shift in concentration of a stressor. In addition, specialized indexes have been developed for benthic macroinvertebrates (and periphyton) tolerance to metals concentrations in sediment (Metals Tolerance Indexes).

Current Versions of the Multi-Metric Index for use in wadeable streams of Puget Sound:

- Kleindl (1995)
- Karr and Chu (1999)
- Morley (2000)
- Wiseman (2003)

2) Periphyton Assessment Tools

- a. Biological Metrics
 - i. Combined Metrics
 - ii. Diatom Metrics
 - iii. Van Dam Diatom Metrics
 - iv. Non-Diatom Metrics

Sampling and assessment using information from the periphyton community has similar utility as the benthic macroinvertebrate community. Several expressions are generated from the taxonomic groups represented in the sample. The use of structural, functional, and pollution tolerance metrics are used to interpret results from periphyton samples at a site confirming presence of a stressor that causes a change in the assemblage from that expected under reference (or unimpaired) stream condition. The periphyton sampling protocol and the benthic macroinvertebrate sampling protocol are described by Adams (2010). The use of multiple biological assemblages is recommended by U.S. EPA guidance as more effective than single assemblage assessments for the ability to detect a broader range of impacts at a site. The assemblages are usually differentially sensitive so do not respond in a measurable way to the same impact.

3) Physical Characteristics

- a. Flow Duration Curves
- b. "Flashiness" Index
- c. Impervious Areas
- d. Low flow during "critical period"

Stormwater typically delivers large volumes of water to the stream channel in a short period of time. Water input in large volumes causes disruption of stream bottom substrate, transport of



aquatic life from the benthos, and loss of useable habitat for indefinite periods of time depending on resulting substrate composition following a storm event or a storm season. Indicators that reflect these impacts describe flow attributes like longevity of increased flow levels, intensity of increased flows, or relationship between stormwater runoff and the landscape processes that promote increased input. Biological response indicators and some of these physical changes are related and useful for identifying impairment.

4) Water Quality Characteristics

- a. 303(d) listings (and location of data collection)
- b. Relationship between biological response and water quality impairment

Relating water quality impairments with biological response is the primary objective in this Technical Approach. Water quality impairments associated with stormwater input are more fully described in Table 1. The focus for describing cause-effect relationships will use variables that are changed by stormwater impacts and some of which will be measured when summer benthic sampling occurs. Describing chemical and physical condition of sites during the summer assumes that impacts from stormwater input earlier in the year are still present and have an effect on the aquatic invertebrate community.

5) Site Selection

- a. Existing Site Locations (how do they inform on site selection)
- b. Response Reaches
- c. Relationship to Landscape Setting (and pollutant types)
- d. Compliance Points and the TMDL Model

Existing biological data will be used to determine the differences among locations in the drainage and if these differences are related to field sampling variability or if a response is due to measurable physical and chemical differences. The existing data should be supplemented with additional data collection so that physical settings and surrounding land uses are represented as part of the potential effect on stream biological conditions throughout the drainage. Local agencies and other partners on this project will be consulted for existing data and local knowledge. Their input will enhance the utility of the final data set and interpretation based on results from past and current biological monitoring effort.

Response reaches are portions of the drainage where physical “breaks” occur such as measurable shifts in stream gradient, distinct differences between contiguous reaches (e.g., channel segment type), or a shift in land use type (e.g., urban versus agricultural). These locations may be suitable for segmenting the drainage for load allocations and the base of which would be considered “compliance points”. The benthic macroinvertebrate monitoring locations should be coincident with water quality compliance points from the TMDL so that information can be integrated and management decisions based on characterization of the same portion of the drainage.

Important elements to consider when establishing new biomonitoring sites:



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- Use information from existing biomonitoring sites;
 - Partition drainage into areas with dominant land use types;
 - Identify major gradient breaks on the mainstem;
 - Identify likely locations for “TMDL Compliance Points”; and
 - Final site locations integrate the above elements (as much as possible).

Interpretation of Biological Information

1) Species Tolerances Increasers/Decreasers

Four model output files are generated when submitting formatted predictor variable matrix and the formatted taxonomic matrix. The following is a description of the “Taxa Response Summary File” that identifies potential tolerances of individual taxa in a collection of sites sampled from the drainage.

The Taxa Response Summary File – This file lists all taxa that the model expected to see (i.e., those taxa in the reftaxa.txt file) as well as any new taxa that occurred at test sites (testtaxa.txt file) but were not observed in at least 1 reference site sample. For each of these taxa, listed is their average probability of capture (assuming sites were under reference condition), the number of test sites at which taxa were predicted to occur, the number of test sites at which taxa were observed, and the ratio of observed sites to expected sites for each taxon. This ratio is labeled as the ‘Sensitivity Index’ and is interpreted as a measure of sensitivity of a taxon to whatever stressors are influencing a taxon within the set of test sites submitted for assessment. A ratio > 1 indicates the taxon was found at more sites than expected and was thus an ‘increaser’ or tolerant taxon. A ratio < 1 indicates the taxon was found at fewer sites than expected and was thus a ‘decreaser’ or intolerant taxon. The magnitude of these values can provide insight into the relative sensitivities of taxa to stressors, although care should be taken to avoid over-interpreting ratios based on small numbers. Results obtained by separately submitting sets of samples that differ in the primary stressors known to be affecting sites may provide insight regarding the relative sensitivities of taxa to different stressors.

2) Stressor Groups

The relationship between stressor groups (e.g., nutrient enrichment, sediment transport, metals toxicity, etc.) and biological response using the RIVPACs tool (and biometrics) improves the utility of biological assessments for management decisions. Some stressor groups will be easier to detect with the RIVPACs tool and biometrics whereas other stressors will be difficult to detect on the short-term. Integrating biological assessment with water quality assessment will improve the ability for this approach to adequately describe potential threats to aquatic ecosystem health from stormwater input.

Several steps in the development of relationships between stressors and biological response are required. The primary steps include:

- Identification bio-indicators that respond to distinct stressors;



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- Ability to predict biological response by determining presence of stressors (physical habitat assessment or water quality characterization); and
 - Verification of the predicted biological responses

3) Develop a CADDIS (Causal Analysis Diagnosis/Decision Information System) Model

Biological impairments included on the 303(d) list are often included in Category 2 or Category 4C. These categories acknowledge that direct measurements of aquatic life conditions are not meeting expectations. In the absence of companion environmental information that could identify specific pollutant(s) responsible for the impairment, a stepwise process for identifying and systematically eliminating potential causes for impaired biological condition has been developed and used by Ecology (Adams 2010). The CADDIS approach (Causal Analysis Diagnosis/Decision Information System) for identification of specific pollutants likely the cause for impairment had been originally developed by U.S. Environmental Protection Agency and adopted by Ecology.

The identification of stressors and stressor groups (chemicals or physical elements in the aquatic environment that have the same effect on biological response) is the next step following 303(d) listing based on biological impairment of a stream segment. Specific parameters that will be measured in this project are identified from several sources: the 303(d) listings, local monitoring effort, existing monitoring data that detected high concentrations of toxics, and specific physical or chemical characteristics known to impair habitat and biota from nearby, similar streams. A simple description for the process is provided in Figure 5. The intent for using this process and following steps in this diagram is to accurately identify pollutant(s) causing biological impairment, and through a series of management actions, use strategies to abate pollution problems and restore healthy biological conditions.

The Management Context of the Stressor Identification Process

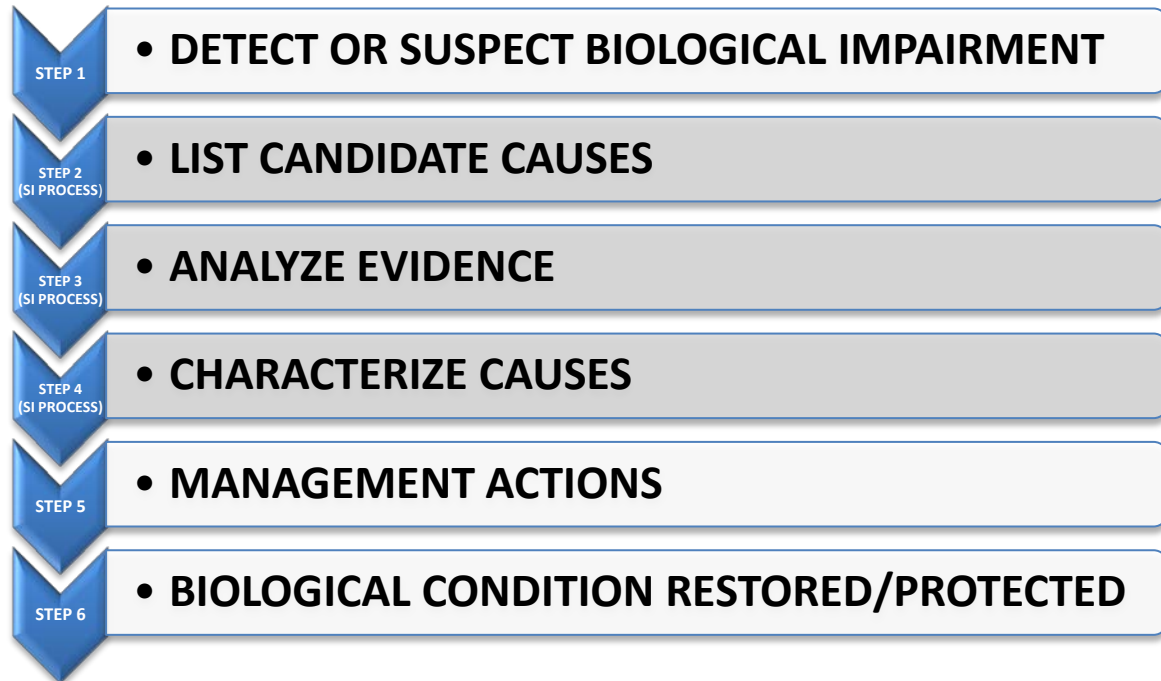


Figure 5. Steps in the Stressor Identification process that identify probable cause(s) for biological impairment.

This stepwise process for diagnosing and identifying stormwater stressor impacts to biological communities in Western Washington is an effective approach for documenting past and current conditions within a watershed. The organization of information can be used for future evaluations and modification of assessment tools in managing stormwater. Important information presently available will make future decisions on how to manage growing impacts in a watershed over time when stored in an accessible location.

Examples for identification of stressors to benthic macroinvertebrate communities and identification of cause-and-effect relationships are provided in the following Tetra tech documents:

Water Temperature, Sediments, Toxics

Wiseman CD, LeMoine M, Plotnikoff R, Diamond J, Stewart A, Cormier S (2009) Identification of Most Probable Stressors to Aquatic Life in the Touchet River, Washington. U.S. Environmental Protection Agency, Cincinnati OH. EPA/600/R 08/145.

Sediment and Nutrients:

Tetra Tech (2009) Groundhouse River Total Maximum Daily Loads for Fecal Coliform Loads and Biota (Sediment) Impairments. Prepared for Minnesota Pollution Control Agency. 375 p.

Lane C, Cormier S (2004) Screening Level Causal Analysis and Assessment of an Impaired Reach of the Groundhouse River, Minnesota. U.S. Environmental Protection Agency, Cincinnati OH.



CADDIS Guidance for Washington State

Adams K (2010) [Guidance for Stressor Identification of Biologically Impaired Aquatic Resources in Washington State](#). Washington State Department of Ecology, Olympia WA. Publication No. 10-03-036.

4) Relative Risk Analysis

Relative risk is often used in medical research to determine the likelihood that a stressor (e.g., smoking) is linked to an effect (e.g., heart disease). Similarly, researchers have successfully used this concept to determine the likelihood that environmental stressors have an effect on responses observed in the biological community. The use of relative risk in determining order in which stressors affect the biological response is based on probabilities of stressor effect among a group of sites.

Calculating relative risk for stressors in the Squalicum Creek and Big Soos Creek drainages would require use of a control group of sites (reference) for each watershed and the remaining sites known to be potentially affected by several different intensities of a stressor or stressor group. The ratio of the probability the stressor is present at a group of treatment sites and has an effect on the biological response versus the probability of the same stressor effect on the group of control (reference) sites is the relative risk measurement. A relative risk score of ≤ 1 indicates the stressor has no significant effect on biota. Relative risk scores of > 2 indicate there is likely a significant effect by the stressor(s) on the biological community. The most common stressors nationally that explain impairment in benthic macroinvertebrate communities are: total phosphorus, total nitrogen, and excess sediments (EPA 2006). Each of these stressors is commonly associated with stormwater input to receiving streams.

5) TMDL Model Results

- Compliance points and BMI conditions
- Relationship between Stressors and BMI Indicators
- How BMI and Periphyton respond to Stormwater Impacts (predictions)

Coupling biological results with TMDL model predictions and thresholds for select parameters integrates multiple types of monitoring information and makes management efforts more effective in a shorter time frame. The biological information generated from assessments at multiple points within the drainage is simply another measure of condition and measurement of progress in controlling pollutants. Identifying stressors related to stormwater impacts where biological response is more sensitive to subtle effects adds to existing assessments that may not detect certain impairments. The aggregation of assessments that measure physical, chemical, and biological conditions to determine potential impacts from stormwater input is consistent with Clean Water Act goals.

Each of the factors in Table 1 is described in terms of negative impacts to the BMI community with presence of stormwater input. Bioassessments at a site will be interpreted by using the response expectations in Table 1. Existing biological information will be interpreted by



examining companion physical habitat and water chemistry table by comparing to categories in Table 1. Biological community condition from suspected stormwater impaired sites compared with conditions at reference sites will identify impairment factors related to a “Stormwater Impact” category and will be interpreted by using the “Response” category. Identification of stressors responsible for current biological conditions will be determined by using Table 2 as a data interpretation tool.

Stormwater impacts outlined in Table 1 result in changes to aquatic habitat and chemical properties in media and surface water that can effect survival rates for salmon eggs (spawning areas), rearing juveniles, and smolt migration. Several pathways for metals bioaccumulation in each of the salmon life stages can increase mortality. Response to stormwater impacts are discussed in Table 1 and with statements that describe how salmon life stages are potentially affected by physical changes to habitat and exposure to toxics. Confirmation of predicted ‘Response’ from Table 1 will be determined through collection and analysis of sediment samples for metals burden and presence of Polycyclic Aromatic Hydrocarbons (PAHs).

Table 1. Changes in streams following stormwater input and how they are harmful to aquatic life.

STORMWATER IMPACT		RATIONALE	RESPONSE
Flow Variation	<p>Substrate Movement Is substrate size subject to transport and at what intensity of stormwater input?</p>	<p>Large pulses of stormwater discharge can mobilize large quantities of substrate.</p> <p>Fine substrate moved by stormwater will smother usable habitat for periphyton growth or reduce size of viable periphyton patches.</p>	<p>“Negative” - with high intensity stormwater input.</p> <p>Salmon spawning habitat could be affected; buried eggs, dislodged eggs, dewatered sand bars during critical low flow period.</p>
	<p>Substrate Size Are substrate size changes a result of stormwater input?</p>	<p>Stable substrate presents better colonization potential.</p>	<p>“Negative” – mobilization or rolling movement reduce habitable substrate.</p> <p>Salmon spawning habitat could be dominated by ‘fines’.</p>
	<p>Water Velocity Do changes in water velocity patterns following stormwater input affect BMI communities?</p>	<p>Velocity changes at select habitat following stormwater input changes morphological characteristics of the channel.</p>	<p>“Negative” – increase/decrease beyond a pre-storm event velocity range will affect living space for BMI.</p> <p>Changes availability and quality of salmon spawning and rearing habitat.</p>
	<p>Flow Volume Do seasonally significant volume changes affect BMI communities?</p>	<p>Changes in flow volume (excessively high in winter or critically low in summer) change timing and duration for usable habitat conditions (e.g., channel condition, BMI community, salmon presence).</p>	<p>“Negative” - during critical low flow - increases likelihood of low DO, higher temps, etc. High volumes – increased volume affects living space for BMI and periphyton.</p> <p>Poor salmon rearing habitat during the critical low flow period.</p>
Factors Influencing Toxics Exposure Potential	<p>Sediment / Water Column In which media do toxics aggregate and present exposure potential?</p>	<p>Stormwater toxics associated with one or more media may increase mortality in BMI community.</p> <p>Stormwater sequestered in periphyton community may present a toxic food base for ‘scaper’ macroinvertebrate taxa.</p>	<p>“Negative” – higher concentrations in one or more of the aquatic media (e.g., sediment, pore water, surface water, or periphyton) will decrease survivability in the BMI community.</p> <p>Salmon rearing habitat will be affected by potential for increased exposure of juveniles. Salmon spawning habitat with metals exposure results in increased egg mortality.</p>



STORMWATER IMPACT		RATIONALE	RESPONSE
Factors Influencing Toxics Exposure Potential <i>(continued)</i>	Residence Time Are toxics resident in media for extended periods of time?	Identity of toxics and characteristic association with specific types of media can increase mortality in the BMI community.	"Negative" – toxics associated with organic/inorganic media will present long-term impacts to the BMI community. Resident metals in salmon spawning and rearing habitat increased potential for bioaccumulation and increased mortality.
	Habitat Association Are there characteristic habitats in a candidate watershed where toxics aggregate in harmful levels?	Among the variety of morphological settings in a stream, there are particular types of habitat vulnerable to concentration and that increase exposure potential for BMIs. Periphyton growth on hard substrates may sequester metals from stormwater and transfer effects to the benthic macroinvertebrate community.	"Negative" – if stormwater input decreases the area of suitable habitat (e.g., increasing fines, increasing toxics with fines) expected BMI taxa presence will decline. Toxics aggregation in salmon rearing and summer stream refugia results in increased mortality.
Point of Toxics Exposure	Mixing Zone Exposure Is there a mixing zone and does toxics exposure present a greater impact to BMI?	Mixing zones downstream of stormwater input may have substantial impacts on BMI communities, but with diminishing effects outside of the mixing zone.	"Positive" – a defined mixing zone is expected to have some impact on aquatic life, but BMI conditions outside of this zone will improve dramatically.
	Suspended Contaminated Sediment Is suspended material carrying a high toxics load?	Sediment transport invokes a "drifting" behavior in many sensitive BMI species. Worse, if the suspension contains toxics the BMI drifters will suffer increased exposure.	"Negative" – suspended sediment will encourage drifting behavior in the more valuable component of a BMI community and may expose aquatic life to high concentrations of stormwater toxics. Contaminated sediments, once deposited in slackwater, will increase mortality of salmon eggs.
	Substrate Deposition Are contaminated toxics redistributed on a routine basis?	Stormwater input may have a broad influence on changes to substrate composition and make colonization difficult for endemic species when the shift extends over broad, spatial areas of the stream bottom.	"Negative" – increased fines and toxics associated with fines broadcast over a large spatial area will reduce the ability of sensitive BMI species from decolonizing. Rearing salmon will be exposed to increased potential for bioaccumulation of toxics where extensive contaminated sediments occur.



6) "Dirty" Water Models (the reverse of a 'clean' RIVPACS model)

Another way to use modeling of biological community condition is to predict the resulting species assemblage under impaired stream conditions. The benefit in using this approach is that management decisions considering 'abating pollution' sources (via BMPs) versus 'no action' will be informed on intensity of resulting impairment to biological communities. This forecasting knowledge determines how much progress a management plan might achieve if implemented. The results for this approach also informs on those stressors contributing greatest impact on biological communities. A brief background on the current RIVPACS modeling ('clean' models) approach and the inverse approach ('dirty' models) is provided.

Multivariate predictive models (i.e. RIVPACS-type models) have been used with great success in assessing the biological conditions of running waters (e.g., Wright 2000, Norris and Nichols 1999, Hawkins et al. 2000, Hawkins and Carlisle 2001), but little work has been conducted examining the potential utility of this modeling approach for forecasting the effects of changing either the amount or type of stressor occurring within aquatic ecosystems (Coyish et al. 2002). Current RIVPACS-type models are 'clean' models in the sense that they predict the biological assemblage that should occur at a site in the absence of ecosystem degradation. These models predict the probability of observing different taxa based on naturally occurring features, such as stream slope, catchment size, and geographical location (Moss et al. 1987). The organisms that are predicted to occur by the model are then compared with those that are collected to derive an assessment of the biological condition of the stream. An assessment score is derived by measuring the deviation between the observed and predicted assemblages. The fewer number of expected taxa that are found as an assessed site condition, the lower the RIVPACS score.

Forecasting the effects of management activities requires 'dirty' models, i.e., models that use stressor variables in addition to naturally occurring factors (predictor variables) to derive estimates of the assemblage expected to occur within different environmental settings. Isolating pollutants and their effects on biological communities is exceedingly difficult when conducting stressor/response research in the natural environment. Stressor "groups" should be identified for further evaluation of continuity in community response. These "groups" may include: physical stressors (e.g., sediment transport, increased turbidity, absence/removal of critical substrates, etc.), water quality stressors (e.g., increased temperatures, nutrient enrichment, dissolved oxygen depletion, etc.), or chemical contaminants in sediment or water column. Hughes *et al.*, in "Human Disturbance Gradient" applications, recommended the following as stressor group designations: habitat structure, flow regime, water quality, toxics and bio-engineered chemicals, energy sources, and biotic interactions (the underlined stressor groups are available with existing data and are routinely generated using the proposed Ecology biomonitoring protocols). Examination of how biotic communities, from repeated locations, respond to stressors should identify the number of "stressor groups" that can be detected using the RIVPACS modeling technique. This approach represents an advance in development of more sensitive analytical tools for interpreting biological information.



Measuring Improvements from TMDL Implementation

Development of biological endpoints (or “triggers”) used to identify pollutants for which a TMDL is developed are unique to a drainage. Just as TMDLs are drainage-specific, biological endpoints related to stressor(s) are unique to a drainage setting. Biological expectations based on the RIVPACs model are generalized over a broad spatial scale, but impacts to water quality and physical habitat are compounded over time. The biological expectation is constant and based on original setting conditions whereas human impacts to resources leave a disturbance-history that is never restorable to the original state.

Several important concepts for detecting stormwater impacts and developing a management strategy are addressed in this Technical Approach. These following concepts should be the focus in development of technical tools and in development of the stormwater TMDLs:

- 7) TMDL Compliance Point Limits
 - a. Couple biological assessments with TMDL expectations
- 8) Relationship between BMI condition (indicators) and TMDL Limits
 - a. Inverse/direct relationship between treatment/response variables
- 9) Biological thresholds description
 - a. Endpoints for biological response (physical habitat, water quality indicators)
- 10) Interpreting biological response to stormwater quality
 - a. Describing biological condition improvements
 - b. Using predictions to determine location and type of impairment
- 11) Use of Multiple Indicators to detect/diagnose stormwater impacts
 - a. Management decisions informed by an integrated monitoring approach
 - b. Sensitivity of BMI to specific stormwater stressors
 - i. Sporadic water quality/physical impacts
 - ii. Continuous effects; stressor influence on BMI condition

Some of the stream characteristics associated with stormwater impact to streams are listed in the following tables. Specific physical and chemical conditions are altered by stormwater input and respond in either a negative or positive direction. The development of technical tools to integrate biological information into the TMDL process can be informed by reviewing elements from the following two tables.



Table 2 lists several factors that are associated with stream dynamics and physical habitat that will influence stream community health. This table serves as a checklist for determining the stressor groups contributed by stormwater input and what combinations of these occur at a site. A healthy aquatic life community in a stream is measured against expectations from similar settings. The Department of Ecology uses the RIVPACS assessment tool (for Western Washington streams) to determine quality of a stream, in part, based on health of the benthic macroinvertebrate (BMI) community. The RIVPACS tool requires that several predictor variables be used to calculate expected BMI species at a site. The predictor variables are related to some of those factors listed in Table 2:

- Wetted width of the stream channel
- % of Cobble and Boulder in the stream channel (substrate sizes >64mm)
- % of Loose Gravel at a site in the stream channel(16mm-63mm)
- Slope of the stream bed



Table 2. Stormwater influence on stream dynamics and habitat that are directly related to changes in aquatic communities.

Stream Reach	PHYSICAL IMPACT			CHEMICAL IMPACT			VULNERABLE HABITAT		
	Flow Variation			Factors Influencing Toxics Exposure Potential			Point of Toxics Exposure		
	Substrate Movement Is substrate size subject to transport and at what intensity of stormwater input?	Substrate Size Are substrate size changes a result of stormwater input?	Water Velocity Do changes in water velocity patterns following stormwater input affect BMI communities?	Sediment / Water Column In which media do toxics aggregate and present exposure potential?	Residence Time Are toxics resident in media for extended periods of time?	Habitat Association Are there characteristic habitats in a candidate watershed where toxics aggregate in harmful levels?	Mixing Zone Exposure Is there a mixing zone and does toxics exposure present a greater impact to BMI?	Suspended Contaminated Sediment Is suspended material carrying a high toxics load?	Substrate Deposition Are contaminated toxics redistributed on a routine basis?
Segment 1									
Segment 2									
Segment 3									
Segment 4									
Segment 5									
Segment 6									



The candidate stream segment will enable the testing of each of the factors listed in Table 2. Existing data may already be used to describe conditions for one or more of the factors listed in this table.

Recommended Technical Approach

There are three components for developing a bioassessment monitoring and analysis strategy to support TMDL development in the Squalicum Creek and Soos Creek watersheds. The following main categories for developing this assessment strategy were presented earlier and are the main components for organization of recommendations.

- STEP 1** Identify biological evaluation tools and methods for analysis;
- Identify sites for biomonitoring in summer 2012 using the following criteria:
 - Use information from existing biomonitoring sites
 - Partition drainage into areas with dominant land use types
 - Identify major gradient breaks on the mainstem
 - Identify likely locations for “TMDL Compliance Points”
 - Final site locations integrate the above elements (as much as possible)
 - Use Ecology Biomonitoring protocols for field collection of benthic macroinvertebrate (BMI) and companion physical habitat and water quality data,
 - Analyze BMI data using the Western Washington Model for generation of RIVPACs scores for each site,
 - Identify sites in the Squalicum Creek and Soos Creek drainages as reference according to the RIVPACs model scores,
 - These sites will be further examined for physical setting characteristics as a guide for determining prescriptions on managing stormwater impacts
- STEP 2** Biological information and water quality information that needs to be combined as part of an integrated assessment for identifying impairment from stormwater input;
- Develop relationships between physicochemical parameters influenced by stormwater and biological response scores (i.e. RIVPACs)
 - Use the CADDIS (Stressor Identification) process as guidance to develop these relationships between BMI assessments and companion physical habitat and water quality conditions
 - Identify how biological conditions respond to stressor groups and couple with setting characteristics
- STEP 3** How to interpret possible outcomes for biological conditions and water quality conditions following assessment
- Identify stressors where response is stronger in either the biological indicators or water quality indicators (sensitivity to the pollutant stressor)
 - Determine if there is a biological response to pollutants used in the TMDL
 - Develop a list of water quality and biological indicators where response is consistent to stressor groups or a specific pollutant.



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Appendix B: PEST1PCB and BNA Analyte Lists

The following list includes the 22 pesticides or breakdown products and the nine PCB Aroclors that will be tested by Manchester Environmental Laboratory under the PEST1PCB list.

PEST1PCB	BNA	BNA (continued)	BNA (continued)
alpha-BHC	Phenol	3-Nitroaniline	Di-N-Octyl Phthalate
beta-BHC	Bis(2-Chloroethyl)Ether	Acenaphthene	Benzo(b)fluoranthene
gamma-BHC (lindane)	2-Chlorophenol	2,4-Dinitrophenol	Benzo(k)fluoranthene
delta- BHC	1,3-Dichlorobenzene	4-Nitrophenol	Benzo(a)pyrene
Heptachlor	1,4-Dichlorobenzene	Dibenzofuran	3B-Coprostanol
Aldrin	1,2-Dichlorobenzene	2,4-Dinitrotoluene	
Heptachlor epoxide	Benzyl Alcohol	Diethyl phthalate	
trans-chlordane (gamma)	2-Methylphenol	Fluorene	
	Bis(2-chloro-1-methylethyl) ether	4-Chlorophenyl-Phenylether	
cis-Chlordane (alpha)	N-Nitrosodi-n-propylamine	4-Nitroaniline	
Endosulfan I (Alpha-endosulfan)		4,6-Dinitro-2-Methylphenol	
Dieldrin	4-Methylphenol	N-Nitrosodiphenylamine	
Endrin	Hexachloroethane	1,2-Diphenylhydrazine	
Endrin Ketone	Nitrobenzene		
Endosulfan II (Beta-endosulfan)	Isophorone	Triethyl citrate	
Endrin Aldehyde	2-Nitrophenol	4-Bromophenyl phenyl ether	
Endosulfan Sulfate	2,4-Dimethylphenol	Hexachlorobenzene	
4,4'-DDE	Bis(2-Chloroethoxy)Methane	Tris(2-chloroethyl) phosphate (TCEP)	
4,4'-DDD	Benzoic Acid	Pentachlorophenol	
4,4'-DDT	2,4-Dichlorophenol	Phenanthrene	
methoxychlor	1,2,4-Trichlorobenzene	Anthracene	
Toxaphene	Naphthalene	Caffeine	
Chlordane (technical)	4-Chloroaniline	4-nonylphenol	
	Hexachlorobutadiene	Carbazole	
PCB Aroclors	4-Chloro-3-Methylphenol	Di-N-Butylphthalate	
PCB-1016	2-Methylnaphthalene	Triclosan	
PCB-1221	1-Methylnaphthalene	Fluoranthene	
PCB-1232	Hexachlorocyclopentadiene	Pyrene	
PCB 1242	2,4,6-Trichlorophenol	Bisphenol A	
PCB 1248	2,4,5-Trichlorophenol	Retene	
PCB 1254	2-Chloronaphthalene	Butyl benzyl phthalate	
PCB 1260	2-Nitroaniline	Benz[a]anthracene	
PCB-1262	Dimethyl phthalate	3,3'-Dichlorobenzidine	
PCB-1268	2,6-Dinitrotoluene	Chrysene	
	Acenaphthylene	Bis(2-Ethylhexyl) Phthalate	



Appendix C: Chain of Custody Form

Chain of Custody Record		Matrix Code Source Code No. of Containers Alkalinity Conductivity pH Turbidity <input type="checkbox"/> Chloride <input type="checkbox"/> Sulfate Fluoride Total Suspended Solids Total Dissolved Solids <input type="checkbox"/> Total Solids <input type="checkbox"/> % Sol <input type="checkbox"/> % Vol Sol TOC DOC BOD5 Oil & Grease (HEM) Ammonia Nitrite/Nitrate Total Phosphate Orthophosphate TPN Chlorophyll <input type="checkbox"/> Filters Fecal Coliform <input type="checkbox"/> MP <input type="checkbox"/> MPN Total Coliform <input type="checkbox"/> MP <input type="checkbox"/> MPN E. Coli <input type="checkbox"/> MP <input type="checkbox"/> MPN % Klebsiella Enterococcus PP Metc Mercury (Hg) <input type="checkbox"/> low level Hachman Individual Elements (please list) <input type="checkbox"/> Total <input type="checkbox"/> Dissolved VOA BTEX TPHG TPHD	Mail Stop: _____ <input type="checkbox"/> preliminary investigation <input type="checkbox"/> Monitoring <input type="checkbox"/> For HW Designation <input type="checkbox"/> For NPDES Date Results needed by: _____ Name/Reference # of QAPP for this project: _____
Relinquished By: _____ Received By: _____ Yr _____ Mo _____ Da _____ Hr _____ Mn _____ Seal ID _____ Condition of Seals _____	Manchester Lab Sample Number		
Comments: _____			

Laboratory Analyses Required

