Stormwater Action Monitoring
Stormwater Effectiveness Studies

Detailed Study Design Proposal &
Quality Assurance Project Plan (QAPP)

Longevity of bioretention depths for preventing acute toxicity from urban stormwater runoff

Prepared For:

Stormwater Action Monitoring
Washington State, Department of Ecology
300 Desmond Dr. SE (FedEx)
P.O. Box 47600 (USPS)
Olympia, Washington, 98504-7600
(360) 407-6158

Prepared By:

Washington State University
Puyallup Research and Extension Center
2606 W Pioneer Ave
Puyallup, Washington, 98371
(253) 445-4500

March 2019

Proposal Author and Contact Information

Jenifer McIntyre, Ph.D.
Washington State University
Puyallup Research and Extension Center
Assistant Professor
2606 W Pioneer Ave
Puyallup, Washington, 98371
jen.mcintyre@wsu.edu
(253) 445-4650

QAPP Author and Contact Information

Lane Maguire
Washington State University
Puyallup Research & Extension Center
Graduate Research Assistant
2606 W Pioneer Ave
Puyallup, Washington, 98371
lane.maguire@wsu.edu
(816) 803-3462

Project Manager and Contact Information

Jay W. Davis
U.S. Fish & Wildlife Service
Washington Fish & Wildlife Office
Resource Contaminants Specialist
510 Desmond Dr. SE, Suite 102
Lacey, Washington 98503
jay_davis@fws.gov
(360) 753-9568

Signature Page

Approved by:

Date

Lane Maguire, Primary Author, Washington State University, Puyallup Research & Extension Center

Date

Jenifer McIntyre, Lead Entity, Washington State University, Puyallup Research & Extension Center

Date

Jay Davis, Participating Entity, U.S. Fish and Wildlife Service

Date

Shelly Fishel, Lab Project Manager, Analytical Resources, Inc.

Date

Marie Holt, Lab Project Manager, Spectra Laboratories

Date

Keunyea Song, Ecology Water Quality Program Project Manager

Date

Brandi Lubliner, Ecology Water Quality Program QA Coordinator

1.0 Table of Contents

List of Tables

List of Figures

List of Acronyms and Abbreviations

ANOVA	Analysis of Variance
ARI	Analytical Resources, Incorporated
BMP	Best Management Practice
COC	Chain of Custody
CWC	Clean Water Control
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
DQA	Data Quality Assessment
DQO	Data Quality Objective
EPA	Environmental Protection Agency
GC/MS	Gas Chromatography/Mass Spectrometry
GSI	Green Stormwater Infrastructure
HPF	Hours Post-Fertilization
LCS	Laboratory Control Samples
LOQ	Limit of Quantitation
MDL	Method Detection Limit
MF	Membrane Filtration
MS/MSD	Matrix Spike/Matrix Spike Duplicate
MS4s	Municipal Separate Storm Sewer System
MQO	Measurement Quality Objective
NOAA	National Oceanic and Atmospheric Administration
NPDES	National Pollutant Discharge Elimination System
NWFSC	Northwest Fisheries Science Center
PAH	Polycyclic Aromatic Hydrocarbon
PSM	Pre-Spawn Mortality
RL	Reporting Limit
RPD	Relative Percent Difference
RSD	Relative Standard Deviation
SWMMWW	Stormwater Management Manual for Western Washington
SAM	Stormwater Action Monitoring
TSS	Total Suspended Solids
USFWS	United State Fish and Wildlife Service
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
WSU-P	Washington State University – Puyallup
WWHM	Western Washington Hydrology Model

Executive Summary

The migration of coho salmon every fall from the ocean to the upper reaches of freshwater streams coincides with increasing rainfall in the Pacific Northwest. Much of this rainfall runs off of asphalt and other impervious surfaces found in urban areas, such as the Puget Sound Basin, and into the very streams where salmon spawn. Exposure to urban stormwater runoff, which contains a complex mixture of contaminants, can be acutely toxic to coho salmon, presently a species of concern under the U.S. Endangered Species Act. Increasing urbanization is expected to worsen the decline of local coho populations. Research has shown that Low Impact Development (LID) techniques, specifically bioretention cells, can mitigate the toxic impacts of urban stormwater runoff. Bioretention cells allow urban landscapes to act more as they would have prior to urbanization by increasing the capture, infiltration, and detention of urban stormwater runoff and filtering many of the pollutants that it contains. In some urban areas, feasibility, size constraints, and installation and maintenance costs may hinder the implementation of bioretention cells. Bioretention cell installations with a shallower soil media depth would decrease costs, expand options for use, and potentially reduce the export of pollutants (e.g., nutrients). The goal of this study is to explore the life expectancy of various depths of bioretention soils. To do so, stormwater runoff will be collected from a busy, urban road site and applied to experimental columns, containing five different depths of bioretention soil media. Runoff will be applied at an accelerated rate in order to simulate 10 water years in two calendar years. The chemical and biological effectiveness of the columns in treating urban stormwater runoff will be assessed using analytical chemistry and the health of two fish species: juvenile coho salmon and zebrafish embryos. The study outcomes are expected to help inform stormwater managers, National Pollutant Discharge Elimination System (NPDES) permit coordinators, and others involved in stormwater management.

3.0 Introduction and Background

3.1 Introduction and Problem Description

Each autumn, adult coho salmon, *Oncorhynchus kisutch*, travel from the ocean to the upper reaches of rivers, where they spawn. In the Pacific Northwest, this migration event, which has spanned six million years, coincides with increasing seasonal rainfall. In urbanized areas, such as the Puget Sound Basin, impervious surface area causes much of this rainfall to run off into the spawning habitats of coho salmon. Urban stormwater runoff contains a complex mixture of metals, polycyclic aromatic hydrocarbons (PAHs), and other chemicals toxic to many aquatic organisms.

Previous studies have shown that adult coho salmon are particularly sensitive to toxic urban runoff and experience a suite of symptoms, including disorientation, a loss of equilibrium, surface swimming and gaping, and eventually mortality, in response to exposure from such toxicants (McCarthy et al. 2008). In most cases, adult female coho returning to freshwater urban streams to spawn die with nearly 100% egg retention, a phenomenon referred to as coho prespawn mortality (PSM) (Scholz et al. 2011). Coho salmon serve as a sentinel species for the impacts of stormwater runoff, in part because of their high sensitivity to water quality, but also because they spend much of their lives in freshwater compared to other salmon species. Increasing urbanization and population growth are predicted to increase the loading of runoff pollution to waterways and exacerbate already high mortality rates for coho salmon, presently a species of concern under the U.S. Endangered Species Act and a keystone species of high ecological, economic, and cultural value (Feist et al. 2017). Life-history models constructed by researchers to estimate the impacts of increasing runoff pollution and PSM on coho salmon predict rapid declines in local coho populations affected by PSM (Spromberg and Scholz 2011).

Research by the National Oceanic and Atmospheric Administration (NOAA) Fisheries, the U.S. Fish and Wildlife Service (USFWS), and Washington State University-Puyallup (WSU-P), has shown that, in addition to adult coho, untreated urban highway stormwater runoff is acutely lethal to juvenile (McIntyre et al. 2015) and newly hatched coho, as well as the macroinvertebrate species that juvenile salmon rely on for prey (McIntyre et al. 2014). Runoff has also been shown to have sublethal effects on salmon, invertebrates, and zebrafish (*Danio rerio*), a small, highly studied freshwater fish that has been used extensively in human health research (McIntyre et al. 2014). Zebrafish are a model species used in human health and environmental toxicological studies particularly for their rapid development and optical clarity, making it relatively easy to determine morphological abnormalities. Sublethal effects observed in zebrafish and other aquatic organisms exposed to stormwater runoff include delays in hatching, developmental delays, negative impacts on lateral line development in larval zebrafish and salmon embryos, abnormal heart development in zebrafish embryos and larvae, alteration of blood hematological parameters, and impaired growth, all of which can have negative consequences for overall organismal fitness (Young et al. 2018; McIntyre et al. 2016, 2018).

Stormwater treatment with Green Stormwater Infrastructure (GSI) technologies, such as bioretention, is increasingly being used to filter stormwater runoff and infiltrate stormwater at the site. Previous studies have demonstrated the effectiveness of bioretention treatment systems in

treating urban runoff and preventing acutely lethal and sublethal effects to aquatic organisms (McIntyre et al. 2014, 2015; Spromberg et al. 2016).

Created by the Clean Water Act in 1972, the NPDES aims to remediate water quality pollution by regulating point sources of discharge into United States waterways. The Washington State Department of Ecology (Ecology) administers the NPDES permits regulating discharge from Municipal Separate Storm Sewer Systems (MS4s) owned or operated by the state's cities and counties, and the State Department of Transportation. The permits require local governments to manage polluted stormwater in order to mitigate the effects of pollution and contamination on downstream waters (Ecology, 2012a, 2012b, 2012c, 2014). GSI technology is a relatively inexpensive and highly effective method of managing stormwater volumes, providing stormwater runoff treatment on-site and allowing municipalities to meet NPDES permit requirements.

Stormwater Action Monitoring Program (SAM), Western Washington's collaborative monitoring program, aims to improve stormwater management through evaluating the efficacy of diverse innovative stormwater management practices, such as bioretention. USFWS and WSU-P are partnering to examine the effectiveness and longevity of toxicity prevention and water quality treatment of the bioretention soil media (BSM) over time at various infiltration depths, including those shallower than 18 inches, the depth currently required by Ecology in the 2012 Ecology Stormwater Management Manual for Western Washington as amended in 2014 (SWMMWW, 2014).

3.2 Regulatory Requirements

The data collected from this study is intended to provide more information on the lifespan of BSM and the critical depths to reduce toxicity to fish, and ultimately will inform Ecology's stormwater guidance, specifically bioretention design (BMP T7.30, "Bioretention Cells, Swales, and Planter Boxes," of Volume V of the 2012 SWMMWW as amended in 2014).

Urban jurisdictions are increasingly looking to incorporate GSI technology, such as bioretention, into new or existing infrastructure in order to comply with National Pollutant Discharge Elimination System (NPDES) regulations.

4.0 Project Overview

4.1 Study Goal

The goal of this study is to optimize bioretention design guidance by evaluating the longevity of standard Bioretention Soil Media (BSM) for preventing toxic impacts of stormwater runoff on aquatic organisms. Specifically, this project will answer the following two questions:

1. How long can the 60:40 (sand:compost) BSM prevent toxic effects to aquatic animals?
2. Can reduced BSM depths be sufficient to provide biologically significant improvements in water quality as standard 18" BSM?

4.2 Study Description and Objectives:

Through chemical and biological analyses, this study will examine the effectiveness and longevity of BSM of varying depths in treating runoff stormwater collected from a busy urban

road site. Incremental BSM depths (18”, 15”, 12”, 8”, and 6”) will be evaluated across 10 simulated water years in approximately two calendar years. Experimental columns, comprised of a 60:40 sand:compost mix by volume overlain with mulch and underlain by a gravel drainage layer, will be constructed to test the effectiveness of the varying bioretention media depths. Influent and effluent will be collected from the columns for analytical chemistry and biological toxicity testing, using zebrafish embryos and juvenile coho salmon. The objective of this study is to determine the minimum media depth that is successful in improving water quality and reducing the deleterious effects of toxic stormwater runoff on aquatic organisms.

4.3 Study Location

Stormwater runoff samples will be collected from a busy arterial road such as the on-ramp to State Route 520 in Seattle, WA with a downspout located in Seattle, WA at the NOAA Northwest Fisheries Science Center (NWFSC. Alternate urban road sites with comparable traffic densities may alternatively be used. The experimental columns that will be used to filter runoff collected from this site will be situated in an approximately 77 square foot shed at the WSU-P Research and Extension Center.

4.4 Tasks Required to Conduct Study

Table 1 summarizes the tasks that will be required to collect the data needed to support the project objectives.

Table 1. Tasks required to conduct study

Task	Objective	Deliverable
Prepare experimental columns	Construct experimental columns and analyze soil media components for chemical composition, leaching potential, and acute toxic potential	Report on chemistry and toxicology of bioretention soil media (BSM) components
Condition experimental columns	Flush columns with clean water[1] and characterize the chemistry of water passing through the BSM prior application of stormwater runoff	Report on chemistry of clean water effluent and WSU-Puyallup lab water
Test bioretention performance throughout accelerated aging	Analyze influent and effluent to assess chemical and biological effectiveness of columns	Five progress reports
Communication and outreach	Share findings of study with stormwater managers, NPDES permit coordinators, and others involved in stormwater management	Fact sheet, two presentations, and a final report

1 – Clean water refers to municipal water that is cleaned on-site at WSU-P thru reverse osmosis and re-constitution with salts.

4.6 Potential Constraints

Climatic conditions, availability of staff, equipment malfunction, and study funding sources are all possible conditions that may impact the project schedule, budget, or scope. If potential constraints do arise, they will be reflected in the project audits and reports (see Section 12.0 Audits) and any necessary corrective actions will be taken. Possible corrective actions are summarized in Section 10.0 Quality Control.

5.0 Organization and Schedule

5.1 Key Project Team Members: Roles and Responsibilities

Key personnel members for the project are shown below in Table 2.

Table 2. Key personnel

Key Team Members	Role	Responsibility
Jenifer McIntyre Washington State University Puyallup Research and Extension Center (206) 445-4650 jen.mcintyre@wsu.edu	Lead Entity	Oversees QAPP writing, experimental design, budget, timelines, and laboratory procedures. Reviews draft and final reports.
Jay Davis U.S. Fish and Wildlife Service (360) 753-9568 jay_davis@fws.gov	Participating Entity	Reviews QAPP, project deliverables, and final report. Serves as the contact person for all communications, notifications, and billings questions regarding IAA No. 1800154.
Keunyea Song State of Washington, Department of Ecology (360) 407-6158 keunyea.song@ecy.wa.gov	SAM Project Manager	Reviews the project scope and budget, tracks progress, reviews approves contract deliverables. Serves as the contact person for all communications, notifications, and billings questions regarding IAA No. 1800154.
Lane Maguire Washington State University Puyallup Research and Extension Center (816)-803-3462 lane.maguire@wsu.edu	QAPP Author	Analyzes and interprets data. Prepares draft and final reports.
Brandi Lubliner Washington State Department of Ecology (360) 407-7140 brandi.lubliner@ecy.wa.gov	Ecology QA Coordinator	Reviews the draft QAPP and approves the final QAPP.
Shelly Fishel Analytical Resources, Inc. (206) 659-6214	Laboratory Project Manager	Ensures samples are analyzed in accordance with the approved QAPP.
Marie Holt Spectra Laboratories (253) 272-4850	Laboratory Project Manager	Ensures samples are analyzed in accordance with the approved QAPP.

5.2 Project Schedule

Major project tasks and deadlines are summarized in Figure 1.

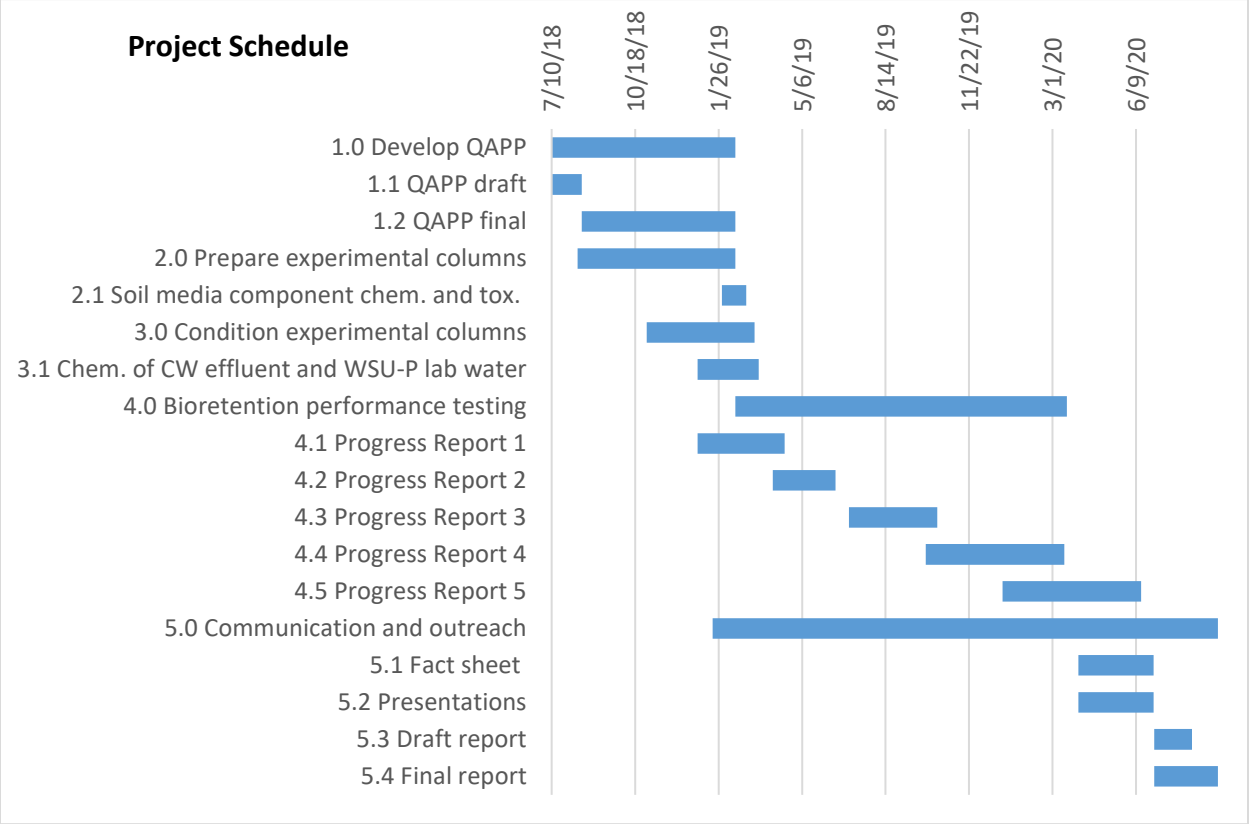


Figure 1. Organizational chart summarizing project milestones

5.3 Budget and Funding Sources

The funding source for the study is the SAM program, which is administered by Ecology. Payment requests will be sent to Ecology and will include a description of the work performed, the progress of the work, and related costs. The total cost of accomplishing the work will not exceed $396,076.00, including any indirect charges. The total budget may not be exceeded without an approved amendment from Ecology. The budget may be shifted between tasks with written approval from Ecology. The project budget is summarized in Table 3.

Table 3. Budget by study tasks and subtasks

Task	Subtask	Budget
1. Develop QAPP	Draft QAPP	$14,000
	Final QAPP	$15,505
2. Prepare experimental columns	Report on chemistry and toxicology of bioretention soil media components	$30,531
3. Condition experimental columns	Report on chemistry of clean water effluent and WSU-P lab water	$33,071
4. Bioretention performance throughout accelerated aging	Progress report 1	$52,476
	Progress report 2	$52,476
	Progress report 3	$52,476
	Progress report 4	$52,476
	Progress report 5	$52,476
5. Outreach and communication	Draft fact sheet explaining results for stormwater managers, NPDES permit coordinators, and other involved in stormwater management.	$5,584
	Two presentations to share findings with stormwater managers, including a presentation to the Stormwater Workgroup and one regional stormwater	$5,584
	Draft final report using SAM template	$19,419
	Final report	$10,000
Total Budget		$396,076

6.0 Quality Objectives

Data Quality Indicators (DQIs) are used to quantitatively and qualitatively describe how well the study data meets the project's objectives. The DQIs will be used to evaluate sources of variability in monitoring results and define tolerable levels of potential error. By helping to minimize error and improve the accuracy of the data, DQIs should increase confidence in the study data and help ensure that the data generated is scientifically and legally defensible (Lombard and Kirchmer 2004). DQIs for measurement data include precision, accuracy, representativeness, completeness, comparability, and measurement range. The DQIs provide the basis for measurement performance criteria (MPCs), which are the acceptance criteria for the DQIs that specify how good the data must be to meet the project objectives.

Table 5 summarizes DQIs for water samples and the QC sample or activity that is used to assess each objective. MPCs for water and sediment samples are summarized in Tables 6 and 7 and are defined as follows:

Precision

Precision is the degree of agreement among repeated measurements of the same characteristic (parameter) under the same or similar conditions. To ensure analytical precision, the relative percent difference (RPD) of laboratory duplicates and check standards can be calculated according to the equation:

$$\text{RPD} = \frac{\text{abs value } (x_1 - x_2)}{\frac{x_1 + x_2}{2}} \text{ x } 100\%$$

where x_1 is the original sample concentration and x_2 is the duplicate sample concentration. RPDs < 20% will be deemed acceptable for water samples. To assess the precision of water quality measurements from experimental columns of the same infiltration depth, the relative standard deviation (RSD) of the triplicate samples can be calculated using the following formula:

$$\text{RSD} = \frac{S}{\bar{x}} \times 100\%$$

where $\bar{x}$ is the mean of triplicate samples, and S is the standard deviation. Smaller RSD values signify more precise measurements.

Prior to the start of the experiment, triplicate samples of sand and compost will be assessed for chemical composition. Additionally, triplicate samples of the each of BSM components will be leached in laboratory water and the leachate collected for analytical chemistry and biological toxicity testing. In the zebrafish toxicity tests, 32 individual fish are used to replicate exposure per treatment. In the coho salmon toxicity tests, three replicates of 8-10 individuals will be used per treatment. These replicates will ensure an adequate level of precision, while minimizing the sacrifice of fish. Triplicate columns for each treatment depth will essentially serve as field duplicates for water sampling.

Accuracy and Bias

Accuracy and bias are used interchangeably. Accuracy is the extent of the agreement between an observed value (sample result) and the true value of the parameter being measured. Instrument calibration and quality control (QC) checks, i.e. field and laboratory blanks and matrix spikes, can be used to assess the accuracy and field and laboratory measurements. Experimental columns for testing bioretention soil media depths will be tested in triplicate to ensure the accuracy of sampling methods. Accuracy can be determined by calculating percent error, which should be less than 10% for all measurements. Percent error will be calculated according to the following equation:

$$\% \text{ error} = (\text{accepted value} - \text{experimental value})/\text{accepted value} \times 100\%$$

Accuracy can also be assessed using matrix spike (MS) and matrix spike duplicate (MSD) samples, in which a known concentration of the analyte of interest has been added. Percent recovery of the analyte of interest, using the equation below, will be used to calculate analytical accuracy.

$$\mathrm{R} = \frac{\mathrm{M}}{\mathrm{T}} \times 100\%$$

where M is the measured value and T is the true value.

Completeness

Completeness is a measure of the number of measurements judged valid, compared to the total number of planned measurements. Completeness should be 90% or better according to the equation:

$$\% \text{ complete} = \frac{(\#\text{of valid results}) * 100}{(\#\text{ of samples tested})}$$

Representativeness

Representativeness is a qualitative term that describes the extent to which a sampling design adequately reflects the environmental conditions of a site. The stormwater collection site represents a busy urban highway in the Puget Sound area. This source of stormwater has been used in prior studies and is known to produce toxicity in the fish being tested. The experimental columns will be situated in a shed on the WSU-Puyallup campus, where a thermal regime will be established so that the thermal conditions of the accelerated water year match that of the simulated calendar year. In this way, the environmental and microbial conditions of the experimental site will more closely reflect those of the actual environmental site in a typical calendar year.

For chemical and biological analyses, collecting stormwater runoff from 60 different storm events, each with varying conditions (varying intensities, duration, and antecedent dry period) over a two-year period will further ensure representativeness from this land use.

Comparability

Comparability is the extent to which data from one study can be compared directly to either past data from the current project or data from another study. The use of standardized sampling and analytical methods and units of reporting will help ensure comparability. Water chemistry values for collected stormwater runoff can be compared to previous research studies, for which there exists a record of expected ranges (McIntyre et al. 2014; Spromberg et al. 2016). To ensure comparability, sampling methods will be consistent with these previous studies and municipal stormwater permit requirements. Furthermore, water chemistry values for influent and effluent collected from bioretention columns will be compared with values from stormwater discharge data characterization studies by the Washington State Department of Ecology National Pollutant Discharge Elimination System (NPDES) Phase I Stormwater Permit and the Washington State Department of Transportation (WSDOT) NPDES Municipal Stormwater Permit (Ecology 2012a, 2014), as well as with values from the Internal Stormwater Best Management Practice (BMP) Database. The procedures used in this experiment will generally be comparable to McIntyre et al. 2014 for influent and bioretention treated effluent water chemistry and toxicity.

Sensitivity

Sensitivity is the ability of the method or instrument to detect the contaminant of concern and other target compounds at the level of interest. Sensitivity will be determined by the quality of the instruments/equipment used and by calibration methods.

Table 5. DQIs for study data

Data Quality Indicators	QC Sample and/or Activity Used to Assess Measurement Performance
Precision-Overall	Column replicates, biological replicates
Precision-Analytical	Lab duplicates, laboratory control spikes (LCS), matrix spike duplicates (MSD)
Accuracy/Bias	Field and laboratory blanks, matrix spikes, instrument calibration
Comparability	Sampling methods comparable to previous, similar research studies
Sensitivity	Instrument calibration, method detection limit (MDL), Reporting Limit (RL)/Limit of Quantitation (LOQ)
Data Completeness	Implement corrective actions for missing data
Representativeness	Collect samples that represent a range of conditions expected during storm flow

Table 6. Measurement performance criteria for water quality parameters

Analyte	Method	MDL	RL	Lab Replicates (RPD)	LCS (% R)	MS/MSD (% R)	Completeness (%)
Fecal coliform	SM 9222 D MF	N/A	N/A	≤20	N/A	N/A	90
Total suspended solids	SM 2540D	0.5 mg/L	0.5 mg/L	≤20	80-120	N/A	90
Dissolved organic carbon	SM 5310 B	0.08 mg/L	0.5 mg/L	≤20	77-119	N/A	90
Total Copper	EPA 200.8	0.2 µg/L	0.5 µg/L	≤20	85-115	70-130	90
Total Zinc	EPA 200.8	0.19 µg/L	0.5 µg/L	≤20	85-115	70-130	90
Total Cadmium	EPA 200.8	0.05 µg/L	0.2 µg/L	≤20	85-115	70-130	90
Total Lead	EPA 200.8	0.079 µg/L	0.1 µg/L	≤20	85-115	70-130	90
Total Arsenic	EPA 200.8	0.05 µg/L	0.2 µg/L	≤20	85-115	70-130	90
Total Nickel	EPA 200.8	0.2 µg/L	0.5 µg/L	≤20	85-115	70-130	90
Alkalinity	SM 2320 B	0.3 mg/L	1 mg/L	≤20	N/A	N/A	90
pH	SM 4500-H+ B	N/A	N/A	≤20	95-105	N/A	90
Ortho-phosphate	EPA 365.3	0.01 mg/L	0.01 mg/L	≤20	80-108	N/A	90
Nitrate + Nitrite	Systea Easy (1-Reagent)	0.003 mg/L	0.01 mg/L	≤20	77-112	N/A	90
Dissolved Copper	EPA 200.8	0.05 µg/L	0.1 µg/L	≤20	85-115	70-130	90
Dissolved Zinc	EPA 200.8	0.19 µg/L	0.5 µg/L	≤20	85-115	70-130	90

Analyte	Method	MDL	RL	Lab Replicates (RPD)	LCS (% R)	MS/MSD (% R)	Completeness (%)
Dissolved Cadmium	EPA 200.8	0.05 µg/L	0.1 µg/L	≤20	85-115	70-130	90
Dissolved Lead	EPA 200.8	0.079 µg/L	0.1 µg/L	≤20	85-115	70-130	90
Dissolved Arsenic	EPA 200.8	0.05 µg/L	0.2 µg/L	≤20	85-115	70-130	90
Dissolved Nickel	EPA 200.8	0.20 µg/L	0.5 µg/L	≤20	85-115	70-130	90
Dissolved Calcium	EPA 200.8	3.4	10	≤20	80-120	70-130	90
Dissolved Magnesium	EPA 200.8	1.9	6	≤20	80-120	70-130	90
Dissolved Sodium	EPA 200.8	27	81	≤20	80-120	70-130	90
PAHs	EPA 8270D-SIM	0.000460-0.00586 µg/L*	0.011 µg/L	30	30-160*	30-160*	90

*Compound specific

Water hardness will be calculated from the sum of dissolved calcium and magnesium concentrations, expressed in $CaCO_3$ equivalents.

Table 7. Measurement quality objectives for soil media parameters

Analyte	Method	MDL (mg/kg)	RL (mg/kg)	Lab Replicates (RPD)	LCS (% R)	MS/MSD (% R)	Completeness (%)
Total Copper	EPA 200.7	0.03	0.1	≤ 20	80-120	75-125	90
Total Zinc	EPA 200.7	0.2	0.6	≤ 20	80-120	75-125	90
Total Cadmium	EPA 200.7	0.03	0.1	≤ 20	80-120	75-125	90
Total Lead	EPA 200.7	0.03	0.1	≤ 20	80-120	75-125	90
Total Arsenic	EPA 200.7	0.4	2.5	≤ 20	80-120	75-125	90
Total Nickel	EPA 200.7	0.5	1.5	≤ 20	80-120	75-125	90
Ammonia	SM 4500 NH3 D	4	4	≤ 20	87-114	N/A	90
Total Nitrogen	Summation**	10,000	10,000	≤ 20	85-115	N/A	90
Nitrate-Nitrite	Easy (1-Reagent)	0.1	0.1	≤ 20	77-112	N/A	90
Total Phosphorous	SM 4500 P E	0.1	0.1	≤ 20	86-110	N/A	90
Organic Matter	ASTM D2974-13	0.5%	0.5%	≤ 20	N/A	N/A	90
Total Organic Carbon	EPA 9060	0.1%	0.1%	≤ 20	85-115	N/A	90
% Solids	SM 2540 G	0.5%	0.5%	N/A	N/A	N/A	90
PAHs	EPA 8270D-SIM	0.401-3.01 µg/kg*	5-10 µg/kg*	30	30-160*	30-160*	90

*Compound specific

**Summation = TKN + Nitrate + Nitrite

7.0 Experimental Design

7.1 Study Design Overview

Highway stormwater runoff will be collected from SR 520 and transported to WSU-P in a stainless steel tote. Experimental bioretention columns (6" diameter) used to treat runoff will be situated in a dedicated temperature-controlled outbuilding at the WSU-P Research & Extension Center. The experimental columns will contain a 60:40 sand:compost mixture, the components of which will be analyzed for chemical composition and leaching potential. Prior to the application of stormwater, experimental columns will be conditioned and 'flushed' with WSU-P lab water.

Infiltration rate (K_{sat}) will be calculated for each treatment, targeting a rate of >50 cm/hour (>20 inches/hour). The target rate of application per column is 12.7 cm/hour (2.3 L/hour). Each column will receive a volume of runoff equivalent to 20:1 contributing:treatment area. The biological effectiveness of the experimental columns will be analyzed using the health of two fish species at the WSU-P Aquatic Toxicology Laboratory. Samples of untreated and treated stormwater will be sent to two Ecology accredited laboratories for analytical analyses: Spectra laboratories (metals + conventional parameters) in Tacoma, WA and Analytical Resources, Inc. (PAHs) in Tukwila, WA. Alkalinity will be measured and hardness will be calculated at the WSU-P Aquatic Toxicology Laboratory. Please refer to Figure 2 for a summary of the study design.

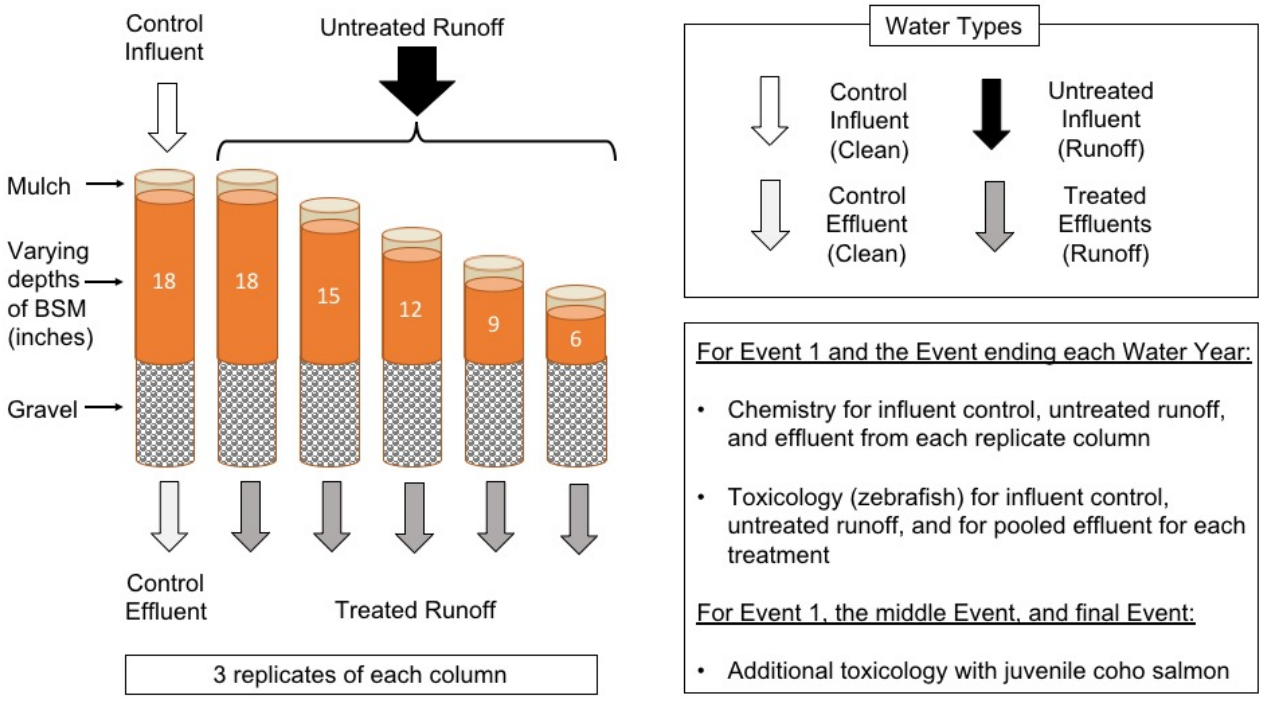


Figure 2. Overview of bioretention treatments, water types, and sampling parameters for Events comprising the 10 water years of runoff treatment.

7.2 Test or Sampling-Site(s) Selection Process

Stormwater runoff samples will be collected from downspouts from an on-ramp to SR 520 in Seattle, WA at the NOAA's Northwest Fisheries Science Center (NWFSC). Highway runoff serves as a concentrated example of urban stormwater runoff, as is illustrated in Table 8, and is highly correlated with pre-spawning mortality events for coho spawners in the Puget Sound region (Feist et al. 2017). Furthermore, the research team has previously sampled the site and a record of the expected range of water chemistry values exists (McIntyre et al. 2014). The approximate site location is shown in Figure 3. This location is subject to change if alternate urban road sites closer to WSU-P become available. Runoff will be collected in a 350-gallon stainless steel tote and transported to the WSU-P Aquatic Toxicology Laboratory within 24-hours of collection. A fiberglass window screen will filter out large particulates in the stormwater during collection.

Table 8. Comparison of Phase I permittee data to WSDOT BMP effectiveness monitoring data (analytical concentration medians) (Ecology 2015; WSDOT 2017).

Analyte	WSDOT: Median Value	Phase I: Median Value
TSS (mg/L)	57	31
Hardness as CaCO3 (mg/L)	24	25.2
Orthophosphate (µg/L)	14.1	21.6
Total Phosphorous (µg/L)	109.5	110
Nitrite-Nitrate (µg/L)	336.3	245
Dissolved Cu (µg/L)	8.025	3.9
Total Cu (µg/L)	26.2	10.4
Total Zn (µg/L)	11.4	70.6
Dissolved Zn (µg/L)	31.2	26.9

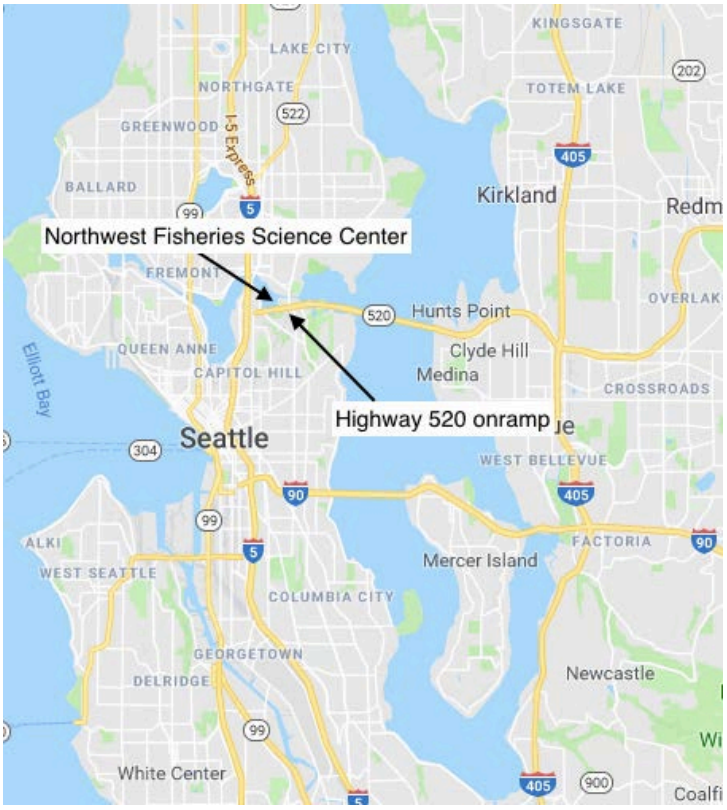


Figure 3. Map of the Seattle metro area indicating the location of the NOAA NWFSC and approximate location of the downspout that drains the SR 520 on-ramp.

7.3 BMP System Design

Each experimental column (diameter = 6 inches, height up to 35 inches) is sized to treat runoff from a contributing area equal to approximately 0.36 m^2 (20:1 contributing:treatment). To accelerate the treatment schedule, the equivalent of 10 water years of runoff will be applied to each column across the study period (<2 calendar years). Runoff will be collected from approximately 60 storm events across the study period to constitute the 10 water years. Each collection will be designated a separate 'event', with approximately six events completing one water year (WY). Assuming 36" of annual rainfall during the calendar year, each column will receive approximately 6" of runoff from the contributing area, or 55.6 L, per event under the accelerated schedule. Therefore, across 10 WY, each column will receive 556 L.

7.4 Column Construction

Experimental columns will be constructed at the WSU-P Aquatic Toxicology Laboratory using 6"-diameter PVC pipe. The columns will contain varying depths of bioretention soil media (18," 15," 12," 9," and 6") in triplicate. Three additional columns will contain 18" of soil media (the depth required by Ecology's SWMMWW, 2012 and draft 2019) for use as clean water controls (CWCs) throughout the course of the study (10 WY) to test the leaching potential of the soil media over time. The bioretention media will be composed of a 60:40 sand:compost mixture, by volume. The soil mixture in each column will be underlain by a 12" gravel drainage layer and overlain by a 2" mulch layer. Above the mulch layer will be a 3" ponding zone which will be used for K_{sat} testing. Soil bioretention components will be sourced and acquired locally. A stainless steel screen will be secured at the base of each column to contain the treatment media.

A PVC reducer, elbow slip, and valve slip will then be attached to the bottom of each column. Untreated stormwater will be pumped from the stainless steel tote used to the transport the stormwater from the sampling site using a peristaltic pump and silicone tubing. Stainless steel tanks (approximately 20-gallon) will be placed below the valve slip of each column to capture treated effluent.

The bioretention soil media will be hand compacted with a 5.5”-diameter tamper to achieve the desired infiltration rate (approximately 20 inches/hour). Infiltration will be initially measured in a test column to develop a compaction method that will yield the desired infiltration rate. Infiltration rate will be measured by closing the valve near the base of the columns and filling the column to the rim with water, from the top of the column. The valve will then be opened, allowing water to drain from the columns. The time required for the water to drain from the rim of the column to the soil surface, as well as the distance between the soil surface and the rim of the column, will be recorded.

7.5 Type of Data Being Collected

Please refer to Figure 2 and Table 9 for an overview of sample and data collection for runoff events treated in the experimental bioretention columns. For each storm event (1-60), we will record pH, conductivity, and turbidity of influent waters (runoff and control water) and effluent waters (filtered through bioretention). For Event 1 and the events ending each WY, influent (n = 1 each) and effluent waters (n = 3 each; 1/column) will be sub-sampled for chemistry analysis (Table 6) and toxicology testing (Table 9).

Table 9. Events for which detailed analytical chemistry and toxicity testing will be conducted.

End of Water Year	Event Number (Approximate)	Number of BSM Depths Tested	Analytical Chemistry	Zebrafish Toxicity Testing	Salmon Toxicity Testing	K_{sat}
-	1	5	Yes	Yes	Yes	
1	6	5	Yes	Yes		Yes
2	12	5	Yes	Yes		Yes
3	18	3	Yes	Yes		Yes
4	24	3	Yes	Yes		Yes
5	30	3	Yes	Yes	Yes	Yes
6	36	3	Yes	Yes		Yes
7	42	3	Yes	Yes		Yes
8	48	3	Yes	Yes		Yes
9	54	3	Yes	Yes		Yes
10	60	3	Yes	Yes	Yes	Yes

BSM = Bioretention Soil Medium

7.6 Baseline Monitoring

Prior to the start of the experiment, triplicate samples of sand and compost will be analyzed for chemical composition, including metals (Cu, Zn, Cd, Pb, As, Ni, Mg, Ca, Na), nutrients (ammonia, total nitrogen, nitrate-nitrite, total phosphorous), organic matter, total organic carbon, total solids, and PAHs. Triplicates of each of the bioretention soil media components (sand,

compost, gravel, and mulch) will then be leached according to EPA method 1312 (EPA 1994). Leached water from each replicate will be assessed for PAHs at Analytical Resources, Inc. (ARI) and for metals (total and dissolved), fecal coliform, total suspended solids, dissolved organic carbon, pH, ortho-phosphate, and nitrite+nitrate at Spectra Laboratories. Alkalinity will be tested at the WSU-P Aquatic Toxicology Laboratory. The remaining leachate will be pooled and assessed for acute toxicity using the zebrafish embryo model at WSU-P.

7.7 Flow Monitoring

To deliver runoff collected from a storm Event, clean water or runoff will be drawn from a common sump using a peristaltic pump. Teflon-lined tubing will be run through multi-channel heads on the peristaltic pump that will deliver water to each column at a rate of 38.33 mL/min for 24 hours. We do not expect ponding to occur at this rate of delivery.

7.8 Precipitation Monitoring

To simulate 10 WY within two calendar years, the research team will aim to collect stormwater from 30 storm events per year (approximately 3 events/month for 10 months of the year). The research team will monitor regional meteorological forecasting to help determine when a storm event is imminent and stormwater can be collected. If forecasts from one or more of the following sources indicate an approaching storm, the field crew will then prepare for sample collection. Any event producing the minimum volume of runoff required for testing will be used (834 L through WY2 and 500 L for the remaining events). Precipitation during the period of runoff collection will be documented from the nearest weather station.

7.9 Column Conditioning

Prior to the application of stormwater runoff, three pore volumes of WSU-P water will be used to condition the experimental columns. One pore volume is approximately 2.8 L. Influent and effluent waters from a fourth pore volume of water will be sampled to analyze the chemical composition (conventional parameters + metals) of water passing through the columns. Samples will not be analyzed for PAHs during column conditioning. Saturated hydraulic conductivity (K_{sat}) will be calculated for each treatment and the values for triplicate samples averaged.

7.10 Bioretention Performance Through Accelerated Aging

The project will simulate 10 water years across approximately two calendar years. To account for the discrepancy in thermal conditions between the simulated water year and the actual calendar year, a thermal regime will be established to more closely align the climatic and microbial conditions of the simulated water year with those of the natural environment in a typical calendar year. Temperature will be regulated in the outbuilding where the columns will be located using a heating and/or cooling unit to approximate temperatures expected from in-ground installations of bioretention.

Volume of clean water or runoff to be applied per column per Event was calculated as follows:

Contributing area (m^2) = Column area (0.018 m^2) x contributing:treatment (20:1) = 0.3646 m^2

Annual precipitation (m) = 0.9144 m

Accelerated annual precipitation (m) = Annual precipitation (0.9144 m) x 5 = 4.572 m

Expected Events per calendar year = 30

Precipitation per accelerated Event (m) = Accelerated annual precipitation (4.572 m) / Events per year (30) = 0.1524 m

Volume per accelerated Event (m^3) = Precipitation per accelerated Event (0.1524 m) x Contributing area (0.3646 m^2) = 0.05572 m^3

Volume per accelerated Event (L) = 0.05572 m^3 x 1000 L/m^3 = 55.7 L

The treatment volume of 55.7 L per replicate will be applied over 24 h in order to avoid an unrealistically high application rate. This is equal to an application rate of 38.6 mL/min (12.7 cm/hour). This rate is within the infiltration capacity of bioretention under the WWHM (0.5-30.5 cm/hour), and is just twice the rate of a 1" rain event on the 20:1 area over an 8 h storm.

After passing through each experimental column, the treated stormwater effluent will be collected into stainless steel sumps. Effluent from the triplicates will be analyzed separately for chemistry but will be pooled for toxicology testing. For testing using juvenile coho salmon, 30 L of treated runoff will be collected from each column and pooled across replicates of each treatment. For zebrafish toxicity testing, 50 mL of treated stormwater runoff will be collected from each treatment replicate, composited in amber glass jars (250 mL), and frozen (-20 ℃) until zebrafish testing. Hydraulic conductivity (K_{sat}) will be calculated once during each water year and again at the end of the experiment.

At the conclusion of WY2, the number of BMS depths being tested will be reduced to three for the remainder of the experiment. Therefore, the initial 8 treatments (influent runoff, effluent runoff for depths 18", 15", 12", 9", 6", influent clean control water, and effluent control for 18"), will be reduced to 6 treatments following WY2. The BSM that remain in the study will be the best performers in terms of preventing acute toxicity while still performing well in terms of chemistry and hydraulic conductivity. If all depths perform equally well at the end of WY2, the three BSM depths continuing in the study will be 18", 12", and 6". WSU-P and USFWS will organize a discussion meeting with Ecology to determine what three depth treatments will move forward.

7.11 Toxicity Testing

Zebrafish Embryos

To evaluate the biological effectiveness of the experimental columns, zebrafish (*Danio rerio*) embryos will be used to evaluate any toxicity associated with bioretention soil media components (Task 2.2) and aging bioretention columns treating stormwater runoff and clean water (Tasks 4.0-4.5) over 18 months of accelerated applications.

Sublethal changes in morphometric endpoints, including hatch time, swim bladder inflation, and eye development, will be used to assess toxicity. Methods will generally follow previously published methods for urban runoff toxicity to zebrafish embryos (McIntyre et al. 2014; protocol

attached). 32 replicates (embryos) will be needed. Zebrafish rearing medium is synthetic freshwater made from 1 g of Instant Ocean Sea Salt dissolved in 1 L of RO water.

For each treatment depth, water toxicity will be assessed using 32 individual embryos (2-4 hours post-fertilization; hpf) placed in individual wells of a 96-well glass-lined microplate. Using a glass pipette, 250 μL of treatment or control water will be added to each well. The well plates will be covered and placed in an incubator (set at 28.5 ℃) in a randomized position. At 24 hpf, treatment water will be replaced and notes made of any obvious developmental delays for each embryo. Dead or severely deformed embryos will be removed at this time. Static renewal of the well plate solution reduces the possibility of DO depletion and negative effects from metabolic waste buildup. It also reduces the possibility of a loss of toxicants through volatilization and/or adsorption to well plates (EPA 2002). At 48 hpf, the hatch rate and survival count of embryos will be assessed.

At test termination of 48 or 96 hours, zebrafish embryos will be dechorionated (if unhatched) and anesthetized in tricaine methane sulfonate (250 µg/L MS-222). Embryos will be mounted in 3% methylcellulose and imaged with a digital camera mounted on a Nikon SMZ800 stereomicroscope. Images will be analyzed using the open-source software image J (http://rsbweb.nih.gov/ij/) to assess sublethal morphometric endpoints. Detailed methods for zebrafish toxicity testing and image analysis can found in Appendices A and B.

Juvenile Coho Salmon

To further evaluate the biological effectiveness of the experimental columns, juvenile coho salmon (*Oncorhynchus kisutch*) will be exposed to stormwater runoff influent and bioretention treated effluent at the beginning of the study (Event 1), the middle of the study (Event ending WY5) and the end of the study (Event ending WY10). Survival will be the endpoint used to assess toxicity based on the acute mortality documented for juvenile coho experimentally exposed to urban road runoff and prevented by bioretention treatment (McIntyre et al. 2015). Triplicate 35-L glass aquaria per treatment (Figure 2) will be filled with influent or effluent waters and maintained at 13 ℃ in water baths. An airstone will be placed in each aquarium to maintain dissolved oxygen levels at ≥ 6 mg/L. Water quality parameters (temperature, dissolved oxygen, pH, conductivity, turbidity) will be recorded for each aquarium. To begin the exposure, juvenile coho will be collected from the rearing tank and, per U.S. Environmental Protection Agency guidelines for acute toxicity testing (EPA 2002), 8-10 fish distributed into each aquarium. Water temperature, pH, dissolved oxygen (DO), and conductivity will be measured daily for each replicate.

Juvenile coho will be monitored daily for moribund or dead individuals, which will be recorded for length and condition. At test termination (96 hours), water quality parameters and any unexpected behavior of survivors (e.g. rising to the surface of the water, increased or decreased activity, change in coloration, loss of equilibrium) will be recorded. Surviving juvenile coho will be euthanized in MS-222 (500 mg/L), and total length and weight recorded. A detailed protocol for coho salmon toxicity testing can be found in Appendix C.

8.0 Sampling & Monitoring Procedures

8.1 Containers, Preservation Methods, Holding Times

Sampling method requirements, including requirements for containers, sample size, preservation, and holding times, for water and soil media samples are summarized in Tables 10 and 11.

Table 10. Water sampling protocol and equipment

Matrix	Analyte	Sample Containers	Preservative	Holding Time
water	Fecal coliform	Sterile specimen cup	Cool 4℃	6 hours
water	Total suspended solids	1 Liter Poly	Cool 4 ℃	7 days
water	Dissolved organic carbon		Cool 4 ℃	Filter ASAP
water	Chloride		None required	28 days
water	Alkalinity		Cool 4 ℃	14 days
water	Ortho-phosphate		Cool 4 ℃	48 hours
water	pH		None required	ASAP
water	Nitrate + Nitrite		Cool 4 ℃	48 hours
water	Total Metals	250 mL HDPE	HNO_3	6 months
water	Dissolved Metals	250 mL	HNO3, 6 degrees C	6 months
water	PAHs	2 each 500 mL Amber glass	Cool 0-6 ℃; 10% dichloromethylene	7 Days Ext/40 days Extracted
water	*D. rerio* acute toxicity	250 mL Amber glass (per treatment)	Freeze in field, store at -20 ℃	6 months
water	Juvenile coho toxicity	450 L Glass carboy (per event)	Cool 13 ℃	<24 h

Table 11. Sediment media sampling protocol and equipment

Matrix	Analyte	Container Type and Size	Preservative	Holding Time
sediment	Total Metals (Cu, Cd, Pb, As, Ni)	1-Gallon Zip Lock bag	None required	6 months
sediment	Ammonia		None required	7 days
sediment	Total Nitrogen		None required	7 days
sediment	Nitrate-Nitrite		None required	7 days
sediment	Total Phosphorous		None required	28 days
sediment	Organic Matter		None required	28 days
sediment	Total Organic Carbon		None required	28 days
sediment	Total Solids		None required	10 days
sediment	PAHs	≥30g in Glass WM, Clear 8 oz.	Cool <6℃	14 days

8.3 Equipment Decontamination

Sumps collecting effluent waters will be decontaminated Events by washing with the following:

- Liquinox
- Municipal water x 3
- Reverse osmosis water x 3

After washing, sumps will covered lightly with aluminum foil and left to sit for a minimum of 24 hours prior to re-use.

8.4 Sample Identification

All water sample containers will be labeled with indelible ink:

- Experimental column identification number
- Date of sample collection (year/month/day: yyyy/mm/dd)
- Time of sample collection (international format [24 hour])
- Field personnel initials

Bioretention soil media component samples will be stored in soil bags that will be provided by the analytical laboratories. Soil bags will be labeled with the following:

- Soil media component type
- Date of sample collection (year/month/day: yyyy/mm/dd)
- Time of sample collection (international format [24 hour])
- Field personnel initials

8.5 Chain of Custody

Chain of custody (COC) forms will be provided by Spectra laboratories and Analytical Resources, Inc. Chain of Custody forms will be maintained for each batch of samples sent to the laboratory and will include the following information: sample ID, date and time sampled, matrix, number of containers, parameters analyzed, number of coolers, cooler temperatures, and the names and signatures of the persons relinquishing and receiving custody of the samples. Copies of the COC forms from the two laboratories can be found in Appendices E and F.

8.6 Field Log Requirements

For each Event tested using the experimental columns located at WSU-P, the following will be recorded on data sheets:

- Date/time start/end
- Climatic conditions (temperature high/low and humidity high/low) in the outbuilding
- Any unusual conditions within the experimental columns (i.e. change in color or odor)
- Water year number
- Event number
- Volume of runoff applied to each column
- Number and volume of samples collected

- Saturated hydraulic conductivity (K_{sat}) for one Event per water year

8.7 Sample Handling, Delivery, and Processing

Stormwater runoff from the urban field site will be collected in a stainless steel tote and transported to the laboratory within 24-hours of collection. Effluent from the experimental columns located in the greenhouse will be collected in stainless steel sumps and placed on ice to preserve the chemical integrity prior to testing. Teflon tubing and a peristaltic pump will be used to subsample stormwater from the stainless steel tote and stainless steel sumps in to aliquots for chemical and biological analysis. Waters will be circulated in the respective containers to prevent settling of particulates.

Spectra and ARI laboratories will provide containers in which to collect samples for chemical analysis. Samples will be transported to the analytical labs within 24 hours in coolers filled with ice to keep water quality samples below 6 ℃.

9.0 Measurement Procedures

9.1 Procedures for Collecting Field Measurements

Saturated hydraulic conductivity will be measured for each treatment column using the following procedure:

- The distance in cm between the rim of the column and the surface of the soil media will be recorded. The initial soil media level will be marked on the outside of the column.
- The valve at the base of the column will be closed and the column filled to the rim with water.
- The valve will be opened and water allowed to drain from the columns.
- The time required for the water to drain from the rim of the column to the initial level of the soil surface will be recorded.
- Values per column will be averaged for each treatment.

9.2 Laboratory Procedures

See Tables 6 and 7 in Section 6.0 Quality Objectives for measurement procedures for all analytical testing.

9.3 Lab(s) Accredited for Methods

Both Spectra Laboratories and Analytical Resources, Inc. are accredited by the Washington State Department of Ecology. Sediment and water samples will be sent to Analytical Resources, Inc. for analysis of PAHs. Water samples will be sent to Spectra Laboratories for analysis of total (Cu, Zn, Cd, Pb, As, Ni) and dissolved metals (Cu, Zn, Cd, Pb, As, Ni, Ca, Mg, Na), fecal coliform, total suspended solids, dissolved organic carbon, alkalinity, pH, ortho-phosphate, and nitrite+nitrate. Sediment samples will be sent to Spectra Laboratories for analysis of total metals, nutrients (ammonia, total nitrogen, nitrate-nitrite, total phosphorous), organic matter, total organic carbon, and total solids.

10.0 Quality Control

10.1 Field QC Required

Replicates

There will be one replicate sample of each column effluent (3 columns per control and each BSM depth) and each influent water type (control, runoff) for chemical analysis. Influent samples of runoff and control water will not be replicated for chemical analysis in order to limit costs as per the statement of work. Replicating influent water samples would require an additional 22 (duplicate) or 44 (triplicate) samples be analyzed across the study (11 sampled Events; Table 9). Lack of replication for influent samples is acceptable because the emphasis of the project is differences between the effluent samples (control column, BSM depths) over time rather than differences between influent and effluent. For biological analysis, replicates will be 32 individual zebrafish and 3 replicates of 8-10 juvenile coho salmon. These replicates will provide an estimation of the precision of the project's results.

Field Blanks

During one of the project's 60 Events, the laboratory will analyze one field blank for each water parameter. Field blanks will be drawn from a stainless steel tote filled with municipal water in the field. Due to the quantity of water transported by the totes, it is not feasible to use distilled water. Results of the field blank QC method will help ensure that the stainless steel tote and peristaltic tubing is not contributing significant residual contamination from previous runoff applications.

10.2 Laboratory QC Required

Analytical QC procedures provide an indication of the performance of the analytical system. The following QC procedures will be implemented by the Ecology-certified laboratories for each Event sampled.

Check Standards

Also known as laboratory control samples (LCS) or spiked blanks, check standards are samples of a known concentration that are prepared independently of the calibration standards and are used to check precision and levels of bias. Check standards will be analyzed for each parameter with every batch of samples sent to the laboratory. Raw values and percent recovery (see Section 6.0 Quality Objectives for formula) will be included in scheduled progress reports.

Analytical Duplicate Split Samples

Laboratory split sample duplicates will be used to measure precision. Analytical duplicate split samples will represent approximately five percent of the total project samples sent to the laboratory. Raw values and relative percent difference (see Section 6.0 Quality Objectives for formula) will be included in scheduled progress reports.

Matrix Spikes (MS) / Matrix Spike Duplicates (MSD)

Matrix spikes will be analyzed by the laboratory and will be used to indicate bias due to interference from components of the sample matrix. Percent recoveries on MS samples will be compared to percent recoveries of LCS samples. MS and MDS samples will be compared to assess precision. MS/MSD will be analyzed for each applicable parameter with every batch of samples sent to the laboratory. Raw values and percent recovery (see Section 6.0 Quality Objectives for formula) will be included in scheduled progress reports.

Laboratory Blanks

Laboratory blanks, containing effectively none of the analyte of interest, will be prepared and analyzed for each parameter with every batch to ensure that laboratory contamination is not an issue. Results will be compared to established acceptance limits.

10.3 Corrective Action

Periodic audits (see Section 12.0 Audits) will attempt to highlight any gaps or anomalies in information. If possible corrective actions, which may include recalibration of measurement systems, reanalysis of samples (within holding time requirements), collection of additional samples (if outside of holding time requirements), retrieval of missing information, and modification of sampling and analytical procedures, will be implemented.

10.4 Equipment Calibration and Maintenance

Equipment will be maintained and calibrated according to manufacturer and/or equipment manuals. The following table provides a guide for calibration of standard equipment used throughout the project.

Equipment	**Check Interval**	**Parameters to Check**
Balances	Weekly	Zero point, accuracy
Thermometers (digital)	6 months	Check accuracy against reference thermometer
Timers	Yearly	Accuracy
Masterflex peristaltic pumps	Yearly	Flow precision
YSI MultiLab Meter	As used	Zero point, cell constant

11.0 Data Management Plan Procedures

11.1 Data Recording & Reporting Requirements

All observational data (e.g. condition of the columns), basic water quality data for interim WYs, and toxicity testing notes (e.g. juvenile coho water quality monitoring and zebrafish morphometric analyses) will be recorded in a field logbook. The results of all chemical and biological effectiveness testing will be stored in an Excel spreadsheet. All data entered into the spreadsheet will be verified and validated to ensure that DQIs are met.

11.3 Laboratory Data Package Requirements

ARI and Spectra laboratories will deliver a laboratory data package as part of the analytical testing that includes:

- A detailed case narrative that discusses potential problems with the analyses
- Corrective actions to be taken, changes to the referenced analytical methods
- QC results
- A list that defines each qualifier

11.4 Procedures for Missing Data

Missing or unqualified data (due to contamination or laboratory error) should be identified during periodic audits (see Section 12.0 Audits). If missing data is identified, it will be reported with the results in the project's reports. If missing data cannot be retrieved or restored, additional samples may need to be taken and analyzed.

11.5 Data Upload Procedures

The complied summary data and raw data files will be sent to SAM project manager at the conclusion of each WY and at the end of the project. At the end of the study, data detailing bioretention cell effectiveness and longevity will be uploaded to the International BMP Database and the reference ID included with the publication of the final report.

12.0 Audits and Reports

Audits and reports will be utilized to ensure that the project plan is being correctly implemented and that the data is of sufficient quality to meet project objectives. If the QC results indicate problems with data during the course of the project, audits and reports will help ensure that corrective actions are implemented. Audits will be conducted soon after work has commenced, so that corrective actions can be implemented early in the project.

12.1 Audits

Audits will be periodically performed to ensure conformance to the QA project plan and to correct for any problems with the project's water quality, toxicology, and soil media data. Audits for water chemistry data will occur after no more than one or two weeks after analytical results for event one and events ending each water year are received from the laboratory. Audits for soil media, soil media leachate, and column conditioning data will similarly occur within one or two weeks of receiving results from the laboratory. Qualitative audits will verify that field staff is following sample collection procedures, equipment and instruments are being maintained and/or calibrated per the manufacturers' requirements, and data management procedures are followed. Quantitative audits will specifically attempt to highlight any gaps or inconsistencies in information. Analytical data will be compared to DQIs to ensure that MPCs (as defined in Section 6.0 Quality Objectives) are being met. If problems with the data are observed during the course of an audit, the QAPP author will be responsible for identifying the issue and, if possible, implementing corrective actions. Corrective actions may include recalibration of measurement systems, reanalysis of samples (within holding time requirements), collection of additional samples (if outside of holding time requirements), assessment of unqualified data, and modification of sampling and analytical procedures. A record will be kept of any detected issues and corrective actions taken.

12.3 Reports

Reports will be produced and distributed throughout the course of the project to present data results, interpretation of data (if possible), information on project status, and results of QC audits. Reports will be forwarded to Washington Department of Ecology, the USFWS, and pertinent contributing parties at WSU-P. Following the commencement of bioretention performance testing, progress reports will include all raw data (from in-house analytical chemistry and toxicity testing), laboratory analytical reports, and chain of custody documentation as appendices. The reports will be submitted in electronic form (PDF for forms or written reports and Excel for raw data). Specific deliverables and due dates are summarized in Table 12.

Table 12. Deliverables and due dates

Deliverable	Target Date
2.1 Report on chemistry and toxicology of leachate from bioretention soil media components	April 31, 2019
3.1 Report on chemistry of clean water effluent and WSU-Puyallup lab water	May 1, 2019
4.1 Progress Report 1, including status of the contract tasks and decisions related to the tasks made during calls, team meetings, coordination with the technical advisory committee (TAC), and communications with Ecology.	June 30, 2019
4.2 Progress Report 2	August 15, 2019
4.3 Progress Report 3	December 15, 2019
4.4 Progress Report 4	May 15, 2020
4.5 Progress Report 5	September 15, 2020

13.0 Data Verification and Usability Assessment

The data verification and usability assessment defines the process that the project will employ to evaluate the quality of the data and the usability of the data for meeting the project objectives.

13.1 Data Verification

Before data quality can be assessed, data will be examined for errors or omissions and compliance with QC acceptance criteria within one to two weeks of receiving the data. Laboratory water and soil quality results will be reviewed by the QAPP author to ensure that methods and protocols, as outlined in this QAPP, were followed correctly. Unacceptable departures from the QAPP will be noted. The QA Coordinator will verify that all data specified in the Section 7.0, Experimental Design, was obtained and that data entries are consistent, correct, complete, and properly recorded. Finally, the QAPP author will ensure that the laboratories (Spectra and ARI) provide Quality Assurance/Quality Control (QA/QC) information, and that established criteria and MPCs for QC results are met (see Section 6.0 Quality Objectives). Any deviations in sampling design, collection procedures, sample handling, and analytical procedures will be evaluated for potential effects on the validity of the data. Specific measures evaluated during verification include:

- Holding times
- Reporting limits
- Accuracy (by evaluating LCS recovery and matrix spikes recoveries)
- Precision (by evaluating field and laboratory duplicate results)
- Blank contamination (by evaluating laboratory and field generated blanks)

Guidelines will be applied when evaluating data that does not meet proposed MPCs (see Section 6.0 Quality Objectives) for QC results. Evaluated data that does not meet MPCs during verification will be flagged and the appropriate actions, as described below, taken:

- Data from samples that exceed the holding times by more than 24 hours will be rejected. Data that exceeds the holding times by less than 24 hours will be qualified.
- Data that falls below proposed reporting limits (as summarized in Tables 3 and 4) will need to be reanalyzed by the laboratory, time permitting.
- Duplicate results that exceed the project MPCs by more than twice the objective will be rejected. Duplicates results that exceed project MPCs by less than twice the objective will be qualified.
- Control standard results that exceed the project MPCs by more than twice the objective will be rejected. Results that exceed project MPCs by less than twice the objective will be qualified.
- Field and laboratory blank values more than twice that of the blank will be rejected. Field blank values within twice that of the blank will be qualified.
- Matrix spike RPD and %R values outside the control limits indicate uncertainty in the measured results and will be qualified.
- The QA coordinator will communicate directly with the laboratories and/or field staff about future corrective actions.

Data, as described above, that does not meet MPCs may need a data validation qualifier to give an indication of potential bias. The qualifiers presented in Table 13 are consistent with EPA QA/G-8 (EPA 2002b).

Table 13. Data validation qualifiers

Data Validation Qualifier	Definition
U	Analyte was not detected above the sample quantitation limit.
UJ	Analyte was not detected above the sample quantitation limit. However, the quantitation limit is approximate and many or may not represent that actual quantitation limit needed to accurately measure the analyte. The associated value is therefore an estimate and may be inaccurate.
J	The analyte was positively identified. The numerical value is an estimate of concentration of the analyte in the sample.

Data Validation Qualifier	Definition
R	Sample results are rejected due to major inability to analyze sample or meet QC criteria. The presence or absence of the analyte cannot be confirmed.

13.2 Data Usability Assessment

After the data have been validated and verified, a Data Quality Assessment (DQA) is conducted to determine whether data are usable for meeting the project objectives. Data that met the MPCs should be usable as long as the quantity of data is sufficient. For data that did not meet MPCs, a determination of data usability must be made. In order to determine whether the quality and quantity of this data is useable for meeting project quality objectives, data will be assessed qualitatively for representativeness and comparability. Calculations and comparisons of the project's quantitative data quality indicators, including precision, accuracy, completeness, will be made as well. Uncertainty and variability in the data or in the procedures and models used to analyze the data may limit data interpretation. These potential limitations on data interpretation will be addressed in progress report deliverables.

14.0 Data Analysis Methods

14.1 Data Analysis Methods

Non-detects. One-half of the value of the method detection limit (MDL) will be substituted for the value of the non-detect. This substitution technique has been used recently by environmental, and particularly stormwater, studies (Ecology 2015). Antweiler and Taylor (2008) indicated that for data sets with less than 70% of data below the detection limit, this substation technique allowed for an adequate determination of summary statistics.

K_{sat} calculation. Saturated hydraulic conductivity will be calculated by the falling head method:

$$Ksat = \left(\frac{As*Lc}{Ac*t}\right) \ln\left(\frac{H1}{H2}\right),$$

where A_s = cross-sectional area of the standpipe, A_c = cross-sectional area of the soil column, L_c = length of the porous medium in the column, t = time for water to fall from the rim of the ponding zone (H_1) to the surface of the mulch (H_2).

Chemical removal efficiency. For each sampled Event, removal efficiency will be calculated based on the concentration of each analyte present in the influent sample. Percent removal will be determined by: % Removal = 1 - (Effluent concentration - Influent concentration)/(Influent concentration) x 100.

Toxicity removal efficiency. For each tested Event, toxicity removal will be assessed relative to the amount of each type of toxicity present as a result of exposure to the influent sample (acute lethal, sublethal).

Performance by depth and time. Univariate repeated measures ANOVA analyses will test differences in the removal efficiencies (chemistry and toxicology) from the different BSM depths over time.

Longevity analysis. At the end of the study, regression models will be developed to determine whether BSM performance can be predicted by depth and time.

All statistics will be performed with $\propto = 0.05$.

14.2 Data Presentation

Chemical and biological data for BSM longevity and effectiveness will be presented in a combination of tables, charts, and graphs in the final reports to illustrate trends, relationships, and anomalies with the data. The data presentation will address limitations of the study (i.e., accelerated WYs don't simulate the summer season, microbe build-up, or plant effects).

15.0 Reporting

Study findings will be sent to the SAM project manager in the form of a draft fact sheet and final report, which will explain the results for stormwater managers, NPDES permit coordinators, and others involved in stormwater management. In addition, two presentations will be created to share findings of the project with stormwater managers, including a presentation to the Stormwater Workgroup and one regional stormwater conference/workshop.

16.0 References

Environmental Protection Agency (EPA). 1994. Method 1312: Synthetic precipitation leaching procedure.

Environmental Protection Agency (EPA). 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms.

Environmental Protection Agency (EPA). 2002. Guidance on environmental data verification and data validation. EPA QA/G-8.n

Feist, BE, Buhle ER, Baldwin DH, Spromberg JA, Damm SE, Davis JW, Scholz NL. 2017. Roads to ruin: conservation threats to a sentinel species across an urban gradient. Ecol Appl, 27(8): 2382-2396.

Lombard, S., and C. Kirchmer, 2004. Guidelines for Preparing Quality Assurance Project Plans for Environmental Studies. Revised 2016. Washington State Department of Ecology, Olympia, WA. Publication No. 04-03-030.
https://fortress.wa.gov/ecy/publications/summarypages/0403030.html

McCarthy SG, Incardona JP, Scholz NL. 2008. Coastal storms, toxic runoff, and the sustainable conservation of fish and fisheries. American Fisheries Society Symposium, 64: 000-000.

McIntyre JK, Davis JW, Incardona J, Anulacion BF, Stark JD, Scholz NL. 2014. Zebrafish and clean water technology: Assessing soil bioretention as a protective treatment for toxic urban runoff. Science of the Total Environment, 500-501: 173-180.

McIntyre JK, Davis JW, Hinman C, Macneale KH, Anulacion BF, Scholz NL, Stark JD. 2015. Soil bioretention protects juvenile salmon and their prey from the toxic impacts of urban stormwater runoff. Chemosphere, 132: 213-219.

McIntyre JK, Edmunds RC, Redig MG, Mudrock EM, Davis JW, Incardona JP, Stark JD, Scholz NL. 2016. Confirmation of stormwater bioretention treatment effectiveness using molecular indicators of cardiovascular toxicity in developing fish. Environ Sci Technol, 50: 1561-1569.

McIntyre JK, Lundin JI, Cameron JR, Chow MI, Davis JW, Incardona JP, Scholz NL. 2018. Interspecies variation in the susceptibility of adult Pacific salmon to toxic urban stormwater runoff. Environ Pollut, 238: 196-203.

Spromberg JA, Baldwin DH, Damm SE, McIntyre JK, Huff M, Sloan CA, Anulacion BF, Davis JW, Scholz NL. 2016. Coho salmon spawner mortality in western US urban watersheds: bioinfiltration prevents lethal storm water impacts. Journal of Applied Ecology. 53: 398-407.

Spromberg JA and Scholz NL. 2011. Estimating the future decline of wild coho salmon populations resulting from early spawner die-offs in urbanizing watersheds of the Pacific Northwest, USA, Integr Environ Assess Manag, 9999.

The State of Washington Department of Eoclogy. 2016. Western Washington hydrology model 2012 user manual.

The State of Washington Department of Ecology. 2015. Western Washington NPDES phase I stormwater permit: Final S8.D data characterization 2009-2013.

The State of Washington Department of Ecology. 2012. Phase I municipal stormwater permit.

The State of Washington Department of Ecology. 2012. Western Washington phase II municipal stormwater permit.

The State of Washington Department of Ecology. 2012. Eastern Washington phase II municipal stormwater permit.

The State of Washington Department of Ecology. 2012. Stormwater management manual for Western Washington, as amended in December 2014.

The State of Washington Department of Ecology. 2014. Washington state department of transportation national pollutant discharge elimination system and state waste discharge municipal stormwater general permit.

The State of Washington Department of Ecology. 2015. Western Washington NPDES phase I stormwater permit: Final S8.D data characterization 2009-2013.

Washington State Department of Transportation. 2017. WSDOT NPDES municipal stormwater permit effectiveness monitoring status report (S7.C and S7.D) water years 2012-2016.

Young A, Kochenkov V, McIntyre JK, Stark JD, Coffin AB. 2018. Urban stormwater runoff negatively impacts lateral line development in larval zebrafish and salmon embryos. Scientific Reports, 8: 2830.

17.0 Appendices

Appendix A: Zebrafish stormwater runoff exposure protocol

McIntyre

Zebrafish stormwater runoff exposure protocol

DAY -1

The day prior to an experiment, prep glassware and set up zfish spawn in the late afternoon (>4pm)

For morphology alone, 15 embryos per replicate is sufficient, for qPCR more embryos (25-30) is often ideal, especially if you expect mortality. For morphology + qPCR, use 45 embryos per replicate.

The minimum number of replicates per treatment is 3, but 4 is ideal for morphometric analysis. More (4-5) are ideal for qPCR. You may want an extra replicate that will be fixed instead of frozen.

Each exposure will have one control treatment and one or more runoff treatments.

For experiments requiring <200 embryos, it is sufficient to set up one female and one male zmod tank into three spawning groups, each containing up to 2 males and three females. For experiments requiring >200 embryos, more zmod pairs will be needed.

Glassware should be scrubbed with simple green, rinsed 3x in tap water, soaked in distilled water, and then rinsed under the hood 3x in acetone then 3x in methylene chloride and left to dry in the hood.

Set up spawn following instructions in the zmod room

DAY 0

At 8:30-9:00 am, change the water in the spawning chambers to prevent dirty eggs.

Remove runoff from freezer and allow to begin thawing on counter in 204. At the same time, remove sufficient volume of embryo water from bottle in incubator into a clean (E) glass bottle and also place on counter.

Collect eggs by 11:30 am, rinse, and screen for unfertilized/bad eggs. If there appear to be sufficient viable eggs to proceed, place in incubator, labeled with your name and the date.

You may place runoff and control water in a warm (not hot) water bath to accelerate thawing and warm above room temperature.

McIntyre

DAY 2 (48 HPF)

Assess embryos one dish at a time. Either analyze in completely randomly, or assess all replicate 1s followed by all replicate 2s and all replicate 3s so that treatments are sampled across the observation period.

Hatch rate. Proportion of embryos that have hatched.

Survival count. Count dead embryos.

If imaging, or preserving embryos in fixative, dechorionate unhatched embryos at this time and remove chorions.

Imaging. Open BTV program and create a new folder. Using a spatula, place a nickel-sized daub of 3% methylcellulose in a plastic petri dish, smoothing to a flat surface. Gently place embryos onto daub with a dropper, removing extra water. Turn embryos so that they face left with their left side exposed, eyes stacked perfectly.
Window>Capture Controls
Video Size > 1280 x 960
Capture Image: Take one image at 3X (whole embryo) and one image at 6.3X (zoomed into eye/heart region).
Video Size > 800 x 600
Capture Movie: Take a 5s movie of periventral region (focused on heart), longer if heart rate is especially slow.

Create a new folder for each new replicate, noting time begun. Be sure there is a new destination folder for the new replicate or it will overwrite the images in the previous replicate.

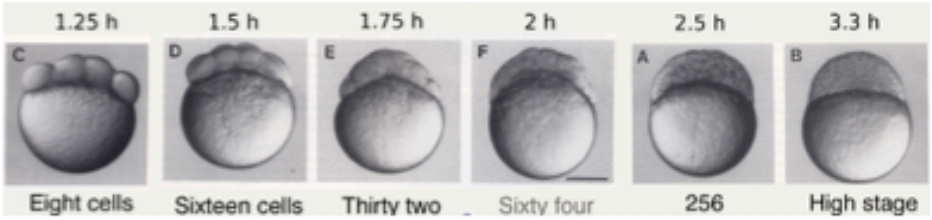
LATER TIME POINTS

HT50. Assess the median hatch time by tracking proportion hatched from few to most. Hatching occurs from approximately 40-78 hpf in controls, but may take much longer in runoff-exposed embryos.

Swim bladder inflation. Proportion with inflated swim bladders (96 h). Alternatively, assess the median time to swim bladder inflation by tracking proportion of individuals with inflated swim bladders across approximately 86-106 h.

Eye development. Continue tracking eye size by imaging the head at 6.3X magnification.

McIntyre



Place eggs in petri dishes between 1.25 h (8-cell) and 3.3 hpf (high cell). Ideally embryos will all be same stage. Choosing embryos at later stages allows best control survival and lower rates of background abnormalities. Prior to sorting, spray embryos well with system water from squeeze bottle to prevent sticking. Transfer embryos with a glass pipette. Do not transfer all embryos to a petri dish at once. E.g., if 15 per dish, transfer 10 to each dish and then another round of 5. This reduces the chance of having different age embryos in different dishes.

Once all petri dishes have correct number of embryos, randomize petri dishes in trays.

Label petri dishes with treatment and replicate number.

Remove residual water around embryos with a plastic pipette then add 10 mL of treatment or control water with a glass pipette attached to the electric pipetter. For exposures with 45 embryos/dish, use 15 mL. For runoff, particulates may have settled in the bottom during storage. To homogenize the sample, invert the jar 10X, then draw and aspirate 10 mL before taking first exposure aliquot. Draw and aspirate as needed during aliquot dispensation to keep particulates in suspension.

Cover petri dishes, randomize position on trays, and place in incubator.

DAY 1

Keep remainder of runoff in pesticide fridglet in 204. Bring day's aliquot to room temperature in covered glass beaker in pesticide incubator.

Water change at 24 hpf: Remove most water with plastic pipette, being careful to minimize egg disturbance. Do not remove all water as the loss of pressure can cause premature hatch. Add new water or runoff with glass pipette so that eggs swirl in petri dish (minimizing fungal adhesion).

Make note of any obvious developmental delays. Remove dead or severely deformed embryos (i.e., no head, no tail). Make notes.

Appendix B: Zebrafish Image Analysis

McIntyre

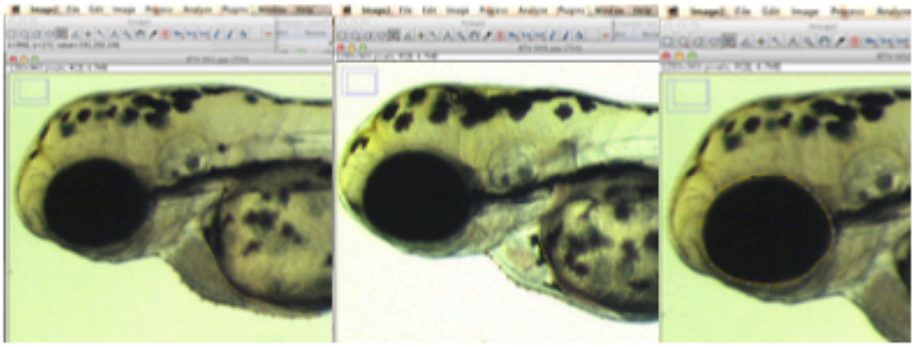
IMAGE ANALYSIS

Sort images in each replicate folder into movies, length, and head folders.

From stills:

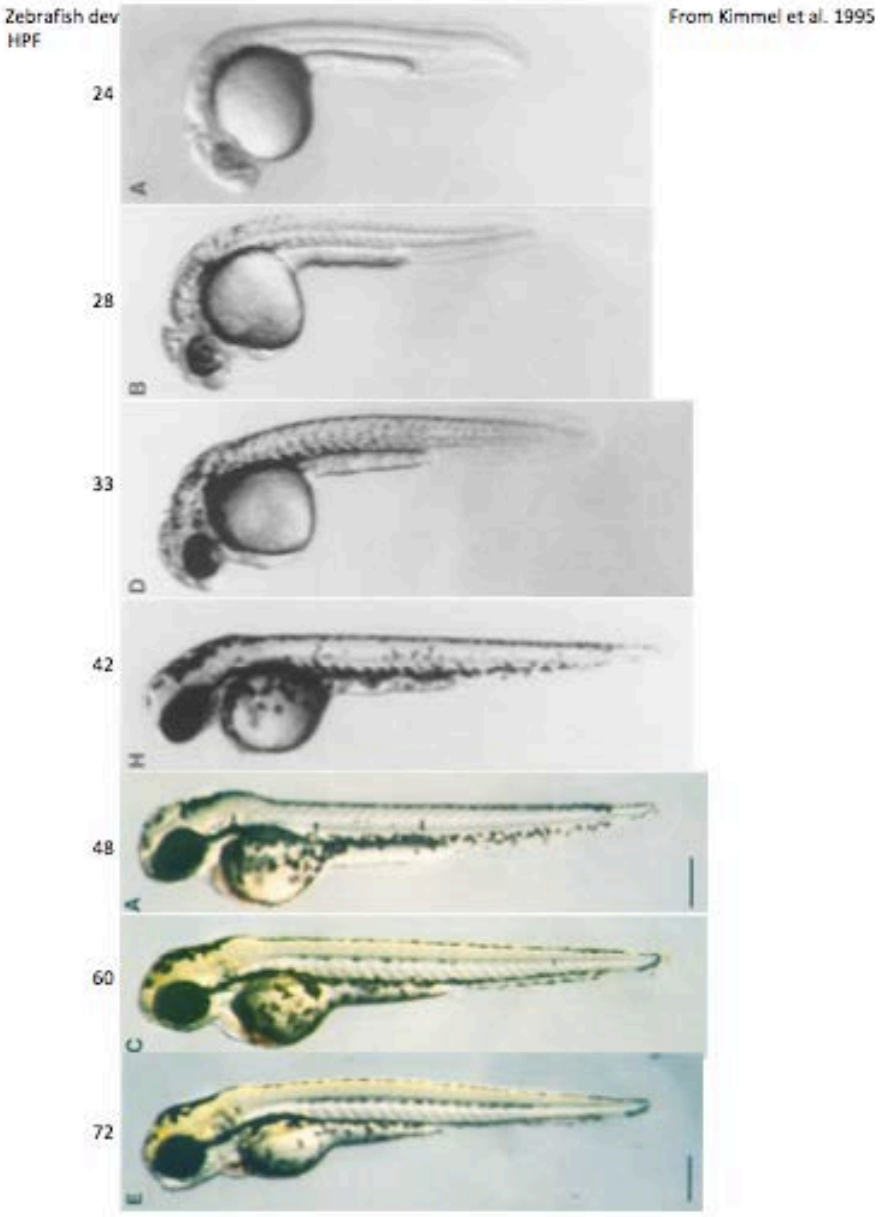
Using Image J, open the first image file in the length folder. Choose the segmented line tool by right clicking on the line tool. Click on the end of the embryo tail, follow the notochord to the ear, and finish the line at the fish head by crossing through the middle of the eye. Double-click to end line. Type ⌘-m (or Analyze>Measure) to measure length. Type ⌘-⇧- O (File>Open Next) for next image. Repeat until all images in folder have been analyzed. Copy the output text and paste into Excel. Make note of which column is the measurements. Clear contents in the output window before moving on to next folder.

Next measure the periventral, pericardial, and eye area from the head folder. Choose the polygon tool in Image J, and trace the outside of the periventral area. Repeat for all images before tracing the pericardial and then eye area.



Periventral area (PVA) Pericardial area (PCA) Eye Area

Developmental delay. As the embryo develops, the head uncurls from around the yolk. Compare positioning of the embryo eye relative to the yolk. Determine if embryo is < 48h, <42 h, or <33 hpf from the following:



Pigmentation. Score individuals for pigmentation. Score = 0 if no light shines through eye when lined up appropriately. Score = 1 if eye is less than fully pigmented. Score = 2 if there is hardly any pigmentation in eye or body.

From video:

For each embryo, assess the presence/absence of pericardial edema and vascular abnormalities described below.

Pericardial edema (PCE). Present when there is no movement of the pericardial sac surrounding the heart as it beats. Usually due to fluid accumulation around the heart.

Unlooped heart (ULH). If the heart chambers are linear or nearly linear instead of looped. Extreme versions of this can be noted as tube heart (TH) with no distinct chambers, and string heart (SH) in which both chambers have collapsed to a skinny string.

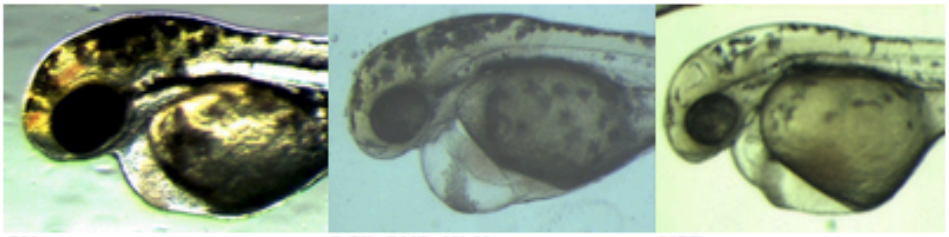
Regurgitation (R). Score = 1 if mild atrial regurgitation. Score = 2 if severe regurgitation (very little net forward motion). Score = 3 if circulatory stasis.

Periventral blood pool (PVP). If blood is pooled in the periventral area (more than control amount).

Yolk sac edema (YSE). Fluid pushing into the yolk in the periventral region without any blood present.

Arrhythmia. Ventricle and atrium do not beat 1:1.

Vascular abnormalities. Note other heart abnormalities including enlarged chambers (LA, LV, LALV), collapsed chambers, cranial hemorrhaging (CH), lack of blood cells (NB), loose blood cells in the pericardium, and tail abnormalities such as blood pooling, margin deformities, and caudal vein disorganization.



CH PCE, PVP, ULH YSE

Heart rate. Count the number of atrial beats. Divide by video length and multiply by 60 to get bpm.

Appendix C: Coho salmon toxicity testing

Toxicity Testing with Juvenile Salmon

Prior to exposure day:

- Plan out the water baths you will use and how you will aerate each exposure tank
- Clean the aquaria and lids you will use for exposures by scrubbing all inside surfaces with soap and hot water followed by thoroughly rinsing with hot water at least three times
- Set aquaria upside down to drip dry
- Chill water in the water baths to the exposure temperature (or colder if you expect exposure waters to need cooling)
- Label aquaria (at least 3 per treatment)
- Ensure sufficient air stones and tubing for aeration
- Ensure with the aquaculture technician that the fish will not be fed the day of the exposure

Exposure day:

- Set aquaria in water bath so that replicates and treatments are randomly distributed
- Carefully fill aquaria making sure to not splash water from one treatment into another (particularly into control water)
- If filling from carboys, use a piece of tubing to hand siphon rather than pouring until the carboy is light enough to handle safely
- Place an airstone into each aquarium and supply a steady stream of air
- Check the temperature of the exposure water and the rearing water, making sure to not contaminate the rearing water
- When the exposure waters are within 1 deg C of the rearing water, the exposure may begin
- Record water quality (temperature, dissolved oxygen, pH, conductivity) for each aquarium

Exposures:

- With a larger net, gently collect a group of juvenile coho from rearing tank and gently release into a control bucket with fresh rearing water
- You want to distribute the fish quickly and gently to reduce stress
- With a smaller net, gently grab a small number of fish (1-5) and invert into an exposure aquarium
- Count each fish aloud to keep track and not dispense too few or too many fish
- Have an assistant mark down which aquaria have received fish and how many
- Proceed to a total of 10 fish per aquarium
- Place the lid on any aquarium with fish; lids must prevent the air stone from slipping out and must not allow any gap of more than ½" to prevent jumpers
- Replace the water in the distribution bucket between fish grabs from the rearing tank

Daily behavior monitoring:

- Fish should be monitored at least daily for mortalities and any obvious changes in behavior
- Healthy fish in an exposure will often spend much of their time relatively inactive near the bottom of the tank
- Sick fish may show a variety of behavioral changes including:
- Rising to the surface of the water
- Increased or decreased activity
- Change in coloration
- Loss of equilibrium
- Record any changes in behavior on the daily monitoring sheet
- Dead fish will sink to the bottom of the exposure tank
- In darkly colored exposure waters, fish may be difficult or impossible to observe
- Check for mortalities by slowly scooping a net along the bottom of the aquarium
- Healthy fish will usually be able to move out of the way, although some may be capture
- Return these fish to the exposure water
- Dead fish should be observed and recorded for length and condition
- Condition includes whether the fish is recently deceased (gills still red or pink) and any other unexpected observations such as wounds or discoloration
- Dead fish should be placed in a plastic Ziploc bag labeled with your name and the date and frozen for later disposal to the municipal trash

Daily water quality monitoring:

- Read the manual for each water quality monitoring device to ensure that it is properly calibrated and that you are operating the device correctly
- Record water temperature, pH, dissolved oxygen, and conductivity for each replicate
- Begin with controls and move to increasingly higher exposure concentrations
- Rinse probes between treatments
- Close aquarium lids when not actively measuring water quality
- Make sure that air stones are still properly situated to provide sufficient aeration

Preparing for test termination:

- Have fresh, buffered MS-222 prepared for euthanizing survivors (80 g/L)
- A dose of 500 mg/L is used to euthanize salmonids
- In 2 gallons of rearing water (8 L), add 50 mL of 80 g/L to achieve 500 mg/L
- If you are necropsying fish to remove tissues:
  - Liquid N_2 (from NOAA at this time)
  - 95% ethanol in squeeze bottle
  - Tools
    - Scale
    - Measuring board
    - Cutting board

96-h acute mortality test

  - Scalpel handles and blades (#11, 21)
  - Scissors
  - Stainless steel forceps and small iris scissors
  - Headlamps for sufficient illumination
- Supplies
  - Gloves
  - Kim Wipes
  - Whirl packs
  - Bile vials
  - Cyrovials + labels
- People

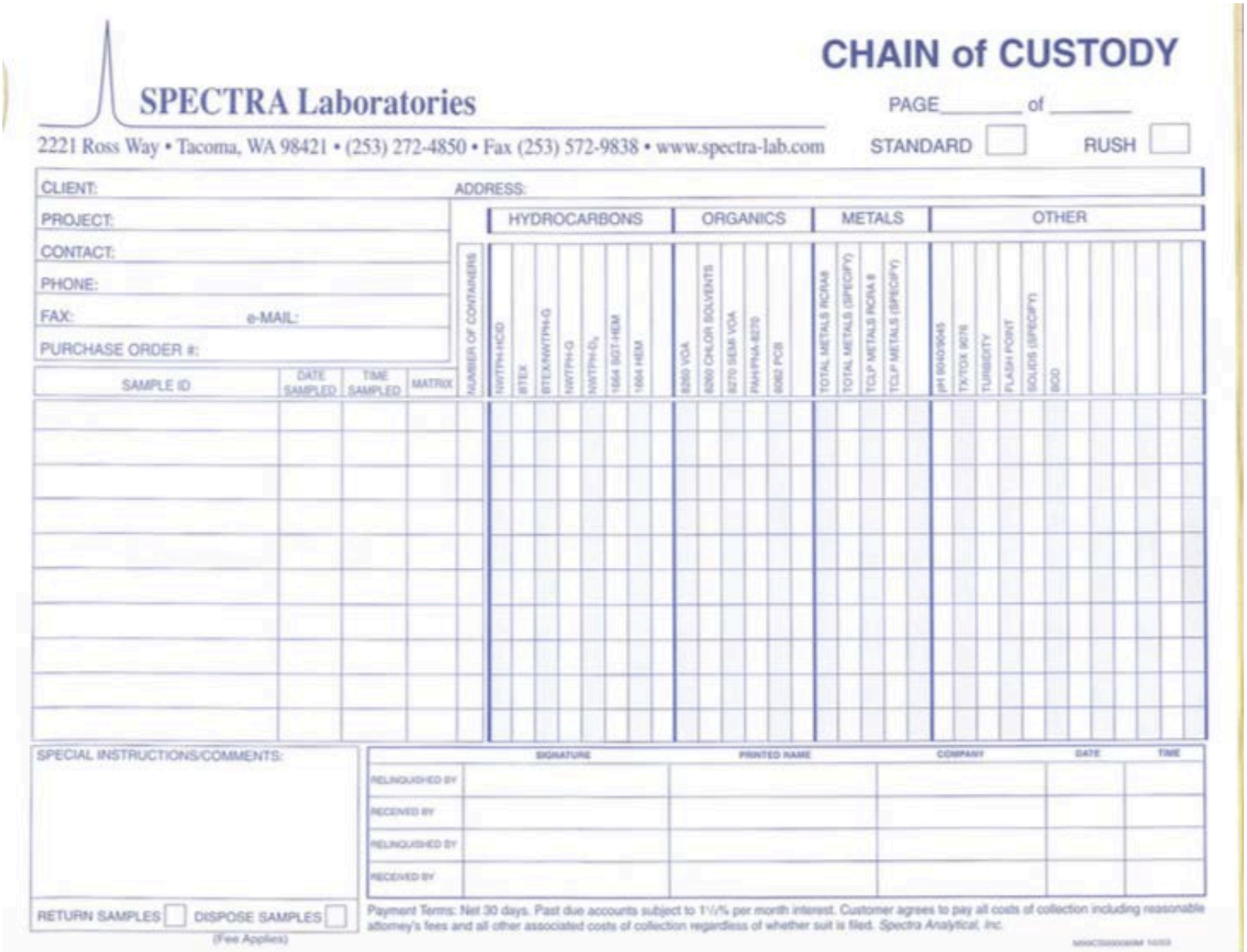
Exposure take-down (96-h):

- Record water quality and note any unexpected behaviors of survivors
- If dead coho, record length on the coho data sheet and dispose of carcass
- One replicate at a time, anesthetize coho in MS-222 until cessation of opercular movement
- Record TL and weight
- Secondary euthanasia: sever caudal vein
- Remove tissues and ***preserve according to planned analyses***

Clean-up:

- All fish carcasses placed in labeled garbage bags for freezing and later disposal
- Drain aquaria; control pump for control water, exposure pump for exposure water
- Drain exposure water through charcoal bucket
- Wash aquaria and lids with soap and rinse 3X with tap water
- Store upside down to drip-dry
- Rinse and replace nets in the fish room
- Discard air stones and tubing
- Wash and leave to dry all dissection tools and boards
- Place chemicals back in their proper storage places
- Place supplies and equipment back in dry lab or storage shelves

Appendix D: Spectra Laboratories example chain of custody form



CHAIN of CUSTODY

SPECTRA Laboratories

2221 Ross Way • Tacoma, WA 98421 • (253) 272-4850 • Fax (253) 572-9838 • www.spectra-lab.com

PAGE_____ of _____

STANDARD ☐ RUSH ☐

CLIENT: ADDRESS:

PROJECT:

CONTACT:

PHONE:

FAX: e-MAIL:

PURCHASE ORDER #:

					HYDROCARBONS								ORGANICS						METALS					OTHER															
SAMPLE ID	DATE SAMPLED	TIME SAMPLED	MATRIX	NUMBER OF CONTAINERS	NWTPH-HCID	BTEX	BTEX/NWTPH-G	NWTPH-G	NWTPH-D_x	1664 SGT-HEM	1664 HEM		8260 VOA	8260 CHLOR SOLVENTS	8270 SEMI VOA	PAH/PNA 8270	8082 PCB		TOTAL METALS RCRA8	TOTAL METALS (SPECIFY)	TCLP METALS RCRA 8	TCLP METALS (SPECIFY)		pH 9040/9045	TX/TOX 9076	TURBIDITY	FLASH POINT	SO1/05 (SPECIFY)	BOD									

SPECIAL INSTRUCTIONS/COMMENTS:

RETURN SAMPLES ☐ DISPOSE SAMPLES ☐ (Fee Applies)

	SIGNATURE	PRINTED NAME	COMPANY	DATE	TIME
RELINQUISHED BY					
RECEIVED BY					
RELINQUISHED BY					
RECEIVED BY					

Payment Terms: Net 30 days. Past due accounts subject to 1½% per month interest. Customer agrees to pay all costs of collection including reasonable attorney's fees and all other associated costs of collection regardless of whether suit is filed. *Spectra Analytical, Inc.*

Appendix E: Analytical Resources, Inc, example chain of custody form

Chain of Custody Record & Laboratory Analysis Request

Analytical Resources, Incorporated
Analytical Chemists and Consultants
4611 South 134th Place, Suite 100
Tukwila, WA 98168
206-695-6200 206-695-6201 (fax)

ARI Assigned Number:	Turn-around Requested:				Date:								
ARI Client Company:	Phone:				Page:	of							
Client Contact:					No. of Coolers:	Cooler Temps:							
Client Project Name:					Analysis Requested								Notes/Comments
Client Project #:	Samplers:												
Sample ID	Date	Time	Matrix	No. Containers									
Comments/Special Instructions	Relinquished by: (Signature)			Received by: (Signature)			Relinquished by: (Signature)			Received by: (Signature)			
	Printed Name:			Printed Name:			Printed Name:			Printed Name:			
	Company:			Company:			Company:			Company:			
	Date & Time:			Date & Time:			Date & Time:			Date & Time:			