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State of Washington

Quality Assurance Project Plan

Pilot Study of an Ambient Monitoring Approach for Evaluating the Biological Integrity of Urban Streams

September 2010

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September 2010

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Signatures are not available on the Internet version.
EAP - Environmental Assessment Program.
EIM - Environmental Information Management database.

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Abstract

The Washington State Department of Ecology (Ecology) will conduct a pilot project to evaluate methods for measuring the biological health of urban streams. Tests will include in-situ toxicity testing with rainbow trout (*Oncorhynchus mykiss*) and planktonic crustaceans (*Daphnia magna*), and bioassessments of benthic macroinvertebrates and periphyton. Indian Creek, a small urban stream in Olympia, Washington, will be the test site for the project.

In addition to biological monitoring, Ecology will use several diagnostic tools to identify chemical stressors in the stream that may adversely affect the instream organisms: benthic invertebrates, periphyton, trout, and *Daphnia*. Two passive samplers (DGT and SLMD) will sample for metals. Passive samplers for polar organics (POCIS) and nonpolar organics (SPMD) will also be used. Results from these samplers will provide a comprehensive list of candidate chemical stressors.

Gene microarray tests will be performed on trout fry and *Daphnia* after in-situ exposure to show responses to chemical stressors. Trout fry tissue will also be analyzed for metals and for two protein biomarkers (metallothionein and vitellogenin) as indications of exposure to specific chemical stressors.

Results from this pilot project will be used to evaluate the effectiveness of these sampling techniques for determining stream quality.

Each study conducted by Ecology must have an approved Quality Assurance Project Plan. The plan describes the objectives of the study and the procedures to be followed to achieve those objectives. After completing the study, Ecology will post the final report of the study to the Internet.

Background

The national Clean Water Act's objective is to restore and maintain the chemical, physical, and biological integrity of the nation's waters. For more than forty years, efforts to achieve this objective have focused on controlling municipal and industrial wastewater discharges to waters of Washington State. Traditional discharge monitoring estimates potential environmental effects using snapshots of pollutant concentrations over time relative to variable receiving stream chemistry and flow. This traditional methodology does not integrate the dynamic nature of waterbodies or effects on biota necessary to directly assess the integrity of a waterbody.

An example of the snapshot approach is collecting grab water samples from individual stormwater outfalls. This approach is problematic for evaluating urban stream health given the large number of stormwater outfalls, highly variable flows, and rapidly changing pollutant concentrations. Also, detecting unknown or illegal discharges with grab sampling is difficult because these events are unpredictable in time and space. This results in an incomplete picture of overall stream health.

The results of this pilot project can help develop an integrated monitoring approach for urban streams that assesses receiving stream water quality for the protection of biological resources. These techniques can be developed for an economy of scale, and field work can be performed by trained city employees or volunteers.

Organisms living in streams are impacted by their environment's pollution. This pilot study will use rainbow trout (*Oncorhynchus mykiss*) and planktonic crustaceans (*Daphnia magna*) placed in a stream (in-situ toxicity testing) to integrate realistic environmental exposures to a broad spectrum of toxic chemicals. Periphyton and macroinvertebrates native to the stream will be collected to evaluate the stream community health effects of pollution.

Study Area Description

The Washington State Department of Ecology (Ecology) will focus the efforts of the pilot project on a small urban stream in Olympia, Thurston County, Washington, called Indian Creek. Indian Creek was chosen because water quality monitoring by the local jurisdictions (City of Olympia and Thurston County) has shown this creek as at least moderately impacted by stormwater runoff and other sources of pollution. Indian Creek is near the Ecology Lacey office, making field sampling easier.

The Thurston County Water Resources Program (Thurston County) will collaborate with Ecology on several parts of the pilot project; they want to apply tools used here to measure stream biological integrity. Thurston County will lead some additional work on a nearby suburban stream, Woodard Creek, with Ecology's help.

Indian and Woodard Creeks are in South Puget Sound (Figure 1). The watersheds for these creeks are adjacent to each other. Indian Creek drains into Budd Inlet and Woodard Creek drains north into Henderson Inlet.

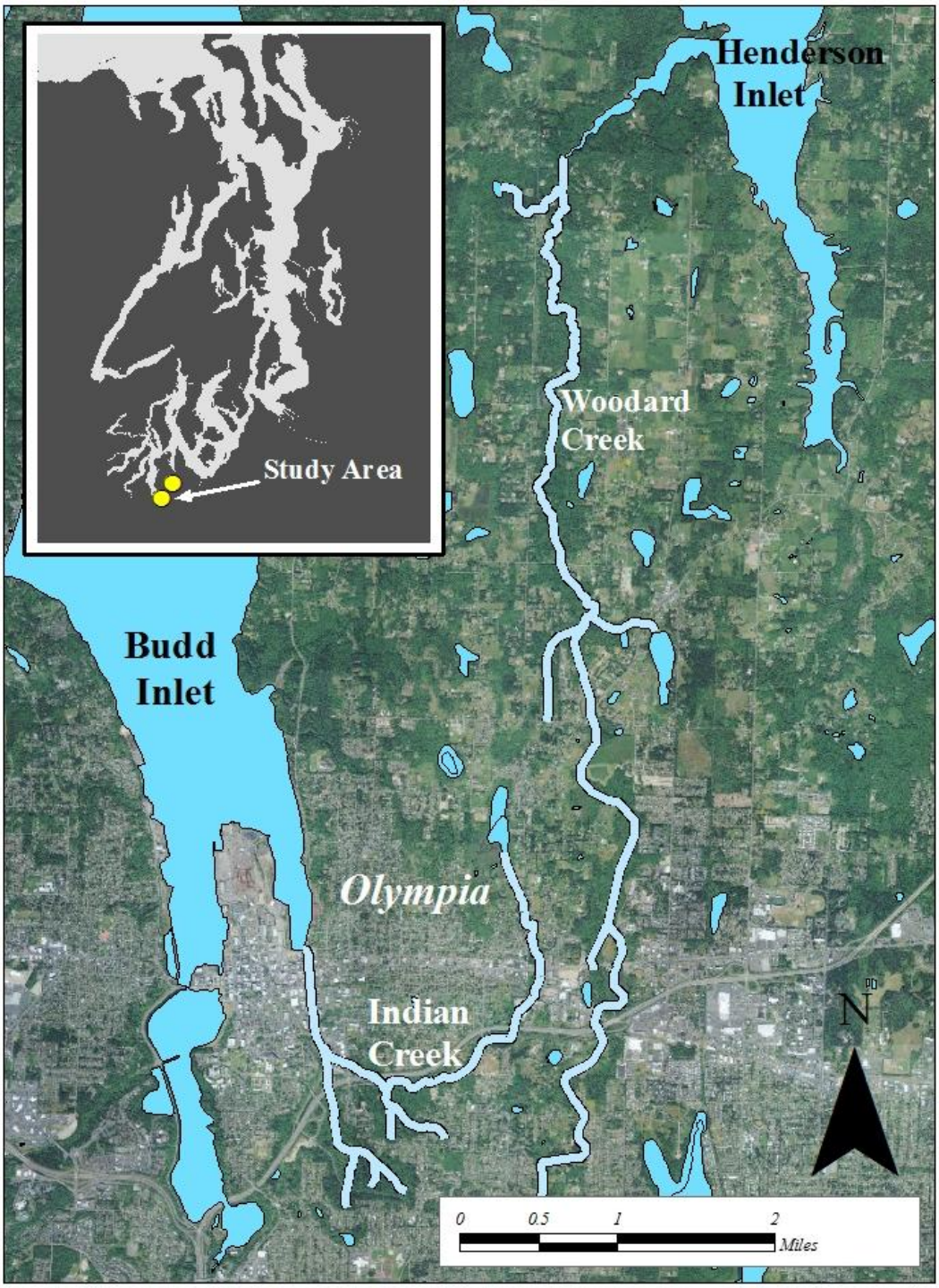

Figure 1. Indian and Woodard Creek Watersheds.

Indian Creek

Indian Creek is a small urban stream located in Thurston County, Washington. The Indian Creek watershed is approximately 1,500 acres and contains 30% impervious surface (TRPC, 2003).

Indian Creek originates from a wetland complex that includes Bigelow Lake then flows through a mix of land uses including urban, industrial, residential, and parks (Figure 2). The creek crosses under Interstate 5 twice and under numerous other roads. It eventually joins Moxlie Creek and is then piped under downtown Olympia to the east bay of Budd Inlet.

Many of the culverts on Indian Creek are too small or have too much height drop to allow for salmon migration. Despite these barriers, resident trout inhabit various reaches of the stream (City of Olympia, 2010).

Historical Data on Indian Creek

Indian Creek has failed State water quality standards for fecal coliform bacteria on numerous occasions. Although bacteria are not a major parameter of concern for this study, these failures indicate that the stream is influenced by pollution.

Thurston County monitored a major stormwater outfall entering Indian Creek from Interstate 5 in 1995 (Thurston County, 1996). They found elevated levels of cadmium, copper, lead, and zinc in the stormwater outfall samples. Lesser amounts of these metals were measured in the receiving water of Indian Creek. This outfall now discharges to the Indian Creek Stormwater Treatment Facility, constructed in 2001, before discharging to Indian Creek. The treatment facility is designed to reduce 50% of the contaminants that enter Indian Creek due to stormwater runoff (City of Olympia, 2010).

A study conducted by Ecology in 1997 detected pesticides and herbicides in Indian Creek just upstream of the confluence with Moxlie Creek. Data from this study is summarized in Table 1 (Davis, 2000). These concentrations are relatively low and do not exceed any Washington State or national standards; however, these chemicals in grab samples indicate that the creek is affected by urban pollution.

A Benthic Invertebrate Index of Biological Integrity (BIBI) was conducted on Indian Creek (near Wheeler Ave.) by Thurston County in July 2009 (unpublished data, 2010). The BIBI test measures the composition of the invertebrate community in a given stream compared to a regional index. The BIBI score for Indian Creek was 34, which indicates moderate biological integrity on the following scale:

- Low Biological integrity = 0-24.
- Moderate Biological integrity = 25-39.
- High Biological integrity = >40.

Ideally, a healthy stream system should be in the high biological integrity category. Therefore, the Thurston County study shows that Indian Creek is impaired.

Table 1. Pesticides and Herbicides Detected in Indian Creek in 1997 (ug/L, part per billion)

Parameter	May 12		July 7		August 26	
Insecticide						
Chlorpyrifos	0.003	NJ				
Herbicides						
2,4-D					0.089	
4-nitrophenol					0.059	NJ
Bromacil	0.011	NJ				
Dichlobenil			0.003	J	0.014	J
2,6-dichlorobenzamide	0.034	J	0.048	J		
Diuron	0.18	NJ	0.1	NJ		
MCPP			0.013	NJ	0.14	
Oxadiazon			0.004	NJ		
Prometon			0.001	J		
Tebuthiuron	0.027	J	0.022	J		
Triclopyr					0.061	
Fungicide						
Pentachlorophenol	0.022				0.31	

J - The analyte was positively identified. The result is an estimate.
NJ - There is evidence that the analyte is present. The result is an estimate.

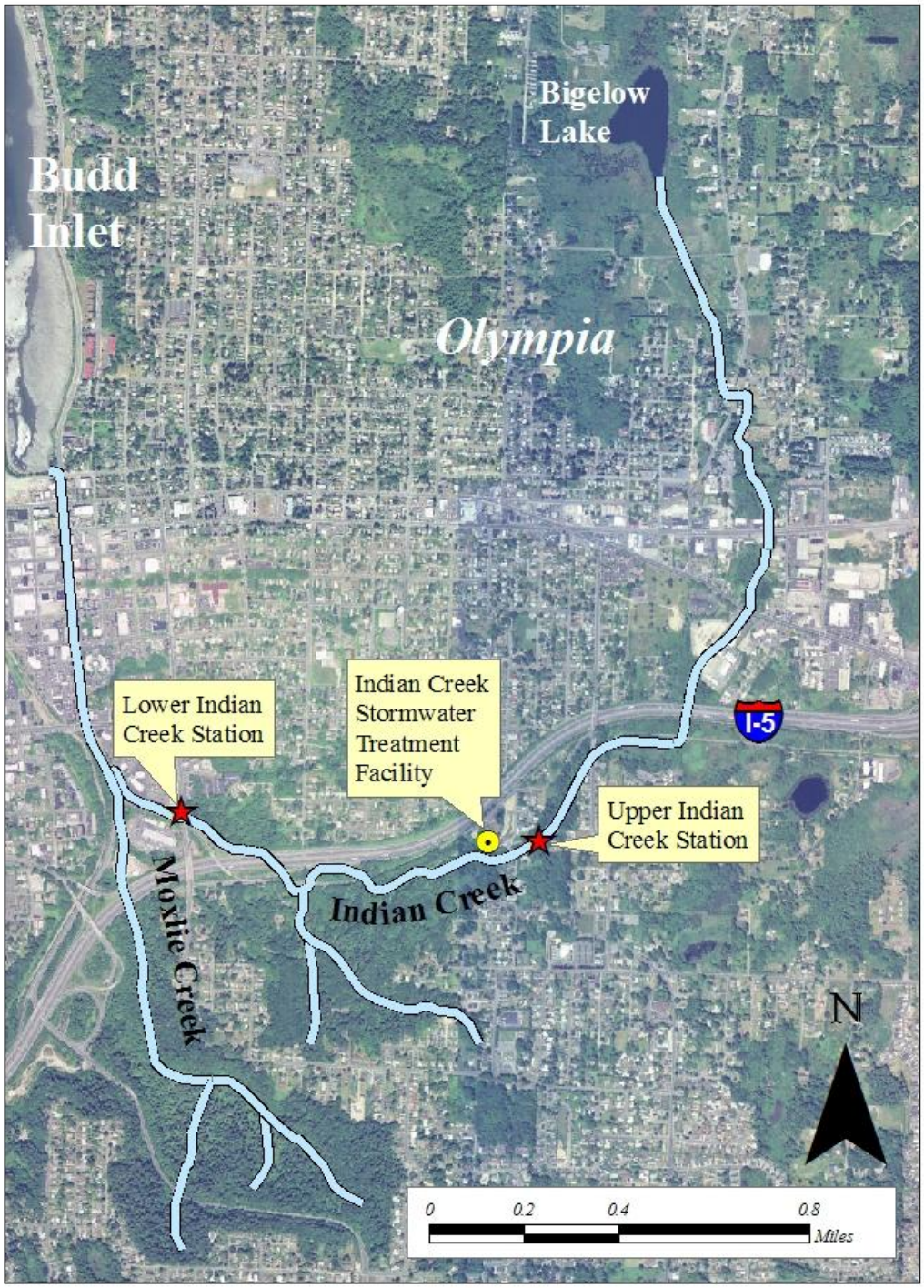

Figure 2. Indian Creek Watershed and Stations for the Ambient Pilot Project.

Woodard Creek

Woodard Creek drains a basin of 5,090 acres, and flows into Henderson Inlet (Figure 1). Industrial and high-density commercial development surrounds the groundwater-fed wetlands that are the creek's headwaters (Sargeant et al., 2006). Most of the creek downstream of the headwaters flows through private rural land. It is suspected that failing septic systems are negatively impacting Woodard Creek in rural areas (Thurston County, personal communication).

Thurston County periodically monitors water quality in Woodard Creek through their Ambient Monitoring Program. Surface water data is available for both Woodard and Indian Creeks at the Thurston County website: www.co.thurston.wa.us/health/ehswat/swater.html.

Project Description

The purpose of this pilot project is to evaluate an integrated method for assessing the biological integrity of streams and their suitability for supporting salmonid early lifestages. The method will include in-situ (in-stream) toxicity testing with rainbow trout (*Oncorhynchus mykiss*) and planktonic crustaceans (*Daphnia magna*) and benthic macroinvertebrate and periphyton community assessments.

In addition to biological monitoring, several diagnostic tools will be used to identify chemical stressors present in the stream that may be the cause of adverse effects to the instream organisms: benthic invertebrates, periphyton, trout, and *Daphnia*. Two passive samplers (DGT and SLMD) will be used to sample for metals. Passive samplers for polar organics (POCIS) and nonpolar organics (SPMD) will also be used. Results from these samplers will provide a comprehensive list of candidate chemical stressors.

Gene microarray tests will be conducted on trout fry and *Daphnia* after in-situ exposure to provide indications of responses to chemical stressors. Trout from the in-situ exposure will be analyzed for metals and for a protein biomarker called metallothionein as an indication of exposure to metals in stream.

Laboratory tests will also be conducted on trout. Trout alevins will be exposed to primary effluent and estradiol. Gene microarray tests will be conducted on these trout after they become fry. Trout from the estradiol exposure test will be analyzed for a protein biomarker called vitellogenin as an indication of response to an endocrine-disrupting chemical.

Some of the diagnostic tools and tests used in the study will be tested for utility and accuracy:

- Side-by-side comparisons of the two types of passive samplers (DGT and SLMD) for metals will indicate the effectiveness of the tests.
- Gene microarray tests will compare gene expression in *Daphnia* exposed to two different temperatures: 12° and 25° C. Gene microarray tests for trout exposed to primary effluent during the laboratory tests will be performed on both whole bodies and livers to evaluate the utility of each tissue type.

Monitoring Locations and Timing

Indian Creek was chosen for the pilot project because recent monitoring by Thurston County and the City of Olympia has shown that the creek is moderately impacted by urban pollution. In order to test the tools for the pilot project, an urban creek with moderate pollution is ideal. Using a moderately impacted stream will give the ability to test tools that detect minor to moderate degradation. Furthermore, there is a risk that using a highly impacted stream would destroy the in-situ test organisms, leaving no organisms left to test for sublethal effects to chemical stressors.

An upstream and downstream location on Indian Creek will be used for the project (Figure 2). The upstream site should be less impacted by pollution than the downstream site. Numerous pollution sources including the Indian Creek Stormwater Treatment Facility drain into Indian Creek below the upstream site. Focusing work on two sites allows for comparisons between sites and also provides two levels of degradation to test the monitoring tools that will be used in the project.

The project will take place during late spring (April to May). Spring usually has dry spells between periods of rain, allowing pollutants to build up and then be discharged in large concentrations to streams. Native rainbow trout reproduction is more robust in the spring than in the fall, making spring the ideal time for testing impacts to early lifestages. Commercial trout embryos used in this study are also of higher quality and more reliable in the spring. In addition, this timing follows a successful Pierce County study using in-situ trout testing in a few urban streams in the spring of 2008.

During the course of this pilot project on Indian Creek, Thurston County will lead some additional work on the nearby Woodard Creek. Their work will include in-situ *Daphnia* toxicity testing, *Daphnia* microarrays, and passive sampling for polar organic compounds.

Several entities will be collaborating with Ecology on different aspects of the pilot project. Collaborators include Thurston County, U.S. Geological Survey (USGS), Washington State University (WSU), and the University of California Berkeley (UC). Ecology will also be contracting with several laboratories including Nautilus Environmental (Nautilus), Environmental Sampling Technologies (EST), and Brooks Rand Labs (Brooks Rand).

Background on Monitoring Actions

Trout Toxicity Testing

Environment Canada developed a toxicity test using the embryo, alevin, and fry lifestages of rainbow trout because of concern over water quality in salmonid spawning streams. Each lifestage is sensitive to different pollutants. A test on all of these lifestages combined is a true chronic test. The biological effects assessed by this testing include mortality, failure to hatch, abnormal development, and reduced growth. A trout egg-through-fry test works well either in the lab or in a hatchbox enclosed in a wire basket full of rocks exposed in a stream (in-situ).

This pilot study will include in-situ tests at the two Indian Creek stations with a concurrent laboratory control test using clean water. For this study, clean water will be moderately hard synthetic water as defined by U.S. Environmental Protection Agency (EPA) standards (EPA, 2002). There will also be separate laboratory tests where trout will be exposed to a mix of chemicals such as primary effluent (the source of effluent has yet to be determined).

Daphnia Toxicity Testing

Thurston County and Washington State University (WSU) will conduct in-situ testing with *Daphnia magna*. Daphnids are among the most common toxicity test organisms in the world because of their reliability and sensitivity. Because of their popularity in toxicity tests, the database of daphnid responses to individual chemicals is quite large. Because daphnid are related to many of the benthic invertebrates, their responses in toxicity tests are also relevant to benthic invertebrate assessments.

Benthic Macroinvertebrates

To assess effects on the insects and crustaceans important as food for salmonid fry and juveniles, instream benthic invertebrate assessments will be conducted. Invertebrates are more sensitive than fish to many pollutants such as metals and insecticides. Benthic invertebrate assessments are now standard tools for determining stream health. The replacement of pollutant-sensitive species with pollutant-tolerant species is easily measured.

Additional invertebrate assessments will be conducted on mesh rock bags that will be deployed near the trout baskets at the monitoring stations, similar to a method used by the state of Maine (Davies and Tsomides, 2002). If the colonization results prove useful, they will give a technique to supplement standard instream bioassessments of benthic invertebrates, especially in deeper streams or other difficult circumstances.

Periphyton

Periphyton are a combination of microbes, algae, and bacteria that live on the substrate in aquatic environments. Periphyton will be collected from native substrates at the same time as macroinvertebrates. Similar to macroinvertebrate assessments, periphyton community assessments also show stream health.

Gene Microarrays

Gene microarray analysis measures the expression of hundreds or thousands of genes from an organism exposed to chemical pollutants. Microarrays for assessing environmental contaminants evolved from microarrays used to study developmental processes or basic physiology. Microarrays note when genes are turned on and when they are turned off. A gene might turn on to resist toxicity or turn off because of interference from a chemical.

Trout

Environment Canada developed a rainbow trout gene microarray which will be used by the United States Geological Survey (USGS) on the fish exposed at the Indian Creek stations, on clean lab control fish, and fish exposed to chemicals in the laboratory. Laboratory fish will be exposed to a known mix of toxic chemicals (specific chemicals have yet to be determined) diluted to just below the threshold for lethality so that the trout microarray can be run for comparison on both whole fish and livers.

Because the fry are so small, using whole fish will save time and money and may allow better assessment of toxicant effects on growth and development. On the other hand, the liver is the site of many known responses to toxicity and using whole fish might raise detection limits too much relative to the liver responses. A comparison should shed some light on whether whole fish or livers work best in microarrays.

Daphnia

UC has developed a *Daphnia magna* gene microarray and will conduct the microarray on the daphnids from the in-situ exposures. They will also conduct the microarray on daphnids exposed in the lab to samples of stream water at 12° and 25° C in order to assess differences in gene expression relative to temperature. Previous daphnid microarray work at UC has involved daphnids exposed at 27° C. Daphnid microarrays are run on whole organisms.

Scientists at UC have discovered patterns of microarray response that are diagnostic of copper exposure. The manufacturing and reading of microarrays has been automated. An economy of scale is possible and much information about chemical effects can be gained.

Trout Biomarkers

A biomarker is a chemical produced in a living organism in response to toxicity. A gene on a microarray which is turned on by chemical exposure is usually the gene which produces the biomarker. Biomarkers include enzymes produced to fight toxicity or enzymes with another purpose whose production is affected by toxic chemicals. Each biomarker responds to specific types of chemicals and can be a valuable diagnostic tool. Biomarker response is longer lived than microarray responses and can provide useful information for some time after chemical exposure. If metallothionein is induced in an organism for example, its presence may indicate that the organism was exposed to metals at concentrations and conditions sufficient to produce toxicity.

Biomarker chemicals analyzed on trout from the pilot study include:

- *Metallothionein:* the enzyme produced by an organism in response to exposure to a toxic metal.
- *Vitellogenin:* a protein produced when an organism is exposed to an endocrine disruptor resembling estrogen. The protein is normally only produced in females during egg production.

Passive Samplers

Passive samplers serve the same purpose as a composite sampler for characterizing average chemical exposure over a time period, except that passive samplers can be deployed for a much longer time period. In addition, passive samplers absorb pollutants like living organisms do and provide a better exposure assessment than chemical analysis of a grab or 24-hour composite water sample. Results of the analysis of passive samplers will help interpret bioassessments, toxicity tests, and microarray results.

By using passive samplers for metals, polar organics (water soluble compounds), and nonpolar organics (fat soluble compounds), the study will cover many pollutants of concern typically found in wastewater and stormwater. The passive samplers that will be used for the pilot study include:

- DGT (Diffuse Gradients in Thin film) for metals including cadmium, copper, nickel, lead, and zinc.
- SLMD (Stabilized Liquid Membrane Device) for metals including cadmium, copper, nickel, lead, and zinc.
- POCIS (Polar Organic Chemical Integrative Sampler) for polar organics including herbicides, nonylphenol, and carbamate pesticides.
- SPMD (Semipermeable Membrane Device) for nonpolar organics including polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides, organophosphate pesticides, nitrogen pesticides, and other organic chemicals.

Special Metals Focus

Two types of passive samplers (SLMDs and DGTs) for metals will be compared during the project. The intent is to show that SLMDs are comparable to the better established DGTs which are more expensive. SLMDs have the potential to be deployed for longer durations than DGTs.

Grab samples will be collected from the streams three times to analyze for the same metals to be measured in the passive samplers. Measuring water concentrations of the metals in grab samples will help interpret passive sampler results and perhaps shed light on the comparisons of the two types of samplers.

The Biotic Ligand Model (BLM) predicts metals toxicity based upon competition for fish gill binding sites. The copper BLM is generally accepted and used by EPA for determining water quality criteria for copper. The BLM does not work as well at predicting toxicity from other metals, but the same chemical principles apply and the copper results will at least reveal the tendencies for the other metals. Grab samples will be analyzed for conventional water quality parameters in order to run the BLM for copper. The BLM may shed light on SLMD and DGT performance as well.

Organization and Schedule

Table 3 lists all of the Ecology employees involved in the pilot project.

Table 2. Organization of Project Staff and Responsibilities for Ecology Staff.

Staff (all are EAP except client)	Title	Responsibilities
Randall Marshall Water Quality Program Phone: (360) 407-6445	EAP Client	Clarifies scope of the project. Provides internal review of the QAPP and approves the final QAPP. Assists with data interpretation and co-authors draft and final reports.
Brandee Era-Miller Toxics Studies Unit SCS Phone: (360) 407-6771	Project Manager/ Principal Investigator	Writes the QAPP. Oversees field sampling and transportation of samples to the laboratory. Conducts QA review of data, analyzes and interprets data, and enters data into EIM. Lead author for the draft and final reports.
Michael Friese Toxics Studies Unit SCS Phone: (360) 407-6737	Field Assistant/ EIM Quality Assurance	Helps collect samples and records field information. Reviews final data in EIM.
Scott Collyard Directed Studies Unit Western Operations Section Phone: (360) 407-6455	Lead for periphyton and macroinvertebrate collection	Leads collection and analysis of periphyton and macroinvertebrate samples and provides final data to project manager.
Dale Norton Toxics Studies Unit SCS Phone: (360) 407-6765	Unit Supervisor for the Project Manager	Provides internal review of the QAPP, approves the budget, and approves the final QAPP.
Will Kendra SCS Phone: (360) 407-6698	Section Manager for the Project Manager	Reviews the project scope and budget, tracks progress, reviews the draft QAPP, and approves the final QAPP.
Robert F. Cusimano Western Operations Section Phone: (360) 407-6596	Section Manager for the Study Area	Reviews the project scope and budget, tracks progress, reviews the draft QAPP, and approves the final QAPP.
Stuart Magoon Manchester Environmental Laboratory Phone: (360) 871-8801	Director	Revises the draft QAPP and approves the final QAPP.
William R. Kammin Phone: (360) 407-6964	Ecology Quality Assurance Officer	Reviews the draft QAPP and approves the final QAPP.

EAP – Environmental Assessment Program.
SCS – Statewide Coordination Section.
EIM – Environmental Information Management database.
QAPP – Quality Assurance Project Plan.

Table 3 lists outside entities, their contact information, and their general responsibilities for the pilot project.

Table 3. Contact Information for Outside Entities Involved in the Pilot Project.

Contact Person	Entity	Responsibilities
Robert Black Aquatic Ecologist Phone: (253) 552-1687	U.S. Geological Survey (USGS)	Trout Gene Microarray analysis.
Tiffany Stilwater Project Manager Phone: (206) 632-6206	Brooks Rand Labs	SLMD and DGT preparation, extraction, and analysis. Analysis of metals water samples.
Cat Curran Washington Lab Manager Phone: (253) 922-4296	Nautilus Environmental	Trout in-situ and laboratory toxicity testing.
Terri Spencer Phone: (816) 232-8860	Environmental Sampling Technologies (EST)	SPMD and POCIS preparation and extraction.
Chris Vulpe Associate Professor Phone: (510) 642-1834	University of California, Berkeley (UC)	Daphnid Gene Microarray analysis.
Barb Wood Environmental Specialist Phone: (360) 754-3355	Thurston County Water Resources	Assistance with daphnid in-situ toxicity testing. Lead on Woodard Creek sampling.
John Stark Extension Director Phone: (253) 445-4519	Washington State University, Puyallup Research and Extension (WSU)	Lead on daphnid in-situ and laboratory toxicity testing.

Table 4. Proposed Schedule for Completing Field and Laboratory Work, Data Entry into EIM, and Reports.

Field and laboratory work	Due date	Lead staff
Field work completed	May 2010	Brandee Era-Miller
Laboratory analyses completed	September 2010	
Environmental Information Management (EIM) database		
EIM user study ID	BERA0008	
Product	Due date	Lead staff
EIM data loaded	December 2010	Brandee Era-Miller
EIM quality assurance	January 2011	Michael Friese
EIM complete	April 2011	Brandee Era-Miller
Final report		
Author leads	Brandee Era-Miller and Randall Marshall	
Schedule		
Draft due to supervisor	January 2011	
Draft due to client/peer reviewer	February 2011	
Draft due to external reviewer(s)	March 2011	
Final (all reviews done) due to publications coordinator	April 2011	
Final report due on web	May 2011	

Quality Objectives

Manchester Environmental Laboratory (MEL), the contract laboratories, and collaborating entities are expected to meet quality control requirements of methods selected for this project. Quality control procedures used during field sampling and laboratory analyses will provide data for determining the accuracy of the monitoring results. Waivers were obtained for the non-accredited methods used in the project. Ecology policy requires waivers for all non-accredited methods under Executive Policy 1-22.

Table 5 shows the measurement quality objectives (MQOs) for the methods selected for the chemical analyses. MQOs for biological toxicity tests are available from the contract laboratories and referenced in the *Sampling Procedures* section of this report.

Table 5. Laboratory Measurement Quality Objectives for Chemical Analyses.

Parameter	Lab Control Samples (% Recovery)	Duplicate samples (RPD)	Matrix Spike (% Recovery)	Matrix Spike Duplicates (RPD)	Surrogate Recoveries (% Recovery)
TOC & DOC	80 – 120	≤20%	75 – 125	20%	NA
TSS	80 – 120	≤20%	NA	NA	NA
Chloride	90 – 110	≤20%	75 – 125	20%	NA
Alkalinity	80 – 120	≤20%	NA	NA	NA
Sulfate	90 – 110	≤20%	75 – 125	20%	NA
Hardness	85 – 115	≤20%	75 – 125	20%	NA
Ca, Mg, Na, & K	85 – 115	≤20%	75 – 125	20%	NA
Cd, Cu, Ni, Pb, & Zn (water)	75 –125	≤20%	75 –125	≤20%	NA
Cd, Cu, Ni, Pb, & Zn (SLMD & DGT)	75 –125	≤20%	75 –125	≤25%	NA
Cd, Cu, Ni, Pb, & Zn (tissue)	85 – 115	≤20%	75 – 125	20%	NA
Pesticides	50 – 150	≤50%	50 – 150	40%	30 – 150[1]
BNAs	50 – 150	≤50%	50 – 150	40%	30 – 150[1]
Herbicides	40 – 130	≤40%	NA	NA	30 – 150[1]
Carbamates	30 – 130	≤40%	NA	NA	30 – 150[1]

RPD - Relative percent difference.
NA - Not applicable.
[1] - Surrogate recoveries are compound specific.

Analytical precision and bias will be evaluated and controlled by use of laboratory check standards, duplicates, spikes, and blanks analyzed along with study samples.

Precision is a measure of the ability to consistently reproduce results. Precision will be evaluated by analysis of check standards, duplicates/replicates, spikes, and blanks.

Bias is the systematic error due to contamination, sample preparation, calibration, or the analytical process. Most sources of bias are minimized by adherence to established protocols for the collection, preservation, transportation, storage, and analysis of samples. Check standards (also known as laboratory control standards) contain a known amount of an analyte and indicate bias due to sample preparation or calibration.

Method blanks will be analyzed along with all samples to measure any response in the analytical system for target analytes. Method blanks have an expected theoretical concentration of zero. Field blanks are used to detect bias from contamination. This may include contamination from containers, sample equipment, environmental surroundings, preservatives, transportation, storage, other samples, or laboratory analysis.

Labeled surrogates will be added to the SPMD and POCIS samples prior to extraction. Surrogates have similar characteristics to target compounds. The recovery is used to estimate the recovery of target compounds in samples.

Sampling Design

Figure 3 details the timeline for each piece of the project. Sampling for the project is estimated to span approximately 40 days with multiple assessments going on simultaneously both in-situ and in the laboratory.

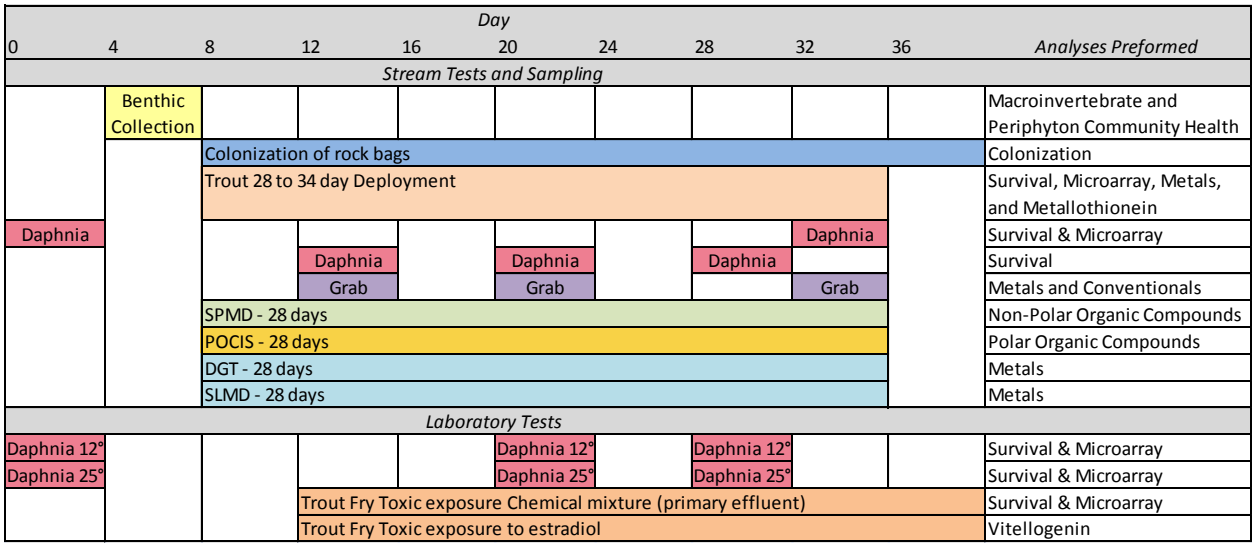

Figure 3. Timeline of Activities for the Ambient Pilot Project.

Day 0 - 4: Complete daphnid in-situ toxicity tests for both a 48- and 96-hour cycle at each site. Send the daphnids from the 48-hour test to the laboratory for gene microarray analysis. Run companion lab tests at 12° and 25° C, using water from the upstream Indian Creek site, and analyze gene microarray.

Day 0 – 36: Take basic water quality parameters (temperature, conductivity, pH, and dissolved oxygen) with a MiniSonde® meter. Take these water quality parameters each time work is done at the sites. Measure flow periodically throughout the project.

Day 6: Analyze macroinvertebrate and periphyton one time at each site.

Day 8: Install trout hatchboxes in stream. Invertebrate colonization begins on mesh rock bags placed in the stream and continues through the course of the project and up to 56 days. Trout toxicity tests will take 28-34 days, depending on stream temperature. Check hatchboxes weekly and clean if siltation of embryos occurs. Install a Tidbit continuous temperature monitoring device on one hatchbox at each site.

Day 9: Deploy passive samplers (SPMD, POCIS, DGT, and SLMD) and keep instream for approximately 28 days. Install a Tidbit continuous temperature monitoring device with the passive samplers.

Day 12: Daphnid in-situ toxicity tests. Take grab samples for analysis of conventional parameters and metals.

Day 20: Daphnid in-situ toxicity tests. Run companion lab tests at 12° and 25° C, using clean water and water from the downstream Indian Creek site. Take grab samples for analysis of conventional parameters and metals.

Day 28: Daphnid in-situ toxicity tests. Run companion lab tests at 12° and 25° C, using clean water and water from the Woodard Creek site, and analyze gene microarray.

Day 32: Daphnid in-situ toxicity tests. Send daphnids from the 48-hour test to the laboratory for gene microarray analysis. Take grab samples for analysis of conventional parameters and metals.

Day 36: Trout in-situ toxicity test ends. Take trout to laboratory for counting and measuring. Whole body trout will be processed for microarray assessment and for metals and metallothionein analysis. Collect invertebrates from the rock baskets that held trout hatchboxes, preserve, then send out for enumeration. Collect passive samplers and ship to testing laboratories for extraction and analysis.

Laboratory toxicity testing: As convenient, during the same time as the trout in-situ test is going, expose trout alevins (same age as in-situ trout) from the laboratory to a mixture of toxic chemicals such as primary effluent for comparison of whole fish versus liver tissue with microarray assessment. Analyze trout plasma for vitellogenin to test for response from the laboratory estradiol exposure.

Monitoring Actions for the project are also presented in tabular format in Appendix B.

Passive Samplers

SPMD

Semipermeable membrane devices (SPMDs) were developed by USGS and are an established technology used to concentrate hydrophobic (non-polar) chemicals from water (Huckins et al., 2006). One SPMD membrane on a spindle is shown in Figure 4.

Figure 4. Standard SPMD Membrane Mounted on a Spider Carrier.

SPMDs contain the lipid triolein. Hydrophobic chemicals are attracted to the lipid (lipophilic) and concentrate over the period of deployment. SPMDs mimic the uptake of chemicals in the fatty tissue of aquatic organisms like fish.

For current study, the following are the target analytes for the SPMD analysis:

- Chlorinated pesticides.
- Organophosphorus pesticides.
- Nitrogen pesticides.
- Semivolatile organic chemicals such as polycyclic aromatic hydrocarbons (PAHs).

More information is available on SPMD and POCIS samplers at the following USGS website: http://biology.usgs.gov/contaminant/passive_samplers.html.

POCIS

Whereas the SPMD is able to concentrate hydrophobic non-polar compounds, a polar organic chemical integrative sampler (POCIS) is able to concentrate hydrophilic, polar organic compounds (Figure 5) and was similarly developed by the USGS (Alvarez et al., 2004).

Figure 5. Three Standard POCIS Membranes on a Deployment Carrier.

Similar to the SPMD, passive sampling is based on membrane diffusion and a sequestering medium. The POCIS sampler consists of resin/adsorbent mix between polyethersulfone membranes. The membranes have a 0.1 um pore diameter, two orders of magnitude larger than the SPMD diameter of 0.001 um. The sequestering mixture contains solutes, bio-bead resins, and carbon-based sorbents which perform well with hydrophilic pesticides.

For current study, the following will be the target analytes for the POCIS analysis:

- Carbamate pesticides
- Herbicides

SLMD and DGT

Stabilized liquid membrane devices (SLMDs) and DGTs (diffusive gradients in thin film) are passive samplers that concentrate bioavailable trace metals of interest for the study: cadmium, copper, lead, nickel, and zinc out of water. Both types of passive sampler will be used in this study and compared for accuracy and utility.

The SLMD consists of a hydrophobic reagent mixture sealed inside a polymeric membrane. The reagent diffuses to the outer surface of the membrane, providing a fresh complexing agent that absorbs metals. More information on SLMD technology is at this USGS website: http://biology.usgs.gov/contaminant/passive_samplers.html.

DGT Research Ltd in Britain manufactures and supplies DGTs. The DGT for metals sampling utilizes a polyacrylamide diffusive layer combined with a chelex binding layer. The use of DGTs is well documented. Brook Rand has more information at: https://brooksrandlabs.sharefile.com/d/s8db84936f104423b.

Grab samples for low level metals will be collected three times during the deployment of SLMDs and DGTs at both Indian Creek sites. Data on metals in creek water will help Brooks Rand with their interpretation of the SLMD and DGT results.

Sampling Procedures

Bioassessment

Biological assessment of Indian Creek will be conducted with macroinvertebrate and periphyton analysis. Both bioassessment tests give an indication of the overall biological health of a waterbody. These assessments will be conducted before the installment of trout hatchboxes and passive samplers to avoid excess disturbance to the stream bottom benthic community prior to collection.

Benthic Macroinvertebrates

Macroinvertebrates will be collected under the supervision of Scott Collyard of Ecology's EA Program. He is specialized in macroinvertebrate biological monitoring and will follow Ecology's macroinvertebrate collection protocols as described in the Ecology publication: *Benthic Macroinvertebrate Biological Monitoring Protocols for Rivers and Streams: 2001 Revision* (Plotnikoff and Wiseman, 2001).

Additional macroinvertebrates will be collected from clean rocks placed in mesh bags that will be deployed in the stream for 28 – 56 days at the monitoring stations. This is similar to a method used by the State of Maine (Davies and Tsomides, 2002).

Stream Collection Method

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 with or a minimum of 1,000 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.

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 with a D-Frame 500-micrometer mesh kicknet. A device fastened to the base of the D-Frame kicknet encloses a one-foot by one-foot area in front of the sampler. 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. Remaining substrate within the sampler will be thoroughly agitated to a depth of 2 to 3 inches (5 to 8 cm).

Net contents are then emptied into a rinse tub by holding the net over a tub, inverting the net and gently pulling the net inside out. Tub contents are then poured into a U.S. Standard No. 35 sieve. The tub should be rinsed and examined to insure all organisms are removed. This procedure is repeated until all eight samples have been collected.

All of the sieve contents are to be placed in the sample bottles. Each sample container is filled no more than 2/3 full to allow room for the alcohol preservative. Labeled sample bottles are then shipped to the contract laboratory for analysis.

Periphyton

Periphyton will also be collected under the supervision of Scott Collyard of Ecology's EA Program. He is specialized in periphyton collection using a method modified from Wyoming's *Manual of Standard Operating Procedures for Sample Collection and Analysis* (WDEQ/WQD, 2005). The draft of this modified periphyton collection method is included in Appendix D.

Periphyton collection includes collecting rocks (2.5 – 4 inch in diameter) or woody debris (0.5 – 2 inch in diameter and 3 – 5 inch in length) from 8 quadrants across a riffle in the stream. The periphyton on the rocks or wood is then gently scrubbed and rinsed off into a container. The rinsate is poured into a 500 mL Nalgene sample bottle and preserved. Samples are kept in a darkened cooler and sent to a contract laboratory for analysis.

Foil templates of the rocks or wood are taken to match the areas where the periphyton was attached. The templates are later used to calculate the total area of periphyton collection.

Trout Toxicity Testing

A Hydraulic Project Approval (HPA), fish transport, and fish stock permits have been obtained from the Washington Department of Fish and Wildlife for use of trout (in-situ) and are kept on file by the Ecology project manager. Permission from the private landowners at the Indian and Woodard Creek project sites has also been granted.

The rainbow trout (*Oncorhynchus mykiss*) in-situ testing will be conducted by Nautilus Environmental (Nautilus). Nautilus uses a method modified from the *British Columbia Field Sampling Manual: 2003. For continuous monitoring and the collection of air, air-emission, water, wastewater, soil, sediment, and biological samples* (BC MoE, 2003).

Nautilus will obtain the trout embryos for the trout in-situ toxicity tests from Trout Lodge in Sumner, Washington.

Hatchboxes containing rainbow trout embryos will be installed in the gravel at the upstream and downstream locations on Indian Creek. The hatchboxes will be closed and placed within metal wire cages (approximately 7 by 14 inches). Local stream gravel will be placed around the hatchbox within each cage to hold the boxes in place. If local substrate is too fine, washed stream gravel (~2 inch) will be used to fill and cover the cages. Whitlock-Vibert hatchboxes will be used.

Additional details on the hatchboxes can be found at the following website: www.fedflyfishers.org/Default.aspx?tabid=4384.

Four hatchboxes containing 30 embryos per box will be used at each site (120 embryos total). The hatchboxes will be placed side by side across a portion of the stream that is out of the main current of the stream (thalweg), but still receives adequate flow through as shown in Figure 6. One depression for all four hatchboxes will be excavated in the streambed using hand tools. The hatchboxes will be placed in the depression and covered with stream gravel, so that conditions in the hatchboxes mimic natural salmonid spawning conditions (eggs are exposed to flowing water in the gravels while being protected during high flow events).

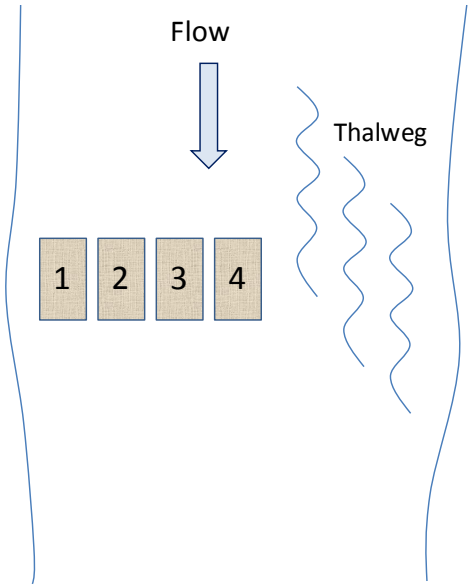

Figure 6. Diagram of the In-Situ Trout Hatchbox Deployment**.**

The eggs/fish in the hatchboxes will be monitored weekly throughout exposure to evaluate embryonic development, hatching success and growth under real-world conditions. This monitoring involves removal, inspection, and reburial of the hatchbox/cage assemblies in the gravel. The test will be terminated once yolk sacs have been absorbed, at which point the fish will be transported to Nautilus where they will be evaluated for characteristics such as deformities and growth.

Once at the Nautilus Laboratory and prior to measurements of length and weight and processing for analysis, the trout will be anesthetized with MS-222 (tricaine methanesulfonate).

Metallothionein and Vitellogenin

Nautilus will analyze metallothionein in trout fry from the in-situ toxicity tests and from clean control fish from the laboratory. For the metallothionein analysis they will use liver and gill tissues from a composite of approximately 10 fish.

Nautilus will analyze trout fry from the laboratory toxicity tests (both clean control and laboratory exposed samples) for vitellogenin. They will extract plasma from approximately 10 fish and pool for analysis.

Metals

Ecology will composite and process whole body trout tissue from the in-situ toxicity tests and send the samples to MEL for metals analysis. Ecology will use instruments clean of metals contamination when processing the trout tissues and use certified jars provided by MEL. Ecology will do this work at the Nautilus Laboratory in Fife, Washington.

Gene Microarray for Trout

Nautilus will prepare whole body trout tissue from the in-situ toxicity tests and whole body and liver tissues from the laboratory toxicity tests at their laboratory in Fife, Washington. USGS staff will then immediately preserve the processed tissues in RNA Later® Buffer at the Nautilus Laboratory.

USGS will transport the preserved tissues back to their laboratory and perform gene microarray analysis. Preserved tissues can be refrigerated for up to 4 weeks at 2-8°C or frozen below -20°C for an indefinite amount of time prior to gene microarray analysis.

Daphnia Toxicity Testing

A small planktonic crustacean called *Daphna magna* (see Figure 7) will be used for the 96-hour acute in-situ toxicity test. John Stark from Washington State University (WSU) and Barb Wood from Thurston County (TC) will lead the *Daphnia* in-situ sampling. They are both experienced in *Daphnia* toxicity testing in laboratory and in-situ testing. For this study they will use a modification of the methods described in Appendix E.

The endpoint for the in-situ acute *Daphnia* test is survival. Ten-day old *Daphnia magna* will be reared at the WSU laboratory. On the morning of deployment, the ten-day old organisms will be placed in glass transport vials at the laboratory for transport to the sampling site. Once onsite, organisms will be transferred into deployment chambers in a bucket using on-site water. Several additional vials of organisms will be transported to the site, left in vials, transported back to lab, and will be kept at 12° C for the duration of the in-situ test. These organisms will serve as control organisms.

Figure 7. *Daphnia Magna* (photo courtesy of Joachim Mergeay).

Physical and chemical measurements (dissolved oxygen, temperature, pH, and conductivity) will be prior to deployment and at the termination of the test. At the end of the 96-hour deployment period, the organisms are collected, placed into a bucket with on-site water, and taken back the WSU laboratory and counted to assess survival.

The in-situ *Daphnia* will be tested five times during the course of the project. Only *Daphnia* from the first and last deployments will be preserved for gene microarray analysis. *Daphnia* for microarray from these in-situ tests will be pulled at 48 hours instead of 96 hours.

As part of the quality assurance for the acute *Daphnia* in-situ testing, samples will also be tested at 12° and 25° C in the laboratory using water from Indian and Woodard Creeks. These temperatures tests are 24 hours in duration.

Organisms from the transport controls, 24-hour temperature tests, and situ tests will be analyzed with gene microarray. *Daphnia* for microarray analysis will be preserved in RNA Later® Buffer in the field following a SOP written by Helen Poynton from EPA. The SOP is included in Appendix F. WSU will do the preservation work at the WSU laboratory. Preserved organisms can be refrigerated for up to 4 weeks at 2-8°C or frozen below -20°C for an indefinite amount of time.

Gene Microarray for *Daphnia*

The *Daphnia* gene microarray testing will be conducted by Chris Vulpe and others at the UC following their internal SOPs.

They will use DNA microarrays to produce gene expression profiles that give an illustration of how pollutants are acting within the exposed organisms. UC Berkeley has co-published several peer-reviewed studies on *Daphnia magna* gene microarray testing for copper and other metals (Poynton et al., 2007, 2008).

Passive Samplers

SPMD

SPMD membranes are prepared and preloaded onto spindles by Environmental Sampling Technologies (EST) in a clean room environment and shipped in solvent-rinsed metal cans filled with argon gas. The SPMD membranes will be kept frozen until deployed.

SPMDs will be deployed and retrieved following EAP *Standard Operating Procedure for using Semipermeable Membrane Devices to Monitor Hydrophobic Organic Compounds in Surface Water, Version 2.0* (Johnson, 2007).

At the sample site, cans containing SPMD membranes will be carefully pried open. Three SPMD membrane spindles from the metal can will be transferred into a sampling canister, and closed by screwing on the lid. Loading the SPMDs into the canisters will be done as quickly as possible because they are known to be potent air samplers. The SPMDs will be fixed atop cement blocks that will sit on the stream bottom, avoiding SPMDs contact with the substrate. SPMDs will be placed in pool areas of the stream to ensure adequate depth of water and attached to a rigid structure by lanyard.

SPMDs will remain submerged until retrieved. Field personnel will wear nitrile gloves and avoid touching membranes. The sampling period will be approximately 30 days. Retrieval will follow reverse order of deployment.

Care must be taken with the cans holding the membranes. Can seals must not be damaged as membranes will need to be resealed in their original container following retrieval to prevent contamination. SPMDs must be maintained at or near freezing until they arrive at EST for dialysis and cleanup.

TOC and TSS grab samples will be collected three times at each SPMD location during deployment.

SPMD membranes will be shipped, under chain-of-custody, to EST by overnight Federal Express, in coolers packed in blue ice. Other water samples will be returned to Ecology Headquarters under chain-of-custody to be transported to MEL the following day.

POCIS

Three SPMD membranes (on three separate spindles) and three POCIS membranes on one deployment carrier fit into one large canister as shown in Figure 8. For this study, the POCIS membranes will be deployed in the same canister as the SPMDs. POCIS are not potent air samplers like the SPMDs and so will go first into the canister to limit air time for the SPMDs.

Figure 8. Large Canister Which Can Fit Both POCIS (left) and SPMD (right) Membranes Together.

SLMD and DGT

SLMDs and DGT samplers and their housing structures will be built in their entirety by Brooks Rand. Figure 9 shows some bare SLMD membranes before they are put into their housing structures for deployment. Brooks Rand and Ecology will deploy the samplers in the stream as complete units following a deployment protocol (currently in draft) supplied by Brooks Rand.

Upon retrieval, the SLMDs and DGTs will be rinsed with ultra-pure reagent water (provided by Brooks Rand), placed in pre-cleaned bags on ice, and shipped overnight to Brooks Rand. SLMDs and DGTs should be extracted within two weeks of collection. The holding time for the extracts is 6 months prior to metals analysis.

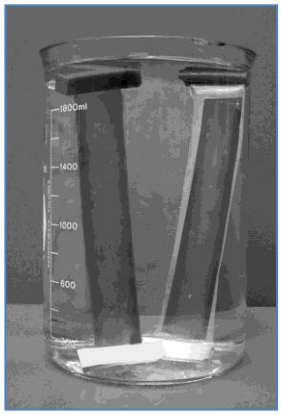

Figure 9. Sheathed Bare SLMD Membranes.

Water Samples for Metals and General Chemistry

All water samples will be collected by hand as simple grabs from mid-channel following the EAP *Standard Operating Procedure for Grab sampling – Fresh water, Version 1.0* (Joy, 2006). Streamflow in Indian Creek is small and well-mixed so that single grabs will be adequate to represent creek water. Powder-free nitrile gloves will be worn by field staff when collecting and handling samples.

Collection of water samples for metals will follow the EAP *Standard Operating Procedure (SOP) for the Collection and Field Processing of Metals Samples, Version 1.3* (Ward, 2007). Both total and dissolved metals will be collected. Samples for dissolved metals will be filtered in the field using pre-cleaned filters from Brooks Rand Laboratory. Field filtering will take place within fifteen minutes of collection. Acidification will be done by the laboratory upon receipt of the samples (within 48 hours).

Table 6 gives the requirements for sample containers, preservations, and holding times for all the water samples being collected for the pilot project.

Table 6. Sample Containers, Preservations, and Holding Times for Water Samples.

Parameter	Container	Preservation	Holding Time
DOC	2 – 60 mL poly bottles; 0.45 um pore size filters	Filter in field with 0.45um pore size filter; 1:1 HCl to pH<2; Cool to 6°C	28 days
TOC	2 – 60 mL poly bottles	1:1 HCl to pH<2; Cool to 6°C	28 days
TSS	1 L poly bottle	Cool to 6°C	7 days
Chloride	500 mL poly bottle (combined in same bottle)	Refrigerate, 0-6°C	28 days
Alkalinity			14 days
Sulfate			28 days
Calcium, Magnesium, Sodium, Potassium, and Hardness	500 mL HDPE bottle	HNO3 to pH<2 by the lab within 24 hours of collection	6 months after preservation
Cadmium, Copper, Nickel, Lead and Zinc	250 mL HDPE bottle*	Field filter for dissolved; HNO3 to pH<2 by the lab within 14 days of collection	6 months after preservation

* Containers and filters provided by Brooks Rand because they are especially clean for low-level metals analysis; all other water chemistry containers will be provided by MEL.

Streamflow Monitoring

Flow will be measured using a Marsh-McBirney flow meter and top-setting rod as described in the EAP *Standard Operating Procedure for Estimating Streamflow: Version 1.0* (Sullivan, 2007). Flow will be taken periodically as time allows during the project.

Hydrolab and Tidbit Data

A MiniSonde® will be used to measure ambient stream temperature, pH, conductivity, and dissolved oxygen each time a project-related activity occurs at the sites, e.g., during passive sampler and in-situ deployment and retrieval. The MiniSonde® will be calibrated and operated following the EAP *Standard Operating Procedure for Hydrolab® DataSonde® and MiniSonde® Multiprobes, Version 1.0* (Swanson, 2007).

Tidbit temperature loggers will be deployed with the passive samplers and trout hatchboxes at each site. Tidbits will be set to log on the half hour.

Laboratory Measurement Procedures

Laboratory reporting limits and analytical methods for passive samplers, water, and fish tissue samples are given in Table 7. A complete analyte list for pesticides, BNAs, carbamates, and herbicides can be found in Appendix C.

Table 7. Laboratory Reporting Limits and Analytical Methods for Passive Samplers, Water, and Fish Tissue.

Analysis	Matrix	Laboratory Reporting Limits	Analytical Method	Laboratory
DOC	Water	1 mg/L	Standard Methods 5310B	MEL
TOC	Water	1 mg/L	Standard Methods 5310B	MEL
TSS	Water	1 mg/L	Standard Methods 2540D	MEL
Chloride	Water	0.1 mg/L	EPA 300.0; Standard Methods 4110C	MEL
Alkalinity	Water	5 mg/L	EPA 310.2; Standard Methods 2320B	MEL
Sulfate	Water	0.5 mg/L	EPA 300.0; Standard Methods 4110C	MEL
Hardness	Water	0.3 mg/L	EPA 200.7; Standard Methods	MEL
Ca, Mg, & Na	Water	0.050 mg/L	EPA 200.7; Standard Methods	MEL
K	Water	0.5 mg/L	EPA 200.7; Standard Methods	MEL
Cd	Water	0.004 ug/L	EPA 1638, modified	Brooks Rand
Cu & Ni	Water	0.04 ug/L	EPA 1638, modified	Brooks Rand
Lead	Water	0.015 ug/L	EPA 1638, modified	Brooks Rand
Zinc	Water	0.05 ug/L	EPA 1638, modified	Brooks Rand
Cd, Cu, Pb, & Ni	Fish Tissue*	0.1 mg/Kg ww	EPA 200.8; Standard Methods	MEL
Zn	Fish Tissue*	5 mg/Kg ww	EPA 200.8; Standard Methods	MEL
Cd	SLMD & DGT	0.04 ug/L	EPA 1638, modified	Brooks Rand
Cu & Ni	SLMD & DGT	0.4 ug/L	EPA 1638, modified	Brooks Rand
Pb	SLMD & DGT	0.15 ug/L	EPA 1638, modified	Brooks Rand
Zn	SLMD & DGT	0.5 ug/L	EPA 1638, modified	Brooks Rand
Pesticides	SPMD & POCIS	66 – 1,000 ng	GCMS, EPA method (modified) SW 846 8270	MEL
BNAs	SPMD & POCIS	500 – 20,000 ng	GCMS, EPA method (modified) SW 846 8270	MEL
Carbamates	POCIS	40 – 200 ng	LCMS, EPA method (modified) SW 846 8321M	MEL
Herbicides	POCIS	125 ng	GCMS, EPA method (modified) SW 846 8270	MEL

Explanations for Table 7:

SLMD - Stabilized Liquid Membrane Device (passive sampler)
DGT - Diffusive Gradients in Thin film (passive sampler)
SPMD - Semipermeable Membrane Device (passive sampler)
POCIS - Polar Organic Chemical Integrative Sampler (passive sampler)
GCMS - Gas Chromatography/Mass Spectroscopy
LCMS - Liquid Chromatography/Mass Spectroscopy
BNAs - Bases, neutrals, and acids
* MEL needs at least ½ a gram of tissue to achieve the stated reporting limits

Trout Biomarker Analyses

Method descriptions for the trout tissue vitellogenin and metallothionein analyses that will be conducted by Nautilus are shown Appendix G, Tables G-1 through G-3.

Trout Gene Microarray

The trout gene microarray tests will be conducted by the USGS following the preparation and laboratory methods presented in Denslow et al. (2007) and Wiseman et al. (2007). The USGS will use a suite of computer software applications called TM4® to interpret microarray results. More information on the TM4® software can be found at the following website: www.tm4.org/madam.html.

The microarray test will be designed to comply with Minimum Information About Microarray Experiments (MIAME): (www.mged.org/Workgroups/MIAME/miame.html).

Daphnid Gene Microarray

The daphnid gene microarray tests will be conducted by Chris Vulpe and others at the UC following their internal SOPs. Their methods are described in some recent publications (Poynton et al., 2007, 2008).

Project Budget

The total cost for the pilot project is approximately $45,252. This estimate includes a 50% cost discount for analysis conducted at MEL. The estimate also includes substantial discounts from some of the collaborating entities and contract laboratories. The cost for MEL analyses is $6,132 and is detailed in Table 8. The total cost for all contract work is $39,120 as shown in Table 9.

Table 8. Costs for MEL Analyses.

Analysis	No. Samples	Field Replicate/QC Samples[1]	Total No. Samples	Price per Unit	Total Price
DOC	6	1	7	35	$245
TOC	6	1	7	33	$231
TSS	6	1	7	11	$77
Chloride	6	1	7	13	$91
Alkalinity	6	1	7	17	$119
Sulfate	6	1	7	13	$91
Hardness	6	1	7	22	$154
BNA[2]	4	3†	7	175	$1,225
Pesticides[3]	4	3†	7	300	$2,100
Herbicides	2	1*	3	140	$420
Carbamates	2	1*	3	130	$390
Ca, Mg, Na, & K	6	1	7	92	$644
Cd, Cu, Ni, Pb, & Zn (tissue)	2	1	3	115	$345
				Total MEL Costs:	$6,132

1 Does not include laboratory quality control (QC) samples, which are included for free.
2 Bases/Neutrals/Acids.
3 Chlorinated, organophosphorus, and nitrogen pesticides.
† Includes air blanks and day zero blanks for the SPMD samples.
* Air blank for the POCIS samples.

Table 9. Cost for all Services by Contract Laboratories and Collaborating Entities.

Contractor/ Collaborator	Type of Contract	General Description of Service	Total Price
Nautilus	Formal Lab Contract	Trout toxicity testing (lab and in-situ)	$15,500
USGS	Cooperative Agreement (50/50 split of total costs)	Trout microarray	$7,000
WSU and Thurston County	Informal agreement (WSU analysis paid for by Thurston County)	Daphnid toxicity testing (lab and in-situ)	-
Agilent & UC Berkeley	Informal agreement (UC analysis free and Ecology purchases lab supplies)	Daphnid microarray	$4,635
Brooks Rand	Formal Lab Contract	Metals passive sampling (DGT/SLMD and water)	$7,955
EST	Formal Lab Contract	SPMD/POCIS preparation and extraction	$2,240
Ecology Contractor	Formal Lab Contract	Macroinvertebrate and periphyton analysis	$1,790
		Total Costs for Contract Work:	$39,120

Quality Control Procedures

Field

Table 10 lists the field quality control samples that will be analyzed for the chemical analyses. Field quality control samples provide an estimate of the total variability of the results (field plus laboratory) and will consist of the collection and analysis of field replicates and field blanks.

All efforts will be made to avoid cross-contamination of samples. Field staff will wear non-talc nitrile gloves throughout the sampling process and carefully follow all SOPs referenced in the *Sampling Procedures* section of this QA Project Plan.

Table 10. Field Quality Control Samples for Chemical Analyses.

Parameter	Matrix	Field Replicate	Field Blank
Cd, Cu, Ni, Pb, & Zn	Water	1/project	3/project (1 per sampling event)
Ca, Mg, Na, & K	Water	1/project	1/project
DOC, TOC, TSS chloride, alkalinity, sulfate, & hardness	Water	1/project	NA
Cd, Cu, Ni, Pb, & Zn	DGT & SLMD	1/project	NA*
Pesticides & BNAs	SPMD	NA	1/project
Pesticides, BNAs, herbicides, & carbamates	POCIS	NA	1/Project

NA - no analysis.
* Laboratory equipment blanks will be used instead of field blanks for the DGTs and SLMDs.

SPMD and POCIS

Prior to deployment, known concentrations of performance reference compounds (PRCs) are spiked into SPMDs by EST. MEL provides the PRCs to EST. PRCs will not be used for the POCIS analyses; analyte constituent concentrations will be estimated from laboratory derived calibrations.

PRC compounds are not normally found in the environment at significant concentrations and slowly release over time. For this study a mix of PCB congeners and deuterated PAHs will be used. The PRC chemicals include: PCB 14, PCB 29, PCB 50, acenaphthylene-d8, and pyrene-d10. The PRC loss rates will be used to adjust uptake (sampling) rates of the target contaminants for SPMDs. Uptake of contaminants and release of PRCs are affected by the turbulence and velocity of water, temperature, and biofouling.

Because SPMDs are potent air samplers, a field blank will be used to account for potential contamination from airborne chemicals. The field blank SPMD is opened to the air for the same amount of time it takes to open and place the SPMD array in the water, and then the blank is resealed and kept on ice. The blank is stored frozen and taken back into the field and opened and closed again to mimic the retrieval process. The blank is processed and analyzed the same as deployed SPMDs. Although POCIS is not as rigorous an air sampler as SPMDs, a POCIS trip blank will be employed to ensure consistent application and comparability between methods.

A field replicate will not be used for SPMD and POCIS samples as a significant cost savings.

Laboratory

The laboratory quality control procedures routinely followed by MEL and the contract laboratories will be satisfactory for the purposes of this project. MEL will follow SOPs as described in the *Manchester Environmental Laboratory Quality Assurance Manual* (MEL, 2006).

The laboratory control samples that will be used for the chemical analyses of this project are listed in Table 11.

Table 11. Laboratory Quality Control Samples**.**

Parameter	Matrix	Method Blank	Laboratory Control Sample	Laboratory Duplicate	Matrix Spike/ Matrix Spike Duplicate	Surrogate Spikes
TSS & Alkalinity	Water	2/project	2/project	2/project	NA	NA
TOC, DOC, Hardness, Sulfate, Chloride, Ca, Mg, Na, K, Cd, Cu, Ni, Pb, & Zn	Water	2/project	2/project	2/project	2/project	NA
Cd, Cu, Ni, Pb, & Zn	SLMD & DGT	2/project	2/project	2/project	2/project	NA
Cd, Cu, Ni, Pb, & Zn	Tissue	1/project	1/project	1/project	1/project	NA
Pesticides	SPMD	1/batch	1/batch	NA	1/batch*	All samples
Pesticides	POCIS	1/batch	1/batch	NA	NA	All samples
BNAs	SPMD	1/batch	1/batch	NA	1/batch*	All samples
BNAs	POCIS	1/batch	1/batch	NA	NA	All samples
Carbamates	POCIS	1/batch	1/batch	NA	NA	All samples
Herbicides	POCIS	1/batch	1/batch	NA	NA	All samples

* MS/MSD extracts will be held frozen at MEL and not analyzed.
NA - not applicable
Batch - One sampling event

SPMD and POCIS

SPMDs require a special group of method blanks which will be prepared by EST for the dialysis and cleanup process. These blanks are in addition to method blanks typically run by labs during analysis. POCIS do not require these special method blanks.

SPMD method blanks include:

- Day-zero dialysis blank to serve as a reference point for chemical compound loss and to represent background during dialysis and cleanup. This blank will contain 3 membranes, as in the field samples, and will be manufactured at the same time as field samples.
- Fresh day-zero blank, prepared just prior to dialysis, contains one membrane.
- Spiking blank, a spiked single membrane, to assess contamination of membranes exposed while spiking the SPMDs at EST after field sampling but before dialysis and cleanup.
- Solvent blank, to assess contamination independent of the SPMDs. This blank does not go through any SPMD process.

In efforts to reduce cost, only the day-zero blank will be analyzed along with study samples. The others will be kept frozen at MEL for analysis in the event that contamination or other problems occur.

Surrogates will be spiked in both SPMDs and POCIS membranes at the EST laboratory prior to dialysis and cleanup, to calculate analytical recovery for each class of compounds. MEL will provide EST a mix of surrogates that will represent all the analyses (pesticides, herbicides, and BNAs) being conducted by MEL.

In addition, MEL will make up and send to EST matrix spikes for the pesticide and BNA analyses being conducted on the SPMD samples. EST will spike one new SPMD membrane in the laboratory with various chlorinated pesticides. A new SPMD will be used to avoid interference with target analytes.

DGT and SLMD

The DGT and SLMD passive samplers also require some special method blanks. Three equipment blanks for both DGTs and SLMDs will be deployed in reagent water in the laboratory at the same time the field samples are deployed. The equipment blanks will stay in the reagent water over the field deployment period and will be analyzed with the field samples.

Three sample blanks for both DGTs and SLMDs will be prepared from fresh passive units and analyzed with the rest of the samples.

Data Management Procedures

Field data will be recorded in a field notebook. Relevant information will be carefully transferred to electronic data sheets and reviewed for potential transfer errors.

The data packages from MEL and the contract laboratories will include case narratives discussing any problems encountered during analysis, corrective actions taken, and an explanation of data qualifiers. The project manager will then review the data packages to determine if analytical MQOs (laboratory control samples, laboratory duplicates, and matrix spikes) were met.

Chemical data and data from the trout and *Daphnia* toxicity tests will be entered into Ecology's Environmental Information Management (EIM) database for availability to the public and interested parties. EIM can handle bioassay toxicity data. Data entered into EIM follow a formal data review process where data are reviewed by the project manager, the person entering the data, and an independent reviewer.

Trout and *Daphnia* toxicity data will also be entered into Ecology's CETIS™ database by Randall Marshall. CETIS™ will help interpret toxicity results for the trout and *Daphnia* tests.

Audits and Reports

MEL participates in performance and system audits of their routine procedures. The results of these audits are available on request.

The Ecology draft technical report will be provided to the client, internal Ecology reviewers, collaborating entities, external reviewers, and other interested parties by March 2011. The final technical report will be completed in May 2011 and will include the following elements:

- Information about the sampling locations, including geographic coordinates and maps.
- Descriptions of field and laboratory methods.
- Tables presenting all the data.
- Discussion of project data quality.
- Summary of significant findings.
- Recommendations for future follow-up work.

Upon completion of the study, most of the data will be entered into Ecology's EIM database. Electronic data and the final report for the study will be available to the public on Ecology's internet homepage (www.ecy.wa.gov).

The collaborating entities for the project may also publish reports relating to their part of the project. Ecology has agreed to let them use any and all data generated from the project.

Data Verification

The project manager will review laboratory data packages and data verification reports. Based on these assessments, the data will either be accepted, accepted with appropriate qualifications, or rejected and re-analysis considered.

To determine if analytical MQOs have been met, the project manager will compare results of the field and laboratory quality control samples to MQOs.

Formal (third party) validation of the data will not be necessary for this project.

Data Quality (Usability) Assessment

Once the data have been reviewed and verified, the project manager, in consultation with the client, will determine if the data are useable for the purposes of the pilot project.

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Appendices

Appendix A. Glossary, Acronyms, and Abbreviations

Glossary

Ambient: Background or away from point sources of contamination.

Biomarker: A chemical found in the body of an organism that indicates exposure to certain chemical stressors in the environment.

Clean Water Act: A federal act passed in 1972 that contains provisions to restore and maintain the quality of the nation's waters. Section 303(d) of the Clean Water Act establishes the TMDL program.

Conductivity: A measure of water's ability to conduct an electrical current. Conductivity is related to the concentration and charge of dissolved ions in water.

Dissolved oxygen: A measure of the amount of oxygen dissolved in water.

Endocrine disrupter: Chemicals that interrupt the endocrine systems of humans and wildlife. The endocrine system controls hormones in the body that moderate normal bodily function.

Impervious surface: A surface that is impenetrable to water. Impervious surfaces include asphalt, concrete, and most rooftops.

Metallothionein: An enzyme produced by an organism in response to exposure to a toxic metal.

Parameter: A physical chemical or biological property whose values determine environmental characteristics or behavior.

pH: A measure of the acidity or alkalinity of water. A low pH value (0 to 7) indicates that an acidic condition is present, while a high pH (7 to 14) indicates a basic or alkaline condition. A pH of 7 is considered to be neutral. Since the pH scale is logarithmic, a water sample with a pH of 8 is ten times more basic than one with a pH of 7.

Point source: Sources of pollution that discharge at a specific location from pipes, outfalls, and conveyance channels to a surface water. Examples of point source discharges include municipal wastewater treatment plants, municipal stormwater systems, industrial waste treatment facilities, and construction sites that clear more than 5 acres of land.

Pollution: Such contamination, or other alteration of the physical, chemical, or biological properties, of any waters of the state. This includes change in temperature, taste, color, turbidity, or odor of the waters. It also includes discharge of any liquid, gaseous, solid, radioactive, or other substance into any waters of the state. This definition assumes that these changes will, or are likely to, create a nuisance or render such waters harmful, detrimental, or injurious to (1) public health, safety, or welfare, or (2) domestic, commercial, industrial, agricultural, recreational, or other legitimate beneficial uses, or (3) livestock, wild animals, birds, fish, or other aquatic life.

Reach: A specific portion or segment of a stream.

Riparian: Relating to the banks along a natural course of water.

Salmonid: Any fish that belong to the family *Salmonidae*. Basically, any species of salmon, trout, or char. www.fws.gov/le/ImpExp/FactSheetSalmonids.htm

Stormwater: The portion of precipitation that does not naturally percolate into the ground or evaporate but instead runs off roads, pavement, and roofs during rainfall or snow melt. Stormwater can also come from hard or saturated grass surfaces such as lawns, pastures, playfields, and from gravel roads and parking lots.

Streamflow: Discharge of water in a surface stream (river or creek).

Surface waters of the state: Lakes, rivers, ponds, streams, inland waters, salt waters, wetlands and all other surface waters and water courses within the jurisdiction of Washington State.

Total suspended solids (TSS): Portion of solids retained by a filter.

Vitellogenin: A protein produced when an organism is exposed to an estrogen-like compound. The protein is normally only produced in females during egg production.

Watershed: A drainage area or basin in which all land and water areas drain or flow toward a central collector such as a stream, river, or lake at a lower elevation.

Wetland Complex: A series of connected wetlands. Wetlands are areas that are inundated or saturated by surface or groundwater at a frequency and duration sufficient to support a prevalence of vegetation typically adapted for life in saturated soil conditions. Wetlands generally include swamps, marshes, bogs, and similar areas.

Acronyms and Abbreviations

DOC	Dissolved organic carbon
Ecology	Washington State Department of Ecology
EIM	Environmental Information Management database
EPA	U.S. Environmental Protection Agency
MEL	Manchester Environmental Laboratory
MQO	Measurement quality objective
QA	Quality assurance
RPD	Relative percent difference
SOP	Standard operating procedures
TOC	Total organic carbon
TSS	(See Glossary above)
USGS	U.S. Geological Survey

Metals

Cd	Cadmium
Cu	Copper
K	Potassium
Mg	Magnesium
Na	Sodium
Ni	Nickel
Pb	Lead
Zn	Zinc

Units of Measurement

°C	degrees centigrade
cfs	cubic feet per second
cm	centimeter
g	gram, a unit of mass
L	liter
m	meter
mg	milligram
mg/Kg	milligrams per kilogram (parts per million)
mg/L	milligrams per liter (parts per million)
mL	milliliters
mm	millimeter
ng	nanogram
µg/L	micrograms per liter (parts per billion)
µS/cm	microsiemens per centimeter, a unit of conductivity

Appendix B. Monitoring Actions

Table B-1. Monitoring Actions for the 2010 Ambient Pilot Projects.

Actions	Responsible	Timing
Instream Biological Assessments		
Benthic invertebrate bioassessment	Ecology	Just after end of 1st daphnid deployment.
Periphyton assessment		
Trout in-situ toxicity testing	Nautilus / Ecology	Just after benthic and periphyton assessments.
Trout gene microarray	USGS	On trout after in-situ deployment.
Fish tissue metals	MEL	
Trout metallothionein	Nautilus	
Daphnid in-situ toxicity testing	Thurston County / WSU	1st action and repeated 4 times during passive sampler deployment with the last timed to end with passive sampler deployment end.
Daphnid gene microarray	UC	On daphnids from 1st and last in-situ.
Laboratory Biological Assessments		
Trout exposed to contaminated water	Nautilus	As convenient during project.
Trout gene microarray on both whole fish and liver	USGS	After trout exposure.
Trout exposed to estradiol	Nautilus	As convenient during project.
Trout vitellogenin		After trout exposure.
Daphnids exposed to downstream samples at 12° and 25°C	Thurston County / WSU	Samples taken on 1st and last daphnid in-situ exposure.
Daphnid microarray on daphnids exposed at 12° and 25°C	WSU / UC	After daphnid exposure.
Passive Samplers and Analysis		
Passive sampler metals - DGT	Brooks Rand / Ecology	For 28 days just after in-situ trout deployment.
Passive sampler metals - SLMD		
Metals analysis - (DGT and SLMD) for Cd, Cu, Ni, Pb, and Zn	Brooks Rand	After passive sampler retrieved.
Passive sampler nonpolar - SPMD	EST / Ecology	For 28 days just after in-situ trout deployment
Extraction SPMD	EST	After passive sampler retrieved
Analysis SPMD - BNAs, PAHs	MEL	When extract delivered to MEL.
Analysis SPMD - pesticides (Cl, OP, N)		
Passive sampler polar - POCIS	EST / Ecology	For 28 days just after in-situ trout deployment.
Extraction POCIS	EST	After passive sampler retrieved.

Actions	Responsible	Timing
Analysis POCIS - herbicides	MEL	When extract delivered to MEL.
Analysis POCIS - pesticides (Cl, OP, N)		
Analysis POCIS - carbamates		
Analysis POCIS - BNAs		
Water Chemistry		
Stream grab sample for metals	Ecology	3 times during passive sampler and daphnid deployments.
Metals analysis - for Cd, Cu, Ni, Pb, and Zn	Brooks Rand	3 times during passive sampler deployment.
Analysis BLM parameters - DOC, pH, Ca, Mg, Na, K, SO_4, Cl, and alkalinity.	MEL	
Biotic Ligand Model (BLM).	Ecology	3 times during passive sampler deployment.
MiniSonde physical and chemical measurements.		On each station visit.

Appendix C. Analyte Lists

Bases, Neutrals, and Acids (BNAs) Analyte List

1,2,4-Trichlorobenzene
1,2-Dichlorobenzene
1,2-Diphenylhydrazine
1,3-Dichlorobenzene
1,4-Dichlorobenzene
1-Methylnaphthalene
2,4,5-Trichlorophenol
2,4,6-Trichlorophenol
2,4-Dichlorophenol
2,4-Dimethylphenol
2,4-Dinitrophenol
2,4-Dinitrotoluene
2,6-Dinitrotoluene
2-Chloronaphthalene
2-Chlorophenol
2-Methylnaphthalene
2-Methylphenol
2-Nitroaniline
2-Nitrophenol
3,3'-Dichlorobenzidine
3B-Coprostanol
3-Nitroaniline
4,6-Dinitro-2-Methylphenol
4-Bromophenyl phenyl ether
4-Chloro-3-Methylphenol
4-Chloroaniline
4-Chlorophenyl-Phenylether
4-Methylphenol
4-Nitroaniline
4-Nitrophenol
4-nonylphenol
Acenaphthene
Acenaphthylene
Anthracene
Benzo(a)anthracene
Benzo(a)pyrene
Benzo(b)fluoranthene
Benzo(ghi)perylene
Benzo(k)fluoranthene
Benzoic Acid
Benzyl Alcohol
Bis(2-chloro-1-methylethyl) ether
Bis(2-Chloroethoxy)Methane
Bis(2-Chloroethyl)Ether
Bis(2-Ethylhexyl) Phthalate
Bisphenol A
Butyl benzyl phthalate
Caffeine
Carbazole
Cholesterol
Chrysene
Dibenzo(a,h)anthracene
Dibenzofuran
Diethyl phthalate
Dimethyl phthalate
Di-N-Butylphthalate
Di-N-Octyl Phthalate
Ethanol, 2-Chloro-, Phosphate (3:1)
Fluoranthene
Fluorene
Hexachlorobenzene
Hexachlorobutadiene
Hexachlorocyclopentadiene
Hexachloroethane
Indeno(1,2,3-cd)pyrene
Isophorone
Naphthalene
Nitrobenzene
N-Nitrosodimethylamine
N-Nitrosodi-n-propylamine
N-Nitrosodiphenylamine
Pentachlorophenol
Phenanthrene
Phenol
Pyrene
Retene
Triclosan
Triethyl citrate

Carbamate Analyte List

Methomyl oxime
Oxamyl oxime
Aldicarb Sulfoxide
Aldicarb Sulfone
Oxamyl (Vydate)
Methomyl
3-Hydroxycarbofuran
Imidacloprid
Aldicarb
Baygon (Propoxur)
Carbofuran
Carbaryl
Methiocarb
1-Naphthol
Promecarb

Herbicides Analyte List

2,4,6-Trichlorophenol
3,5-Dichlorobenzoic Acid
4-Nitrophenol
Clopyralid
2,4,5-Trichlorophenol
Dicamba I
2,3,4,6-Tetrachlorophenol
MCPP (Mecoprop)
MCPA
Dichlorprop
Bromoxynil
2,4-D
2,3,4,5-Tetrachlorophenol
Triclopyr
Pentachlorophenol
2,4,5-TP (Silvex)
2,4,5-T
2,4-DB
Dinoseb
Bentazon
Ioxynil
Picloram
Dacthal (DCPA)
Acifluorfen (Blazer)
Diclofop-Methyl
Chloramben

Pesticide MS Analyte List

2,4'-DDD
2,4'-DDE
2,4'-DDT
4,4'-DDD
4,4'-DDE
4,4'-DDT
4,4'-Dichlorobenzophenone
Acetochlor
Alachlor
Aldrin
Alpha-BHC
Atrazine
Azinphos-ethyl
Azinphos-methyl (Guthion)
Benefin
Benthiocarb
Beta-BHC
beta-Cypermethrin
Bifenthrin
Bromacil
Butachlor
Butylate
Captan
Carboxin
Chlorothalonil (Daconil)
Chlorpropham
Chlorpyrifos O.A.
Chlorpyriphos
cis-Chlordane
Cis-Nonachlor
cis-Permethrin
Coumaphos
Cyanazine
Cycloate
Dacthal (DCPA)
Delta-BHC
Deltamethrin
Di-allate (Avadex)
Diazinon
Diazinon O Analog
Dichlobenil
Dichlorvos (DDVP)

Dieldrin
Dimethoate
Diphenamid
Disulfoton (Di-Syston)
Disulfoton Sulfone
Disulfoton Sulfoxide
Diuron
Endosulfan I
Endosulfan II
Endosulfan Sulfate
Endrin
Endrin Aldehyde
Endrin Ketone
EPN
Eptam
Ethalfluralin (Sonalan)
Ethion
Ethoprop
Fenamiphos
Fenamiphos Sulfone
Fenarimol
Fenvalerate (2 isomers)
Fipronil
Fipronil Desulfinyl
Fipronil Sulfide
Fipronil Sulfone
Fluridone
Fonofos
Gamma-BHC (Lindane)
Heptachlor
Heptachlor Epoxide
Hexachlorobenzene
Hexazinone
Imidan
Kelthane
lambda-Cyhalothrin
Linuron
Malathion
Metalaxyl
Methidathion
Methoxychlor
Methyl Chlorpyrifos
Methyl Paraoxon
Methyl Parathion
Metolachlor
Metribuzin
Mevinphos
MGK264
Mirex
Monocrotophos
Naled
Napropamide
Norflurazon
Omethoate
Oryzalin
Oxychlordane
Oxyfluorfen
Parathion
Pebulate
Pendimethalin
Phenothrin
Phorate
Phorate O.A.
Phosmet O.A.
Prometon (Pramitol 5p)
Prometryn
Pronamide (Kerb)
Propachlor (Ramrod)
Propargite
Propazine
Resmethrin
Simazine
Simetryn
Sulfotepp
Tebuthiuron
Terbacil
Tetrachlorvinphos (Gardona)
Tokuthion
Tralomethrin
Trans-Chlordane
Trans-Nonachlor
Trans-Permethrin
Treflan (Trifluralin)
Triadimefon
Triallate
Trichloronate
Tricyclazole

Appendix D. Periphyton Collection Method

Draft Washington State Standard Operating Procedure for the Collection of Periphyton

Introduction

Periphyton are benthic algae that live attached or in close proximity to various substrates associated with the stream bottom. The structure, diversity, and abundance of periphyton are highly dependent on the diversity and availability of substrates in the stream. Periphyton algae often form visible filaments or colonies in the form of mats or biofilms attached to substrate. Two basic types of periphyton are found in Washington streams: diatoms (Division Chrysophyta, Class Bacillariophyceae) and soft-bodied algae. Soft-bodied algae are represented by four major divisions: green algae (Chlorophyta), blue-green algae (Cyanophyta), gold/brown algae (Chrysophyta) and occasionally red algae (Rhodophyta).

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).

Sampling Time - Index Period

The recommended sample period for periphyton follows the sample period for benthic macroinvertebrates (see Macroinvertebrate Sampling Index Period Standard Operating Procedure (SOP)). It may be necessary to sample outside the recommended index period to coincide with flows in ephemeral, intermittent, or dewatered streams.

Sampling Methods - Field Procedure

The field procedure(s) for collecting periphyton will vary depending on the chosen targeted habitat. The targeted habitat represents the most common and stable habitat in the stream reach. Field selection of the targeted habitat where samples are collected will be based on the following prioritization: 1) riffles with dominant coarse substrate (Epilithic habitat); 2) woody snags in streams with dominant fine-grained substrate (Epidendric habitat); organically rich 3) pea gravel/sand (Epipsammic habitat) or 4) silt (Epipelic habitat) depositional areas along stream margins, and 5) emergent or 6) submerged vegetation (Epiphytic habitat). Equipment and supplies needed to conduct the periphyton sampling and subsequent subsample processing will be assembled and ready for use.

Required items include:

Aluminum foil	Plastic beaker (500 mL)
Digital caliper	Plastic petri dishes (47 mm)
Distilled or deionized water	Plastic sample bottles (500 & 1000 mL Nalgene®)
Dry ice	Plastic tape (electrical preferable)
Envelopes	Plastic trays
Filtration apparatus that includes hand pump (with gage), tubing, filter base, and filter funnel	Pocket calculator
Forceps	Pruning shears
Funnel	Ruler (with metric increments)
Glass microfiber filters (47 mm @ 0.7 micron)	Scissors
Graduated cylinders	Sealable plastic bags
Hand saw (folding)	Spatula
Labels	Serological volumetric pipettes (10 mL disposable) with rubber bulb
Lugol's solution	Toothbrush (soft and firm-bristled)
Pens and permanent markers	Top-setting or survey rod

Sampling Method for Epilithic (Coarse Substrate) Habitats

1. Randomly select eight sampling locations within the riffle. If also sampling for macroinvertebrates using a Surber sampler, samples will be collected in close proximity to (but not within) the randomly selected Surber sample locations. See Macroinvertebrate Sampling SOP for description of selecting random sample locations.

2. Carefully remove 1 or 2 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 rock. If possible, select rocks that are similar with respect to size, depth, and exposure to sunlight. A total of 8 to 16 rocks are collected at each sample 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.

3. Measure water depth and velocity at each of the eight locations using a topsetting rod and velocity meter; record on the datasheet. NOTE: Additional measurements of depth and velocity are not required if the sampler is already measuring these parameters for the macroinvertebrate sample. Assuming the sun is directly overhead, determine the relative degree of riparian shading (e.g., shaded, partial, or full sun) at each randomly selected sample location and record on the datasheet.

4. 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.

5. 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.

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

Subsample Processing Procedures

Each composite sample processed in the field is used to extract subsamples for chlorophyll α 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 α 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.

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 aliquots represent the chlorophyll α subsample. Determine the number of aliquots filtered and record the chlorophyll α 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 resealable 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.

    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 make them aware of plans to ship (via overnight shipping service) coolers containing dry ice and frozen subsamples. Make sure you 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); record on the datasheet.

7. Preserve the ID subsample with 5 to 10 percent Lugol's solution (see Sample Preservative-Lugol's Solution for preparation). Five percent should be sufficient for most samples, although up to 10 percent 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 subsample 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]

Sample Preservative-Lugol's Solution

Prepare Lugol's solution by dissolving 20 g potassium iodide (KI) and 10 g iodine crystals in 200 mL distilled water containing 20 mL glacial acetic acid. Store Lugol's solution in an opaque plastic bottle.

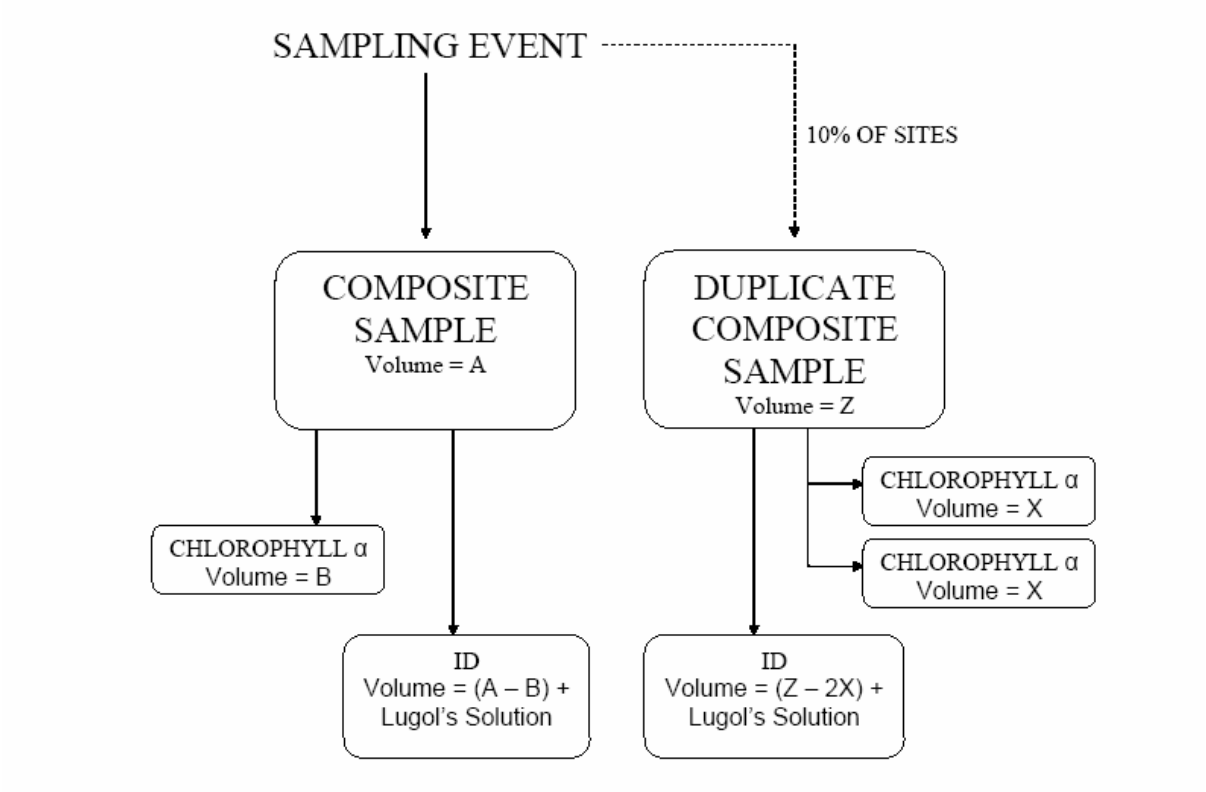
Quality Control

Following the processes described under Sampling Methods-Field Procedures, at least 10% of all collected composite samples must consist of duplicate composite samples (e.g., 2 duplicates for 11 to 20 samples, 3 duplicates for 21 to 30 samples).

Duplicate composite sampling consists of two samplers each with the same equipment, collecting simultaneously alongside 1) randomly selected locations for Epilithic samples, 2) woody snag locations for Epidendric samples 3) shallow depositional locations for Epipsammic/Epipelic samples or 4) locations of emergent or submerged vegetation for Epiphytic samples.

Following the processes described under Subsample Processing Procedures, the sampler that collected the duplicate composite sample, extracts two chlorophyll _ subsamples from the duplicate composite sample. The remaining duplicate composite sample volume will be used for the duplicate ID subsample. Duplicate composite samples are collected to check the variability between field samplers while the two duplicate chlorophyll _ subsamples provide an indication of precision and the quality of the duplicate composite sample homogenization.

Following is an illustration of the duplicate composite sample/subsample processes:



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Appendix E. *Daphnia* In-Situ Toxicity Testing Procedures

In-Situ Toxicity Testing Procedures (provided by Barb Wood of Thurston County)

Acute In-Situ Bioassays

In-situ testing consists of test chambers constructed from 5.1 cm x 12.7 cm clear liner tubes (cellulose acetate butyrate) capped with two polyethylene closure caps. Two long rectangular windows (6 cm x 2.5 cm) are covered with 74 micron mesh to contain organisms and exclude predators while allowing exposure to test media.

***Daphnia magna* – 100% Ambient test – 96 hours**

We will follow this EPA procedure: EPA/600/4-90/027F Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms Section 9 (pg. 45-75). MODIFIED.

NOTE: This process requires removal of neonates from stock cultures 24 hours before test set-up.

- On day of test set-up, remove <24-h neonates from stock cultures. Pool neonates and feed 1:1 YTC and *Selenastrum* 2 hours before use.
- Label 20 ml test tubes with a number, starting with one. Each test site requires a total of 4 replicates. Mark an additional 4 test tubes for travel control data. Generate random test positions using TOXCALC. Mark assigned position below replicate number.
- Fill test tubes half full with MHSW.
- Introduce 1 to 2 test organisms/ replicate by submerging 2 mm internal diameter (i.d.) pipette just under water surface, avoiding any air bubbles. Continue until there are a total of 10 organisms/replicate. Verify that 10 organisms are in each test and control replicate using a fiber light.
- Place test tubes in order of randomized position into a test tube rack. Cover and place in ice cooler with blue ice for transport to the field site. *NOTE*: Organisms should be chilled to field water temperature slowly over a minimum of 2 hours.
- At in-situ test set-up, collect and record the physical and chemical measurements using the YSI 600R multi-meter; D.O. (%, mg/L), temperature (ºC), pH, and conductivity (µS /cm).

Optional: Collect a grab sample in an EPA- approved container by rinsing three times with sample water, submerging container at least 12 inches below the surface, and allowing container to fill. Expel all air and seal with no headspace.

Termination of In-Situ Test

- At in-situ test termination, collect and record the physical and chemical measurements using the YSI 600R multi-meter; D.O. (%, mg/L), temperature (ºC), pH, and conductivity (µS /cm).

- Collect in-situ chambers and place into bucket with sample water for travel back to the laboratory.

At the Laboratory

- Slowly remove an end cap from chamber. Rinse sides of chamber to assure all organisms are collected.

- Note and record any mortalities and abnormal behavior in test organisms collected from the control and test water sites. Record findings on test data sheet.

In-situ test acceptability is no less than 80% survival in the control test site. If no control site was used in the field, in-situ test acceptability is no less than 90% in the travel controls.

- Analyze survival data using the statistical program TOXCALC or CETIS™.

Data Sheet

In-Situ Ambient Toxicity Test

Daphnia magna -- *Acute Test* -- 96 Hour

Client	Test Site
Analyst	Control Site
Date:	

Parameter	**Test Site**		**Control Site**		**NOTES**
TIME	Set	Pulled	Set	Pulled	10 -<24h *Daphnia magna*/chamber
					Travel controls are held @< 4 ° C
TEMP (C)					and fed at 48 hours
O hour					
96 hour					
D.O. %					
O hour					
96 hour					
D.O. (mg/L)					
O hour					
96 hour					
pH					
O hour					
96 hour					
Cond (µS/cm)					
O hour					
96 hour					

SITE	**Replicate #**	**Survival**	
Control	1		
	2		
	3		
	4		
Test	1		
	2		
	3		
	4		
Travel Control	1		
	2		
	3		
	4		

Appendix F. Preservation of *Daphnia Magna* Tissue

Preservation of *Daphnia magna* tissue for RNA Isolation using RNA*later* by Helen Poynton of the U.S. Environmental Protection Agency

Supplies needed

- RNA*later:* Applied Biosystems, part # AM7020 (100 ml) or AM7021 (500 ml).
- Cryogenic vials: Corning round bottom, self-standing, 2.0 ml capacity, (Fisher Scientific) part#: 03-374-21 (or equivalent).
- Fine-tip transfer pipet: Samco, (Fisher Scientific) part # 13-711-30 (or equivalent).
- Weigh boats: Fisher scientific, part # 08-732-112 (or equivalent).

Set-up

1. Place 1.0 ml of RNA*later* in a 2.0 ml cryogenic vial. RNA*later* is stable at room temperature and does not have to be refrigerated.
2. Prepare several blunt-end transfer pipettes for daphnid collection by cutting off the tip of the pipet.

Collection of organisms in the field

1. Open in-situ chambers at water surface to access animals, but do not allow the animals to escape.
2. Remove 5 adult daphnids with a pipet and place in a small weigh boat. Using a fine-tip transfer pipet remove the excess water from the weigh boat.
3. Open the cryovial containing the RNA*later*. Withdraw about 0.25 – 0.5 ml of RNA*later* with a transfer pipet.
4. Holding the weigh boat over the cryovial, add the RNA*later* to the weighboat and "pour" the daphnids into the cryovial.
5. Replace the cap on the cryovial and invert several times to completely submerge the daphnids and allow for RNA*later* penetration of tissues.
6. Place on ice.
7. Repeat until all daphnids are collected. Store all samples overnight at 4^{o} C.

Storage and shipping

Sample must first be incubated overnight at 4^{o} C. After overnight incubation, whenever possible, samples should be stored at -20^{o} C or -80^{o} C, but they may be shipped overnight on ice.
In general, samples preserved with RNA*later* may be stored in the following manner:

- Indefinitely at -80 ^{o} C or -20 ^{o} C. Samples will not freeze at -20 ^{o} C, but RNA will remain intact.
- 1 month at 4 ^{o} C.
- 1 week at 25 ^{o} C.
- 24-h at 37 ^{o} C.

For more details and for protocols on RNA Isolation, see Applied Biosystems "RNA*later* Tissue Collection: RNA Stabilization Solution" Product manual, available at:
www.ambion.com/techlib/prot/bp_7020.pdf

Appendix G. Trout Biomarker Methods (Nautilus Environmental)

Table G-1. Trout Vitellogenin.

Method Reference	Plasma Vitellogenin Quantification. OECD 21-day Fish Assay #230, Biosense Rainbow trout Vitellogenin EIA Kit.
Description	Plasma vitellogenin levels are quantified and compared between non-exposed and exposed fish. Induction of plasma vitellogenin indicates exposure to estrogenic compounds.
Tissue Assayed	Blood plasma.
Amount of Tissue per Replicate per Treatment	• ~7-10 trout fry pooled for one sample (~10 µl plasma yield); 1 biological replicate = 3 fry resulting in an n=3-5 per biological replicate. • Need a minimum of 4 biological replicates/treatment.

Tissue Treatment and Storage	• Remove tail, collect blood via caudal vessels with microhematocrit capillary tube (heparinized).
	• Centrifuge blood in microhematocrit centrifuge, 3 mins @ ~13 000 xg.
	• Store in microfuge tube with aprotinin (protease inhibitor, 0.13 units per sample)
	  ○ -80°C (indefinitely).
	  ○ thaw on ice prior to assaying.

Table G-2. Trout Metallothionein.

Method Reference	Metallothionein (MT) Determination in Liver or Gills. Viarengo et al. 1997, Mar. Env. Res. 277, 69-84; Linde et al., 2006, Biochem. Mol. Biol. Ed., 34, 360-363.
Description	Gill or liver MT levels compared between non-exposed and exposed fish. Induction of MT indicates exposure to elevated heavy metals. The concentration of MT is determined by evaluating the SH group content with the colorimetric Ellman's reagent, using GSH as a reference or purified MT (if available).
Tissue Assayed	Gill or liver.
Amount of Tissue per Replicate per Treatment	• ~10 fry pooled for one sample. • Need a minimum of 4 biological replicates/treatment.
Tissue Treatment and Storage	• Place in plastic bag on ice for a maximum of 4 hours, or freeze on dry ice/liquid nitrogen and store at -20°C until analysis.

Table G-3. Trout Metallothionein (ELISA Method).

Method Reference	Metallothionein (MT) Semi-Quantitative Determination in Liver or Gills. Biosense Laboratories, Prod. No. B00400402, Biomarker ELISA Component Kit Semi-Quantitative – Mab GAM-HRP
Description	Gill or liver MT levels compared between non-exposed and exposed fish. Induction of MT indicates exposure to elevated heavy metals. The ELISA is based on detection of MT using a suitable antibody in an indirect capture ELISA format.
Tissue Assayed	Gill or liver.
Amount of Tissue per Replicate per Treatment	• ~10 fry pooled for one sample. • Need a minimum of 4 biological replicates/treatment.
Tissue Treatment and Storage	• Place in plastic bag on ice for a maximum of 4 hours, or freeze on dry ice/liquid nitrogen and store at -20°C until analysis.