Quality Assurance Project Plan

Field test of plants and fungi on bioretention performance over time

Prepared by

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Field Test of Plants and Fungi on Bioretention Performance over Time

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WSU-P = Washington State University – Puyallup

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Introduction

The Regional Stormwater Monitoring Program (RSMP) is a collaboration of western Washington municipal stormwater permittees, state and federal agencies. The Stormwater Work Group (SWG) oversees the implementation of the RSMP. The RSMP was designed to meet MS4 permittee stormwater monitoring needs. Further, the RSMP provides a structure that allows permittees to pool resources and to conduct effectiveness studies to improve municipal stormwater management. The goals of RSMP effectiveness studies are to measure the effectiveness of various stormwater management activities, best management practices (BMPs), and to communicate findings to the regions' professionals.

The 2013-2018 Western Washington Phase I and Phase II Municipal Stormwater General Permits (permits) require the use of Low Impact Development (LID) where feasible, and bioretention is believed to be the most commonly utilized LID BMP in Western Washington. The US Fish and Wildlife, Washington State University in Puyallup (WSU-P), and the National Oceanic and Atmospheric Administration (NOAA) Fisheries are partnering to test if plants and/or fungi affect hydraulic conductivity, effluent water chemistry, toxicity, or soil microbial diversity of bioretention using the Washington State Department of Ecology's (Ecology) recommended mixture (60% sand, 40% compost). Funding for this project comes from the municipal stormwater permittees via the RSMP. Results are intended to address the RSMP's goal to determine "what soil amendment and bioretention mixes combined with plant selection provides optimum removal of nutrients, bacteria, and metals?"

Recent bench and field scale bioretention studies have indicated a significant potential for pollutant exports from bioretention cells that meet the Stormwater Management Manual for Western Washington (SWMMWW) specifications (Herrera, 2014). Replicated studies using small scale bioretention cells (herein referred to as "mesocosms") indicate that the role of plants in bioretention systems is not well understood (McIntyre, 2014). Further research is needed to evaluate whether adding plants as an installation amendment provides additional pollution control benefits. Wood-decomposing fungi can be used in bioretention mulch to achieve unique environmental services (Thomas et al., 2009; Taylor et al., 2014). This project will evaluate the combination of plants and fungi using four treatment programs in the bioretention cells under field conditions to improve stormwater quality. Specifically, this project will test for hydraulic conductivity, water quality before and after treatment, and the toxicity of stormwater effluents on zebrafish (*Danio rerio*). Four treatments (no plants + no fungi; plants + no fungi; no plants + fungi; plants + fungi) will be evaluated in triplicate.

Bench and field-scale studies, including two in the Puget Sound region, indicate that wood - decomposing fungi can be used in bioretention mulch to achieve important environmental services. A study of two underdrained rain gardens in the Dungeness watershed of the Olympic Peninsula found that inoculating the wood mulch layer with fungi removed 24% more fecal coliform from runoff than the control (Thomas et al., 2009). A 2012 EPA study by Fungi Perfecti LLC and Washington State University (WSU) found that the mushroom-forming fungus *Stropharia rugoso-annulata* grown on alder (*Alnus rubra*) wood chips yielded a 20% improvement in *E. coli* removal relative to the wood chips alone ($p<0.05$) at the hydraulic

loading rate of 0.5 L/min (0.43 $m^3/m^2 \cdot d$) under laboratory conditions (Taylor et al., 2014).The research also indicated that *S. rugoso-annulata* is resilient to the year-round environmental conditions of a Puget Sound stormwater bioretention setting such as alternating wet and dry intervals and temperature extremes from 0 to 40 °C. Earlier work on *S. rugoso-annulata*, also indicates that this species will degrade polycyclic aromatic hydrocarbons (PAHs) in contaminated soil, with reductions of up to 70%, 86% and 84% for benzo(a)anthracene, benzo(a)pyrene, and dibenzo(a,h)anthracene, respectively (Steffen et al., 2007). Fungal biomass has also been studied as an effective sorptive agent with the ability to bind and retain significant amounts of copper from aqueous solutions (Simonescu and Ferdes, 2012). Replicated field data is needed to determine whether field performance justifies incorporating this fungus as a part of the wood mulch in bioretention BMP applications.

Stormwater runoff can be acutely toxic to aquatic animals causing mortality in fish and invertebrates (McIntyre et al. 2014) and sublethal toxicity such as cardiovascular toxicity in developing fish (McIntyre et al. 2015). Bioretention research by WSU/USFWS/NOAA has shown that bioretention soil media (BSM) effectively eliminated acute lethal and sublethal toxicity from road runoff for one (McIntyre et al. 2014, 2015) to four (Spromberg et al. 2016) storm events. Chemical analyses for these experiments showed significant improvement in water quality, particularly for Zn as well as PAHs. It is unknown how bioretention will perform in terms of biological effectiveness for toxicity prevention during constant stormwater loading conditions as bioretention systems age. Previous toxicological studies have used small-scale bioretention mesocosm cells that have been episodically exposed to freeway runoff, but have been irrigated with fresh water during the intervals between runoff tests (McIntyre 2014). In contrast, the present study will compare influent and effluent toxicity from bioretention mesocosm cells that are aged for two years in the field under natural runoff regimes. In this way, we will be able to track chemical and biological performance over time.

This Quality Assurance Project Plan (QAPP) describes the objectives of the study and the procedures to be followed to ensure the quality and integrity of the collected data and ensure the results are representative, accurate, and complete within the scope defined by the study.

Project Description

For the proposed work, stormwater will be treated under field conditions using runoff from a highly urbanized watershed that includes a segment of the Interstate 5 corridor in Seattle, WA. The runoff will be treated on-site using four different types of bioretention columns (bioretention soil media (BSM) only; BSM + plants; BSM + fungi; BSM + plants + fungi), evaluated in triplicate. Through this project we will better understand the interplay of soils, plants, and fungi in the treatment of urban stormwater runoff. We will learn if plants and fungi as soil amendments will improve the water quality of effluent from bioretention cells. Furthermore, this project will increase the understanding of the fate of metals and polycyclic aromatic hydrocarbons (PAHs) in bioretention soil. After two years of real-time stormwater exposure, soil samples will be analysed from each treatment at various depths. An improved understanding of the movement of metals and PAHs through BSM will help to estimate whether the lifetime of BSM (as inferred by pollutant migration through the soil strata) can be extended by plant or fungi soil amendments.

The time series of hydraulic properties contributes to our understanding of water movement in the bioretention cells. By studying the hydraulic properties over time we can determine if plants or fungi affect the hydraulic conductivity or the water holding capacity of the BSM Influent and effluent flow data, combined with water quality and soil monitoring will enable modeling efforts to estimate the lifetime of a bioretention cell. This data will allow us to design and dimension bioretention cells and maintenance intervals in greater detail as well as to better predict and avoid hydraulic failure.

We will address the effectiveness study question posed by the Stormwater Work Group:

“What soil amendment and bioretention soil mixes combined with plant selection combines optimum removal of nutrients, bacteria, and metals?”

Under this project we will evaluate nutrients, bacteria, metals and PAHs. A toxicity monitoring component of the research will also evaluate the subtopic posed by the Stormwater Work Group:

“Where and when are nutrient and metal outputs from LID of concern?”

For this project , we hypothesize that:

- Fungal amendment will increase the nutrient retention capacity of the bioretention soil mix
    - The measured nutrients in the effluent will be lower in fungal-amended cells than in the cells without fungal amendment (see section: **Water Quality Sampling**)
- Amendment with plants and fungi will improve the retention of metals in the BSM
    - The measured metals in the effluent will be lower in plant and fungal-amended cells than in the cells without plants and/or fungal amendment (see section: **Water Quality Sampling**)
- Fungal amendment will show improved reduction of PAHs in effluent.

    - The measured PAHs in the effluent will be lower in fungal-amended cells than in the cells without fungal amendment (see section: **Water Quality Sampling**)
- Pollutants move slower through soils with plant and fungal amendments and therefore bioretention cells with amendments have a longer lifetime than bioretention cells without amendments
    - Soil samples collected from cells with plant and fungal amendment will have lower pollutant concentrations with depth than cells without amendment (see section: **Soil Sampling**)
- Removal of fecal bacteria will be improved by fungal amendment
    - The measured fecal indicator bacteria in the effluent will be lower in fungal-amended cells than in the cells without fungal amendment (see section: **Water Quality Sampling**)
- The presence of plants will prevent loss of hydraulic conductivity but will not detrimentally alter effluent chemistry or toxicity.
    - The measured hydraulic conductivity in the planted cells will remain constant over time, or will decrease less than the hydraulic conductivity of the unplanted cells (see section: **Water Quality Sampling**)
    - The measured water quality parameters in the effluent of planted cells will not be greater than the corresponding unplanted control cells at comparable points in time (see section: **Water Quality Sampling**)

Organization and Schedule

Key personnel members for the plant and fungal soil amendments project are shown on Table 1 and project milestones are shown in Table 2.

Table 1: Key personnel for the plant and fungal soil amendments project.

Name	Title and Organization	Role
Dr. Jenifer McIntyre (206) 445-4650	Assistant Professor, Principal Investigator, WSU	Reviews and approves QAPP. Oversees construction, budget, timelines, field sampling and laboratory procedures. Leads sampling and toxicology training of project personnel. Reviews the draft report and final report.
Alex Taylor (360) 890-5306	Graduate Student, WSU	Conducts field research, analyzes and interprets data, and prepares draft and final report.
Dr. Thorsten Knappenberger (334) 844-3997	Assistant Professor, Co-Principal Investigator, Auburn University	Prepares QAPP, assists in training on sampling procedures and design and analysis of soil physics experiments. Acts as Quality Assurance Coordinator to review data for compliance with QA requirements.
Dr. John Stark (253) 445-4505	Director, WSU	Reviews and approves QAPP. Provides executive review of project scope, timeline, budget, resources, and personnel.
Randall Marshall (360) 407-6445	Ecology Water Quality Program QA Officer	Reviews and approves QAPP
Brandi Lubliner (360) 407-7140	RSMP Coordinator and Ecology Contract Manager	Reviews and approves QAPP, project deliverables and final report.
Cheronne Oreiro (206) 695-6214	Project Manager, Analytical Resources, Inc.	Ensures samples are analyzed in accordance with the approved QAPP
Aaron Young (425) 885-1664	Laboratory Project Manager, AmTest Laboratories	Ensures samples are analyzed in accordance with the approved QAPP

Table 2: Project Milestones

Project Milestone	Date
QAPP approved	October 2016
Bioretention medium procured and analyzed	October 2016
Bioretention cells prepared and installed	October 2016
Baseline testing of bioretention cells	October 2016
Performance monitoring finished	October 2018
Post-experimental testing	December 2018
Final report	December 2018

Quality Objectives

This section discusses the data quality objectives (DQOs) developed to ensure the study objectives are achieved in a qualitative and quantitative manner. The DQOs define the appropriate type of data and tolerable levels of potential errors. The DQOs for this study include the following:

- Data will be generated using established protocols and previously published methods for sampling, sample handling and process, laboratory analysis, and record keeping.
- Data will be representative of the composition of highly urbanized watershed runoff, and will be of known precision, accuracy, and bias.
- Data reporting and analytical sensitivity will be clearly established and adequate for characterizing runoff, soil properties, toxicology, and microbial activity.

The DQOs provide the basis of the measurement quality objectives (MQOs). MQOs provide the quantitative thresholds for data, based on data quality indicators specifically established for analytical and instrument performance. MQOs serve as performance measures described in terms of:

- Sensitivity
- Representativeness
- Precision
- Bias/Accuracy
- Comparability
- Completeness

Measurement Quality Objectives

Sensitivity

Sensitivity is the measure of the concentration at which an analytical method can be positively identified and analytical results reported. The sensitivity of a method is commonly reported as the method detection limit (MDL) or the reporting limit (RL). The QAPP specifies both MDLs and RLs (Table 3 and Table 4), and requires reporting of values between these two limits with "estimation" or "J" flags for conventional water quality parameters and metals. The MDLs listed for each analyte define the lowest concentrations of interest within budget of this project. Qualification of results based on this goal is discussed in the Data Verification section.

For the toxicity study, sensitivity will be gauged by the ability to detect a difference in survival or sublethal metrics between the influent runoff and the laboratory controls. Survival of the organisms in the laboratory controls must be at least 90% for each test.

Representativeness

Representativeness is the extent to which a measurement actually represents true environmental conditions. One component of representativeness is selection of the treatment

site and the influent stormwater that will be monitored. The study site provides for an urban location with a diverse drainage area that includes a busy urban freeway- a watershed that is typical in the dense urban areas of the Puget Sound basin.

Representativeness is particularly difficult to define for stormwater quality and LID treatment in a relatively short-term study because runoff quality and treatment efficacy can change depending on the storm size, phase of the storm, antecedent conditions, season, temperature, etc. The representativeness of this study is also limited to the evaluation of a limited number of plants, bioretention soil types, and the testing of soil cells rather than whole rain gardens, and the relatively infrequent monitoring of storm events (quarterly).

For both the chemical and toxicological analyses, representativeness will be attained by collecting and analyzing runoff from eight rain events over a two-year period with a variety of intensities and durations.

Sample representativeness will be ensured by collecting runoff from eight independent storm events in order to assess treatment performance across a range of storm event conditions with respect to rainfall volume, rainfall intensity, and antecedent dry period. The influent stormwater collected during each storm event will be a composite of three samples (collected as described in sub-section on Water Quality Sampling). Three independently collected influent composite samples will be analyzed for each storm. To meet this goal, we will endeavor to sample during storm events with:

- Target storm depth: A minimum of 0.4 L/m^2 (0.15 inches) of precipitation over a 24-hour period.
- Antecedent conditions: A period of at least 6 hours preceding the event with less than 0.1 L/m^2 (0.04 inches) of precipitation.
- Minimum duration: At least 2 hours.

The goal of this study is to determine relative differences in performance of bioretention cells that are exposed to the same conditions, rather than to determine the performance of any specific treatment to all possible conditions. Therefore, composite samples will be collected for each storm event, but this sample will not necessarily constitute an event mean concentration for each parameter.

Precision

In this study, overall project data quality will be based on total precision and analytical precision. Total precision is the measure of the variability in the results of replicate measurements due to random error that is introduced during sample collection and processing in the field *and* the laboratory analytical procedure. Total precision will be estimated based on the pooled relative standard deviation (RSD_p) of the field triplicates from all sampling events. The RSD_p of these samples will be calculated using the following formula:

$$RSD_p = \frac{S_p}{\bar{x}} \times 100\%$$

where $\overline{x}$ is the mean of all values, and S_p is the pooled standard deviation:

$$S_p = \sqrt{\frac{(n_1 - 1){S_1}^2 + (n_2 - 1){S_2}^2 + (n_3 - 1){S_3}^2 + \cdots + (n_k - 1){S_k}^2}{(n_1 - 1) + (n_2 - 1) + (n_3 - 1) + \cdots + (n_k - 1)}}$$

Where *n* is the number of individuals in the set (3), *S* is the standard deviation of the set, and s_p will be based on $(n_1 - 1) + (n_2 - 1) + (n_3 - 1) + \cdots + (n_k - 1)$ degrees of freedom.

When individual values are less than or equal to 5 times the reporting limit, they will not be included in the RSD_p calculation. The specific MQOs for total precision are defined in Table 3 for water quality parameters.

Analytical precision is the measure of the variability in the results of replicate measurements due to random error that is introduced from just the laboratory analytical procedure. Analytical precision will be assessed based on the relative percent difference (*RPD*) of laboratory duplicates that are run with each batch of samples. The *RPD* of these samples will be calculated using the following formula:

$$RPD = \left(\frac{C_1 - C_2}{C_1 + C_2}\right) \times 200\%$$

where C_1 and C_2 are concentration values. For values that are greater than 5 times the reporting limit, the relative percent difference must be less than or equal to the indicated percentages in Table 3 for water quality parameters. The absolute difference between the duplicates must be $\pm$2 times the reporting limit if the duplicate concentrations are both within 5 times the reporting limit. If either of the duplicate concentrations is at or below the reporting limit, precision will not be assessed.

Four replicates will be used for the zebrafish toxicity tests to maximize precision while minimizing the sacrifice of animals. Precision for each metric can be calculated as the coefficient of variation CV, the standard deviation/mean. Ideally, the CV should be less than 10%.

Table 3: Measurement quality objectives for water quality parameters.

Analyte	Method	Method Detection Limit (µg/L)	Reporting Limit (µg/L)	Lab ReplicatesRPD(%)	Lab Control/Surrogate (%)	Matrix Spike Recovery (%)	MS/MSD (%)
Total Zinc	EPA 200.7	0.59	2.0	≤20	90-110	70-130	51
Total Copper	EPA 200.7	3.26	5	≤20	90-110	70-130	60
Dissolved Zinc	EPA 200.8	0.084	0.5	≤20	90-110	70-130	25
Dissolved Copper	EPA 200.8	0.005	0.10	≤20	90-110	70-130	25
Total Suspended Solids	SM2540D	1.0	1.0	≤20	80-120	N/A	30
Suspen. Sediment Conc.	ASTM D3977	0.1	0.2	≤20	80-120	N/A	32
Total Organic Carbon	SM5310B	106	500	≤20	80-120	75-125	30
Dissol. Organic Carbon	SM5310B	106	500	≤20	80-120	75-125	25
Chem. Oxygen Demand	EPA 410.4	2	10	≤20	80-120	75-125	36
Total Phosphorous	SM4500-P F	1	5	≤20	80-120	75-125	20
TKN	SM4500-Norg	10	100	≤20	80-120	75-125	23

Analyte	Method	Method Detection Limit (µg/L)	Reporting Limit (µg/L)	Lab ReplicatesRPD(%)	Lab Control/Surrogate (%)	Matrix Spike Recovery (%)	MS/MSD (%)
Ammonia	EPA 350.1M	2.3	5	≤20	80-120	75-125	20
Nitrate + Nitrite	EPA 353.2	6	10	≤20	80-120	75-125	20
Ortho-phosphorous	SM4500-PE	2.5	5	≤20	80-120	75-125	20
pH	SM4500HB	0.1 unit	0.1 unit	≤20	80-120	N/A	10
Alkalinity	SM2320B	1.0	1.0	≤20	80-120	N/A	20
Calcium	EPA 200.7	4	50	≤20	90-110	70-130	20
Fecal Coliform	SM9222D	1 CFU/ 100mL	1 CFU/ 100mL	≤20	N/A	N/A	50
E. coli	SM9222DG or SM9221F	1CFU/ 100mL	1CFU/ 100mL	≤20	N/A	N/A	50
PAHs	EPA 8270D-IM	0.01-0.02* ug/L	0.1-0.2*	≤40	30-160	30-160*	40

* Compound specific

Table 4: Measurement quality objectives for sediment quality parameters

Analyte	Method	Method Detection Limit	Reporting Limit (µg/g)	Lab ReplicatesRPD(%)	Lab Control/Surrogate (%)	Matrix Spike Recovery (%)	MS/MSD (%)
Metals: As, Cd, Cr, Cu, Ni, Pb, Zn	EPA 200.8	0.05 (µg/g)	0.20 (µg/g)	≤20	90-110	70-130	36
PAHs	EPA 8270D-SIM	1-4 (µg/kg)	5-10 (µg/kg)	≤40	30-160	30-160	40

Bias/Accuracy

Bias or accuracy is a measure of confidence that describes how close a measurement is to its "true value." Methods to determine and assess accuracy of water chemistry measurements include: instrument calibration, and various types of QC checks (e.g., sample split measurements, spike recoveries, continuing calibration verification checks, internal standards, field and laboratory blanks, external samples), and performance audit samples.

Accuracy will be estimated by reanalyzing a sample to which a material of known concentration has been added (a laboratory control sample [LCS] and a matrix spike [MS] sample), and the results will be expressed as percent recovery R of the added pollutant:

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

where M is the measured and T is the true value. Table 3 and 4 list the acceptable percent recoveries for the parameters. Water blanks (distilled water), equipment rinse blanks, and method blanks will assist in determining bias and reasons for poor accuracy.

For the toxicity study, measurements will be made by the same individual to maximize precision. Bias will be minimized by an expert review of the measurement methods.

Comparability

Comparability is the degree to which data can be compared directly to similar studies. Standardized sampling techniques, standard analytical methods, and units of reporting with comparable sensitivity will be used to ensure comparability. Use of standard operating

procedures for field sampling (pH and specific conductance analyses), decontamination procedures, and laboratory analyses in accordance with the AmTest Laboratory Quality Manual ver. 11.5 (AmTest Laboratories, 2015) will provide comparability across studies. Analytical methods include U.S. Environmental Protection Agency (EPA)-approved field and laboratory methods. Staff obtaining the samples will be trained to follow standard protocols for each parameter as described in this plan. The procedures used in this experiment will generally be comparable to McIntyre et al. (2014) for influent and bioretention treated effluent water quality and toxicity, Lucas and Greenway (2011) for bioretention soil hydraulic conductivity.

For the toxicity experiments, methods used for this study are similar to the Fish Embryo Acute Toxicity Test (OECD 236) published by the international policy body Economic Cooperation and Development (OECD 2013). Thus, toxicity determined in this study will be comparable to toxicity assessments for the same organism in other tests.

Completeness

Completeness will be calculated by dividing the number of valid values by the total number of values. Valid sample data consists of unflagged data and estimated data. A qualitative assessment will be made as to which estimated data may need to be excluded from this calculation prior to annual reporting. If less than 90% of the samples submitted to the laboratory are judged to be valid, then additional samples will be collected until at least 95% are judged to be valid.

Experimental Design

Site Description

The experiments will be conducted at the Washington State Department of Transportation Lake Union Ship Canal Research Facility, located at 650 NE 40th St., Seattle WA (Figure 1). The site is located under the north end of the Ship Canal Bridge in a fenced area with access restricted to authorized personnel only. The site receives runoff from a 12.8 hectare (31.6 acres) drainage area including 9.2 hectares (22.7 acres) of pavement and 3.6 hectares (8.9 acres) of roadside landscaping.

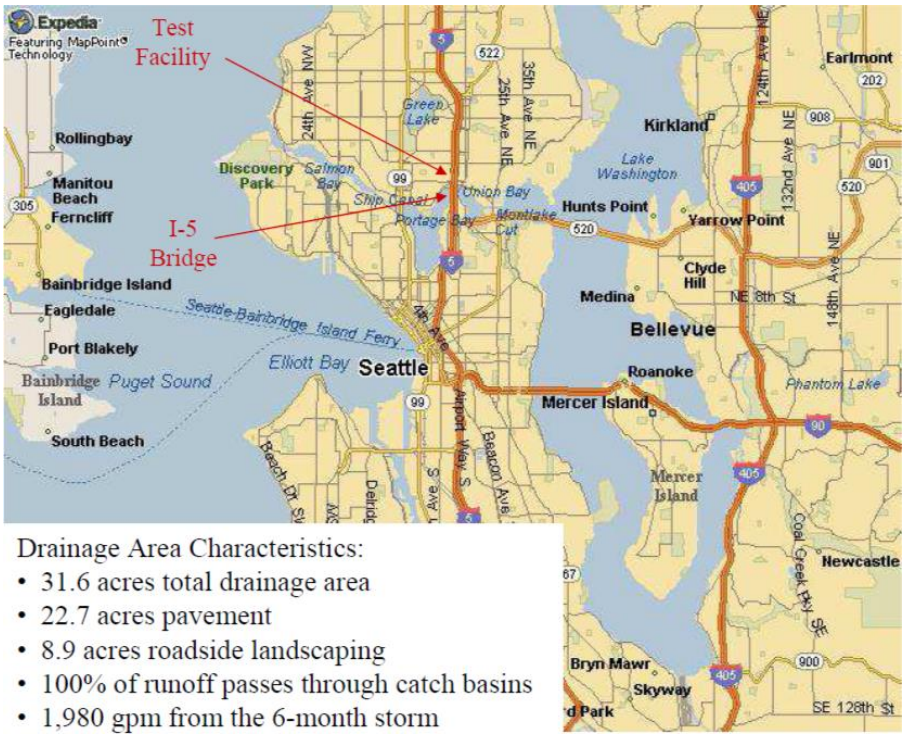
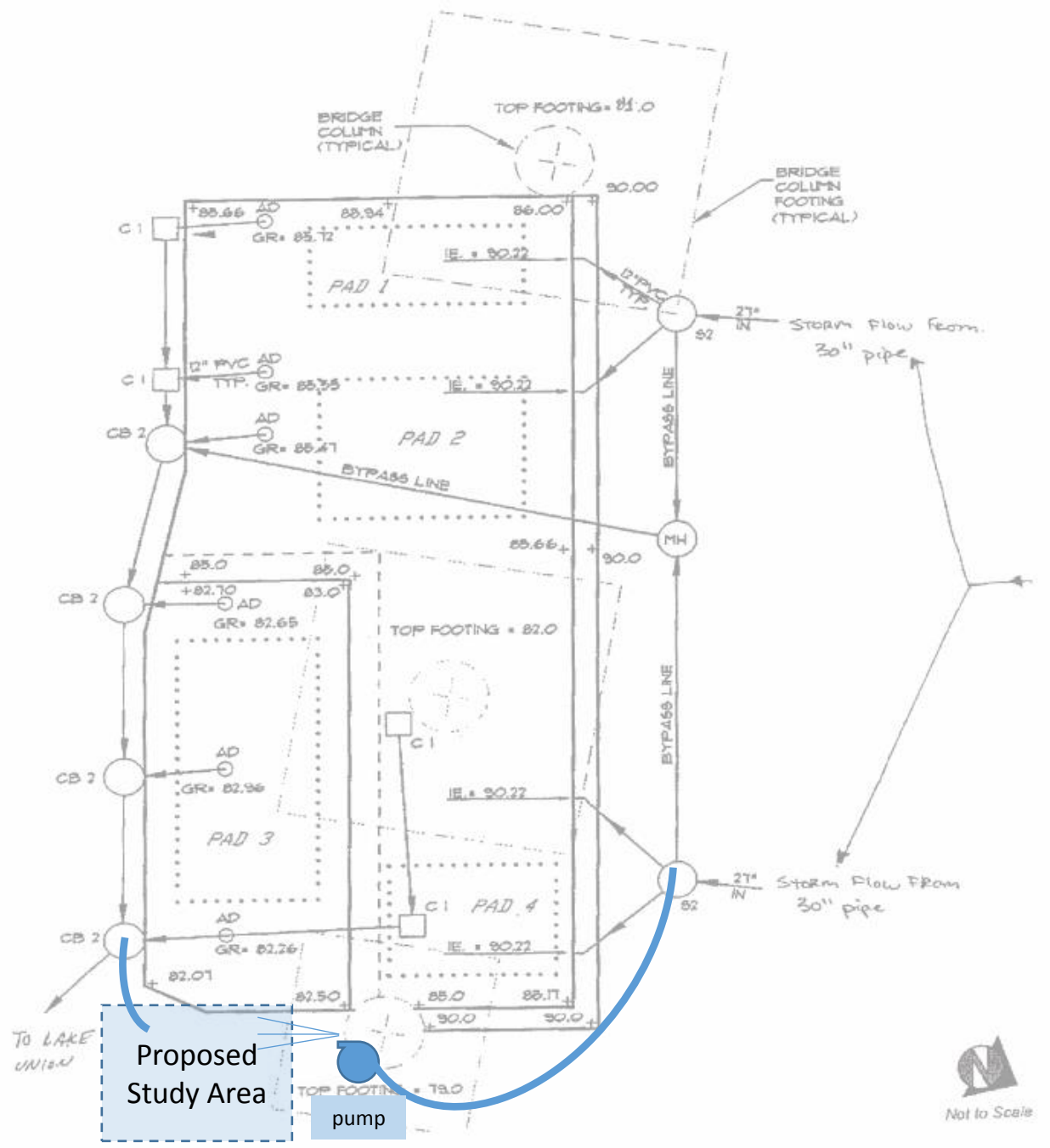


Figure 1: Map of the Seattle metro area indicating the location of the test site with urban drainage area characteristics.

Stormwater captured from I-5 and the surrounding drainage area at the WSDOT Ship Canal field testing location (Figure 2), will be used for this project. Storm flows will be diverted from a flow splitter at the right side of the figure below to this project's study area located just east of the site to allow for sunlight exposure to the bioretention mesocosm cells. Treated effluent will be discharged to a catch basin west of the study area. In 2015, the research facility was surveyed for installation of a treatment technology (Appendix A).



Influent Source and Distribution

Stormwater will be pumped from the flow splitter that diverts influent into flows that supply test bays 3 and 4. The pumped stormwater will be distributed evenly to 12 soil columns. Ports at the inflow and outflow of each bioretention mesocosm cell will allow water sample collection from each cell individually.

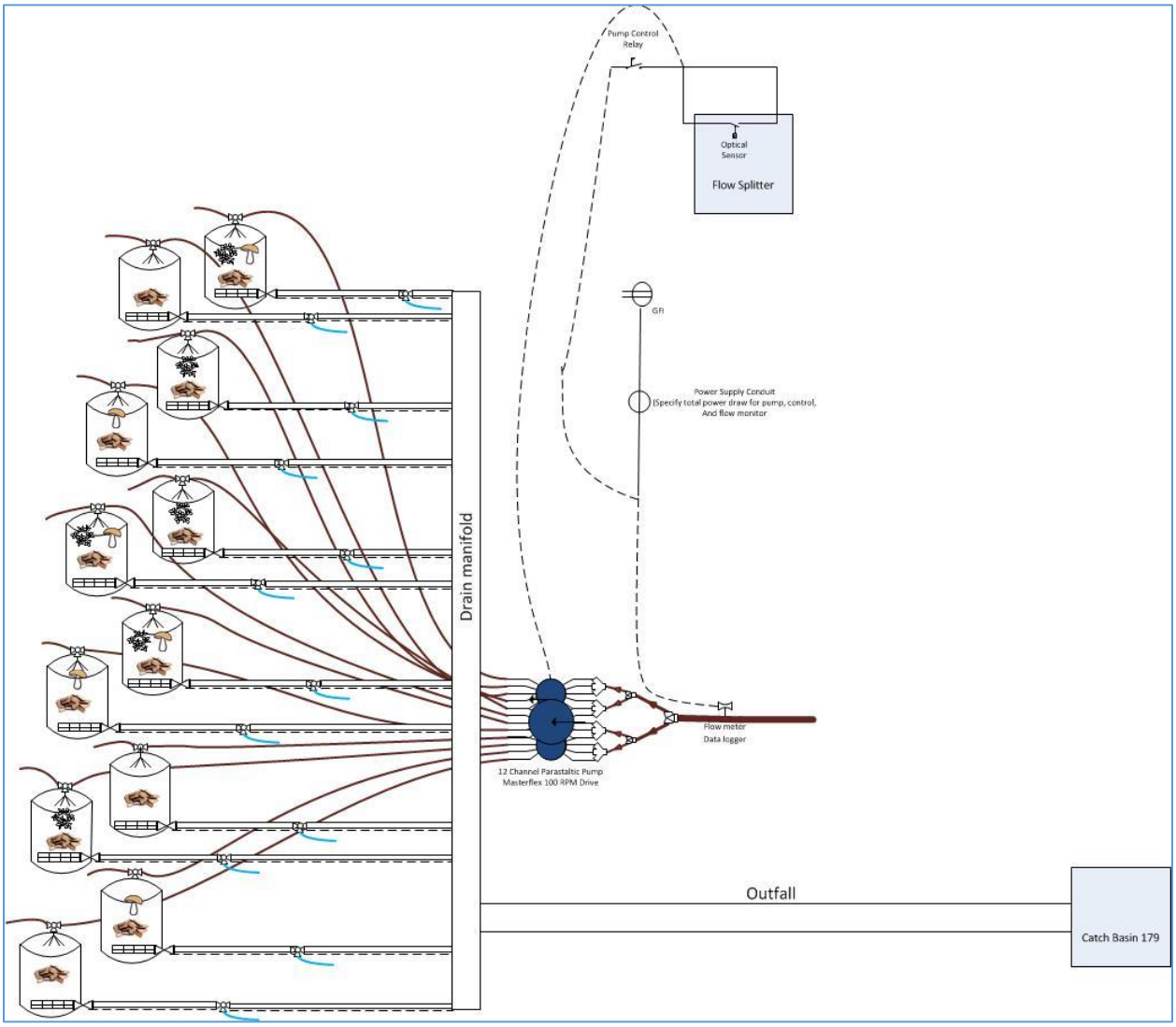


Figure 4: Piping and Instrumentation diagram for the study.

Two peristaltic pumps will operate in tandem on a float switch such that the pumps will be turned on whenever water is present in the flow splitter. An in-line flow meter and totalizer on the source pipe leading to the pumps will allow measurement of flow start and stop times (runoff duration) and totalized flow leading to all bioretention cells. This totalized flow value will be divided by 12 to ascertain the total volume of water that entered each bioretention cell during a given storm event as well as for the duration of the study.

The peristaltic pumps will be equipped with channel heads so that stormwater will be evenly distributed to the 12 columns. The pump will operate at a constant pumping rate for the duration of the study. The pump rate will be calibrated such that each bioretention cell is dosed with stormwater at rate equivalent to the 6 month design storm loading whenever water is present in the flow splitter. The pumped flow rate is computed based on a 20:1 impervious area to treatment area ratio as follows:

Each bioretention cell will be housed inside an 18 gauge 304 stainless steel 208 L (55 gallon) drum (Skolnik Industries Inc., Chicago IL) with a cross-sectional area (bioretention treatment area) of 0.256 m^2 (2.76 ft^2). At a 20:1 ratio, the surface area for each cell will treat a runoff area of 5.13 m^2 (55.2 ft^2). The six month design storm for Seattle, WA is 3.30 cm (1.30 in. or 0.108 ft) over 24 h (SMMWW).

The six month design storm for each bioretention cell is therefore $5.13\ m^2 \times 0.033\ m = 0.169\ m^3$ (5.97 ft^3) over a 24 hour period. When divided over a 24 hour period, the design flow rate is approximately 7.04 L/hour/cell = 117 mL/min/cell (0.248 ft^3/hour/cell).

The pump will therefore draw 1404 mL/min (0.371 gal/min) into the main inlet pipe, which will be evenly distributed between the 12 bioretention cells at a rate of 117 mL/min (1.85 gal/hour).

Sampling Procedures

Baseline Monitoring

We will analyze the BSM for metals and nutrients before the experiment. We will develop water retention curves from the gravel and the soil mix before they are installed into the bioretention cells. Toxicity testing of effluent from clean water infiltration of the bioretention media and drainage layer will qualify toxicity (or lack thereof) of the materials to aquatic biota.

Precipitation Monitoring

The bioretention mesocosm cells will receive runoff continuously from the peristaltic pump whenever water is present at the site. To aid in decision making about when to sample influent and effluent at the site, we will assess precipitation at the project site by using hydrology modeling coupled with recorded rain gauge data provided by the University of Washington and Seattle Public Utilities through the Seattle Rain Watch program (http://www.atmos.washington.edu/SPU/). Historical precipitation data is available in high geographic resolution from the program's network of regional weather stations. Hourly precipitation data is archived on the Seattle Rain Watch website for a period of two months for each weather station. Data will be downloaded and archived on a monthly interval for the proximal weather stations and geographic modeling techniques will be used to estimate precipitation at the project site's watershed area. Notably, the nearest weather station is at the UW Harris Hydraulics Laboratory, approximately 0.8 km (0.5 miles) from the project site. Detailed regional meteorological forecasting is available in real time to allow field staff to monitor the volume of rain events for sampling decision-making.

Satellite imagery and model predictions will serve as the basis for determining whether a rain event will be sampled. Weather information from one or more of the following sources will be evaluated for the project area on at least a daily basis from:

- Seattle Rain Watch at www.atmos.washington.edu/SPU/
- National Weather Service Forecast Office operated by the National Oceanic and Atmospheric Administration (NOAA) www.wrh.noaa.gov/sew
- AccuWeather at www.accuweather.com/en/us/united-states-weather
- KOMO news at www.komonews.com/weather

If a qualifying rain event appears imminent, field crew will prepare to sample by deploying collection equipment and notifying the laboratory.

Flow Monitoring

Flow to the bioretention cells will be controlled by a float switch that will be tethered alongside the peristaltic pump inlet pipe inside the flow splitter that supplies test bays 1 and 2. The pump inlet pipe and float switch will be immersed in stormwater whenever runoff is present at the site. The float switch will govern power to the peristaltic pumps. When water is present at the site, the peristaltic pumps will run at the established flow rate (117 mL per minute per tubing channel). An in-line flow meter/datalogger at the pump inlet pipe will record flow rate and totalized flow for the duration of the project period. The independently recorded flow rate will be used to check the performance of the peristaltic pumps and will be used to ensure that the pump draws water into the system at the designed flow rate (1404 mL/min). Flow rate data will be downloaded from the data logger during monthly visits to the project site during non-storm event days. Flow rate data will be compared monthly to rainfall data to confirm that the system is operating or to identify operation errors quickly.

Infiltration Testing

On a quarterly basis infiltration will be measured in each bioretention mesocosm cell:

1. With the under-drain closed the mesocosm will be filled up to the rim with water.
2. Open the mesocosm under-drain and allow water to drain down to the soil surface.
3. Record the time required for the water to drain from the rim to the soil surface and record the distance in cm between the soil surface and the rim.

This procedure will be repeated 3 times. Field personnel will record all the pertinent information on standardized field forms (see example in Appendix B) which will be scanned and stored in the project database.

Water Quality Sampling

For each sampling event, influent and effluent samples will be collected from each of the 12 bioretention mesocosm cells. This will result in triplicate data for each treatment. Due to the relatively low flow rate for bioretention cell loading, the following sampling procedure will be followed such that influent and effluent samples can be collected contemporaneously.

Glass sample collection bottles will be placed at the influent and effluent sampling tubes illustrated in Figure 4. At least one extra glass influent collection bottle and one effluent collection bottle will be brought to the site alongside the required 24 glass sample collection bottles to serve as a field blanks. Field blank containers will be selected at random at the start of the sampling period and will be situated in the field alongside sample collection bottles. The field blanks will be filled with de-ionized water at the laboratory after sample collection as a field blank to assess incidental contamination from insufficient cleaning of glassware, magnetic stir bar, or ambient conditions at the site.

Stormwater influent and effluent sample collection bottles will be fitted with a screw-on cap for each bottle which will have a hole that is the same diameter as the sample tubing so that the tubing can be held in place for sample discharge into the bottle while not allowing the outside of the tubing to touch the water sample. Each cap will be screwed down loosely to the sample

bottle to allow displaced air to escape as the bottle fills. At each junction on the pipes where the water sampling tubes connect, flow-diverting "Y" splitter valves will allow flow to be discharged to the sampling tubing rather than to the standard operation manifold.

Before sampling begins, all of the flow-diverting "Y" splitter valves will be engaged for 2 minutes to flush the tubing prior to sample collection. After flushing each sample collection tube, the tubes will be attached to sample collection bottles. There will be 12 2-liter influent collection bottles (one for each bioretention mesocosm cell), and 12 7-liter effluent collection bottles (one for each bioretention mesocosm cell). Each collection bottle (the 2-liter influent collection bottles and the 7-liter effluent collection bottles) will be placed on ice in the field so that collected water can cool during the approximate 1.25 hour sample collection period. At the start of sampling, the flow-diverting "Y" splitter valves on the influent sampling ports will be engaged one-by-one to divert flow into the influent sample collection bottles. The influent sample collection bottles will be allowed to fill for five minutes each, such that each influent sample bottle receives approximately 585 mL of influent (117 mL/min x 5 minutes). After five minutes the influent sampling diverter valves will be closed to re-direct water through the bioretention cells, and the effluent sampling diverter valves will be engaged to start effluent sample collection. Effluent sample collection will follow an analogous process except that the effluent bottles will be allowed to fill for 20 minutes. This will add approximately 2,340 mL to each of the effluent sample bottles (117 mL/min x 20 minutes). This entire process will be repeated three times so that influent collection bottles each receive 1,755 mL (117 mL/min x 5 minutes x 3 sampling intervals) and effluent collection bottles each receive 7,020 mL (117 ml/min x 20 minutes x 3 sampling intervals). In this way, samples of influent and effluent are collected simultaneously over approximately 1.25 hours.

Field personnel will record all the pertinent information on standardized field forms (see example in Appendix C) which will be scanned and stored in the project database.

Table 5: Influent and effluent sampling intervals.

Sample	Time (minutes)	Volume per Bottle per Sampling Interval (mL)	Running Total Volume Collected per Bottle (mL)
Influent	0-5	585	585
Effluent	5-25	2,340	2,340
Influent	25-30	585	1,170
Effluent	30-50	2,340	4,680
Influent	50-55	585	**1,755**
Effluent	55-75	2,340	**7,020**

Because the influent is not expected to vary significantly between the 12 soil columns, the laboratory will only receive triplicate influent samples (instead of 12 individual influent samples). The 12 influent samples that are collected in the field will therefore be selected at

random and composited in the field to a create three composite influent samples of approximately 7,000 mL each (1,755 mL x 4 = 7,020 mL) by pooling the entire contents of four randomly selected influent sample bottles into clean 7,000 mL bottles. This will be repeated three times to create three representative influent samples for testing at the laboratories as individual field replicates. The influent will be regarded as constant between each treatment type (soil, soil + plants, soil + fungi, soil + plants + fungi) and will be represented in the analysis as the value of the composite sample mean and sample error. In contrast, each effluent sample will be submitted to the lab as an individual replicate. In total, each storm event will produce 15 samples for the analytical labs.

After each composite influent sample is prepared as previously described and each individual effluent sample is collected in the chilled 7,000 mL glass bottles, the collected stormwater will be transported to NOAA NWFSC where it will be subdivided into the laboratory sample bottles as outlined in table 5, below. Each of the 7,000-mL glass collection bottles will be equipped with a clean Teflon magnetic stir bar to continuously mix the collected stormwater during sample subdivision. New Teflon tubing and a siphon pump will be used in each carboy to subsample stormwater in to aliquots for analysis.

Each set of 12 laboratory sample bottles will be pre-labeled with indelible ink and stored on ice. Following sample collection, composite creation, subdivision into aliquots, and storage in individual ice chests, samples will be directly delivered to the analytical labs as follows:

- Water quality samples except PAHs - AmTest Laboratories (13600 NE 126th Pl, Kirkland, WA).
- PAHs – Analytical Resources, Inc. (4611 S. 134th Place, Suite 100, Tukwila, WA 98168).
- Water samples for toxicity – WSU-P (2606 West Pioneer, Puyallup WA 98371). Samples will be frozen on dry ice in the field and maintained frozen at -20 °C until used.

Soil Sampling

Data from the data loggers (soil water content, soil matric potential, soil electrical conductivity, influent flow rate and totalized flow) will be downloaded at each site visit. These data will be immediately checked for evidence of an equipment malfunction or other operational problem. The data will then be added to a database.

Soil samples will be collected for this project to address three different study questions and therefore samples will be collected under three different regimens that correspond to each question. Soil samples will be collected at quarterly intervals for PCB analysis only by King County personnel (King County, 2016) for two years from the top 12 inches of soil from all 12 bioretention cells using a small diameter hand-push auger. All 12 bioretention cells will be sampled to maintain consistent treatment across the bioretention cells, however only the samples from the 6 columns without introduced fungi will be analyzed for PCBs (King County, 2016). A separate sampling regime will be used for PAHs and metals. For these analyses, samples will be collected during installation and also at deconstruction after two years of field operation.

During installation the starting BSM will be sampled for PAH and metal concentration prior to installation. Due to uncertainty about the source of metal leaching from BSM media, the two components (sand and compost) will be analyzed separately. A composite sample of each material (sand and compost) delivered from the supplier will be collected by taking 10 samples from various locations and depths around the delivered material pile, homogenizing, and subsampling in triplicate. Since PAHs are not anticipated at detectible concentrations in sand, the PAH samples will be collected from the finished 60/40 (sand/compost) mixture during installation into the soil columns. Composite grab samples will be collected as the BSM is added to each drum. Small grab samples will be collected from each drum at multiple layers. Each grab sample from each layer will be split into three 250 mL soil containers supplied by the laboratory. Each soil container will therefore represent a replicate composite of all the starting BSM soil in the 12 soil columns.

Following the two-year water runoff study period, soil samples will be collected for PAHs and metals (zinc and copper) from each bioretention cell. Material for metals analysis will be collected from three depths: 0 – 15 cm (0 – 5.9 in.); 15 – 30 cm (5.9 – 11.8 in.); 30 – 45 cm (11.8 – 17.7 in.). A comparison of these values from the start and end of the project will allow us to assess accumulation and movement of metals. Soil samples will be collected in 250 ml containers from each from each layer of each bioretention cell during installation. During deconstruction, each bioretention cell will be sampled in three locations at each depth using a 2” diameter x 12” length soil recovery auger (AMS Inc., American Falls, ID) according to ASTM D4700-15: Standard Guide for Soil Sampling from the Vadose Zone.

For each layer sampled during deconstruction, three soil samples from a given depth will be pooled to create a composite for each bioretention cell layer. Three layers will be sampled (1 composite per layer per bioretention cell) for a total of 36 soil samples. Samples will be collected in soil sample containers provided by the laboratory, stored and transported according to ASTM D4220: Standard Practices for Preserving and Transporting Soil Samples.

Due to low mobility of higher molecular weight PAHs in soil (Greeney et al, 1987), the PAH samples will only be collected from the upper BSM soil layer, 0 – 15 cm (0 – 5.9 in.). Analysis of PAH concentration in the BSM across treatments will improve understanding of the role of plants and introduced fungi in soil PAH metabolism.

Table 6: Water Sample Analytical Matrix.

Bottle	Water Parameter	Method	Sample Size	Container	Holding Time	Preservation
1	Total Metals (Zn, Cu)/Hardness	EPA 200.7/ SW6010C	250 mL	HDPE	6 months	HNO3, 6 °C
2	Dissolved Metals (Zn, Cu)	EPA 200.8	250 mL	HDPE	6 months	Filter, HNO3, 6 °C
3	Total Suspended Solids	SM2540D	500 mL	HDPE	7 days	6 °C
4	Total Organic Carbon	SM5310B	40 mL	Amber	28 days	H2SO4, 6 °C
	Dissol. Organic Carbon	SM5310B	40 mL	Amber	28 days	Filter w/in 48 hours, H2SO4, 6 °C
	Chem. Oxygen Demand	EPA 410.4	150 mL	Amber	28 days	H2SO4, 6 °C
5	Total Phosphorous	SM4500-PE	250 mL	HDPE	28 days	H2SO4, 6 °C
	TKN	SM4500-Norg	250 mL	HDPE	28 days	H2SO4, 6 °C
	Ammonia	EPA 350.1M	250 mL	HDPE	28 days	H2SO4, 6 °C
	Nitrate + Nitrite	EPA 353.2	250 mL	HDPE	28 days	H2SO4, 6 °C
6	Ortho-phosphorous	SM4500-PE	50 mL	HDPE	48 hours	6 °C
	pH	SM4500HB	250 mL	HDPE	8 hours	6 °C
7	Alkalinity	SM2320B	250 mL	HDPE	14 days	No head-space, 6 °C
8	Fecal Coliform	SM9222D	125 mL	Corning	8 hours	Sodium thiosulfate, 6 °C
9	E. coli	SM9222DG or SM9221F	125 mL	Corning	8 hours	Sodium thiosulfate, 6 °C
10	PAHs	EP 8270D-SIM	Two 500 mL	Amber	7 days	6 °C
11	PCB*					
12	D. rerio acute toxicity	McIntyre 2014	450 mL	Amber	6 months	Freeze in field, store at -20 °C
	Total		**5,470 mL**			

*Water sample for PCB analysis will be collected by King County personnel (King County, 2016)

Table 7: Soil Sample Analytical Matrix.

Bottle	Soil Parameter	Method	Sample Size	Container	Holding Time	Preservation
1	Total Metals (Zn, Cu)	EPA 200.8	250 mL	HDPE	6 months	6 °C
2	PAHs	EPA 8270D	8 oz	Clear glass	6 months	6 °C

Toxicity Testing

Toxicity of the urban runoff influent and bioretention treated effluent for 8 rain events over a 2-year study period will be evaluated for mortality and sublethal cardiovascular toxicity in zebrafish (*Danio rerio*). Effluent from the three bioretention mesocosm cells representing each treatment type (plants, no plants, fungi, no fungi) will be combined into a composite sample, along with a single influent composite sample for each toxicity test. Thus, each toxicity test will consist of five samples:

1. No plants / no fungi effluent
2. No plants / yes fungi effluent
3. Yes plants / no fungi effluent
4. Yes plants / yes fungi effluent
5. Influent

Methods will follow previously published methods for urban runoff toxicity to zebrafish embryos (McIntyre et al. 2014). Embryos will be exposed to thawed runoff for 48 hours beginning at 2 to 4 hours post-fertilization (hpf). Four replicates of 15 embryos will be used to assess the impact of runoff on survival, hatch timing, and morphometric endpoints. Runoff samples will be thawed the same morning that testing begins. Frozen runoff was previously shown to not alter the impact of highway runoff on survival or cardiovascular metrics in *D. rerio* embryos exposed for up to 96 h. The ability to work with frozen runoff is important because embryos cannot always be reared on a cycle that is timely for toxicity testing. A detailed protocol can be found in Appendix E.

Embryos will be imaged at test termination and morphometrics (physical attributes) will be measured from recorded digital images and videos using the open-source software ImageJ (https://imagej.nih.gov/ij/). WSU will perform laboratory controls for each toxicity screen. The laboratory control will be four replicates of 15 embryos that are exposed to zebrafish rearing water.

Re-useable glassware for toxicity testing will be decontaminated by washing it with Simple Green®, cleansed in reverse osmosis (RO) water for a minimum of three rinse cycles and then rinsed under a hood three times in acetone and then methylene chloride. Clean glassware will be allowed to air dry under the hood.

Quality Control

Quality control procedures are identified below for field and laboratory activities. The overall objectives of these procedures are to ensure that data collected for this project are of a known and acceptable quality, and that data quality objectives are met.

Field Quality Control

Quality control procedures that will be implemented for field activities are described below.

Instrument Maintenance and Calibration

Field Notes

During each pre- and post-storm site visit to each monitoring station, the following information will be recorded on a waterproof, standardized field form (see example in Appendix B):

- Mesocosm media tank identification
- Date/time of visit and last sample collected (if sampled)
- Name(s) of field personnel present
- Weather and flow conditions
- Sample volume (if sampled)
- Presence of obstructions in system and remedial actions taken
- Unusual conditions (e.g., oily sheen, odor, color, turbidity, discharges or spills, ponded water, etc.
- Modifications of sampling procedures

Additionally, photographs will be taken at each site visit of each drum to document plant health, mulch condition, and the overall operation of the system. At each site visit data from the flow monitor will be downloaded and qualitatively compared with storm rain gage data to determine if operational failures occurred. Manual calibrations of the peristaltic pump will also be performed to assure that tubing is not occluded and that each bioretention cell receives the equivalent dosing at the desired flow rate (117 mL/min).

During each soil sampling field visit, the following information will be recorded on a waterproof standardized field form (see example in Appendix D):

- Mesocosm media tank identification
- Date/time of visit
- Name(s) of field personnel present
- Weather and flow conditions
- Number of samples collected/composited
- Sample depth
- Sample duplicated?

- Unusual conditions (e.g., oily sheen, odor, color, turbidity, discharges or spills, ponded water, etc.)
- Modifications of sampling procedures

Field Duplicate Split Samples – Soil

Field duplicate split samples will be collected at a sufficient frequency to represent at least 5 percent of the total number of project samples analyzed. Soil sample field duplicate split samples will be collected by mixing the sample in a pre-cleaned stainless steel bowl with a pre-cleaned stainless steel spoon until the mixture is homogenous. The sample will subsequently be split in two and placed in separate soil bags. Duplicate sampling stations will be selected randomly.

All duplicate samples will be submitted to the laboratory and labeled as separate (blind) samples. The resultant data from these samples will then be used to assess variation in the analytical results that is attributable to environmental (natural), sub-sampling, and analytical variability.

Sample Handling, Delivery, and Processing

Ice will be placed around the glass bottles that will be used to collect the influent and effluent from each of the bioretention cells over the 1.25 hour sample collection period. After each targeted storm event, all samples will be minimally processed in the field to prevent potential contamination from trace pollutants in the atmosphere. During delivery to the NOAA laboratory for sample aliquot preparation, all water quality sample containers (i.e., glass collection bottles and the 12 analyte sample bottles for each bioretention cell) will be transported in coolers with ice and kept below 6 degrees Celsius. The volume of ice should be equal to or greater than the volume occupied by samples (twice the volume of ice to samples is recommended during warm temperatures) (USGS, 2003). The temperature of the samples will be measured upon sample delivery and recorded on the chain of custody form.

In the NOAA laboratory, sample bottles for each bioretention cell will be pre-labeled with all pertinent information. The 12 sample bottles will then be filled from the glass collection bottle. Each glass carboy will be equipped with a clean Teflon magnetic stir bar to continuously mix the collected stormwater during sample subdivision. New Teflon tubing and a siphon pump will be used in each carboy to subsample stormwater in to aliquots for analysis.

Sample Identification and Labeling

Each water and soil sample will be identified with a unique label. Bottles from water quality monitoring will be labeled with the following information using indelible ink and labeling tape:

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

All bags from soil monitoring will be labeled with the following information using indelible ink and labeling tape:

- Mesocosm ID number
- Soil column horizon (e.g., 0 to 7.6 cm)
- Date of sample collection (year/month/day: yyyy/mm/dd)
- Time of sample collection (international format [24 hour])
- Field personnel initials

Sample Containers and Preservation

Clean, decontaminated water sampling containers will be obtained from the analytical laboratory in advance of each storm event. Spare sample containers will be carried by the sampling team in case of breakage or possible contamination. Sample containers and preservation techniques will follow U.S. EPA (2007) guidelines. After samples are processed, laboratory personnel will clean glass collection bottles and magnetic stir bars with a five step process:

1. Liquinox detergent rinse
2. Reagent grade water rinse
3. 10 percent hydrochloric acid rinse
4. Reagent grade water rinse
5. Rinse with ultra-grade acetone and allow to air dry

Teflon tubing used for dispensing the stormwater samples into the analytical collection bottles will be new tubing for each sampling event and will not be re-used.

Chain-of-Custody Record

A chain-of custody record will be maintained for each sample batch listing the sampling date and time, sample identification numbers, analytical parameters and methods, persons relinquishing and receiving custody, dates and times of custody transfer, and temperature of sample upon delivery.

Laboratory Quality Control

Quality control procedures that will be implemented in the laboratories are described in the following subsections.

Control Standards

Control standards for each parameter will be analyzed by the laboratory with every sample batch. A laboratory sample batch will consist of no more than 20 samples and may include samples from other projects. Raw values and percent recovery (see formula in the Quality Objectives section) for the control standards will be presented in each laboratory report.

Matrix Spikes

For applicable parameters, matrix spikes will be analyzed by the laboratory with every sample batch. A laboratory sample batch will consist of no more than 20 samples and may include samples from other projects. Raw values and percent recovery (see formula in the Quality Objectives section) for the matrix spikes will be presented in each laboratory report.

Laboratory Duplicate Split Samples

Laboratory split-sample duplicates for each parameter will be analyzed for specifically labeled QA samples submitted with every sample batch. This will represent no less than 5 percent of the project submitted samples. Raw values and relative percent difference (see formula in the Quality Objectives section) of the duplicate results will be presented in each laboratory report.

Field Blanks

One field blank for each water parameter will be analyzed with every sample batch. Field blanks will consist of commercially available distilled water introduced in the field into a randomly selected glass carboy that is handled in the field as if it were a stormwater sample container. This test will assure that glass carboy, stir bar, and sample dispensation processes are not a source of contamination.

No field blanks will be used for soil parameters.

Data Management

A relational database will be developed to store data gathered as part of this project: water quality, hydraulic conductivity, data logger, precipitation, pumped flow rate and totalized flow, quality control, and toxicity data. The database will also include all the metadata associate with this project. Statistical analysis will be performed in R. Data tables, meta data, and statistical scripts will be compiled to an R package and the package will be available in the WSU website after the project. Additionally, the tables will be exported in csv and excel file format.

Audits and Reports

During this study, routine audits of the compiled data will be performed to ensure this QAPP is being implemented correctly. In addition, the data from this study will be summarized in annual reports. The activities are described in more detail in the following subsections.

Audits

Audits will be performed to detect potential deficiencies in the hydrologic, water quality, toxicity, and soil data that will be collected for this project. Audits for soil hydrologic data will occur after each site visit. In connection with these audits, the project Quality Assurance Coordinator will examine the new data collected from each monitoring location in relation to data from prior monitoring to identify potential QA issues. This audit will specifically include an examination of the data record for gaps, anomalies, or inconsistencies in the flow data. Any data generated from calibration checks that were performed at a particular monitoring location will also be entered into control charts and reviewed to detect potential instrument drift or other operational problems. In the event that QA issues are identified on the basis of these audits, the Quality Assurance Coordinator will immediately perform a site visit to troubleshoot the problem and to implement corrective actions if possible. Any QA issues that are detected through these audits will be documented in the electronic data record.

Audits performed for water quality and soil data will occur within four weeks of receiving results from the laboratory. This review will be performed to ensure that all data are consistent, correct, and complete, and that all required quality control information has been provided. Results from these audits will be documented in standardized quality assurance worksheets (see example in Appendix F) that will be prepared for each batch of samples. In the event that a potential quality assurance issue is identified through these audits, the Quality Assurance Coordinator for the study will review the data to determine if any response actions are required. Response actions might include the collection of additional samples or the reanalysis of existing data. Any QA issues that are detected through these audits will be documented in the quality assurance worksheets.

Reports

Annual reports will be prepared through the course of this study to present compiled data, analysis results, and major study conclusions. Each report shall include all monitoring data collected during the preceding water year (October 1 – September 30). The second year report will also integrate data from the first year into the analysis of results, as appropriate. The reports will be submitted in both paper and electronic form (PDF) and include the following specific information:

- A 2-page summary of the project setup, intent, results, analysis, and implications written for a lay-person audience
- Results from hydrologic monitoring performed in connection with each bioretention mesocosm cell

- Results from water quality and soil sampling performed in connection with each bioretention mesocosm cell
- Results from toxicity tests
- Graphical and tabular summaries for the collected data
- Results from any statistical analyses that are performed on the data
- Major conclusions from monitoring performed over the water year

Appendices with tabular compilations of all raw monitoring data, field data sheets, laboratory analytical reports, chain of custody documentation, and the Data Quality Assurance Memorandum (see Data Quality Assessment section)

Finally, the relational database will be provided to the RSMP Coordinator and will be available from WSU.

Data Verification

Data verification will be performed to determine the quality of the compiled data. This process involves a detailed examination of the associated quality control results to determine if the MQOs specified in the Quality Assurance section have been met. The specific procedures that will be used to verify and validate hydrologic and chemistry data are described in the following sections.

Water Quality and Soil Data Verification and Validation

Water quality data obtained for the study will be reviewed by the Quality Assurance Coordinator to verify that all samples were collected in accordance with the procedures identified in this QAPP and that all required quality assurance/quality control (QA/QC) information was provided by the laboratory. The Quality Assurance Coordinator will then examine the data to determine if there were any errors or omissions. EPA functional guidelines for data validation of MS/MSD, control limits and other method parameters will be used first and then the data usability review will examine results relative to the MQOs.

For soil data, values associated with minor quality control problems will be considered estimates and assigned J. Values associated with major quality control problems will be rejected and qualified R. Estimated values may be used for evaluation purposes, while rejected values will not be used.For water quality data, sample is interpreted to represent the mean concentration during the sampling period, but not necessarily the mean concentration for the whole storm event. However, laboratory error can lead to compromised data which is not representative of the target. Therefore, the water quality data collected for this study will be labeled with unique quality assurance flags for both laboratory and field data QA issues. Data qualifiers will be consistent with typical laboratory qualifier conventions as shown below:

- J = Value is an estimate based on analytical results. Used when measurement quality objectives for field duplicates, laboratory duplicates, matrix spikes, laboratory control samples, holding times, or blanks have not been met.
- REJ = Value is rejected based on analytical results. Used when major quality control problems with the analytical results.
- U = Value is below the reporting limit. Used based on laboratory method reporting limit.
- UJ = Value is below the reporting limit and is an estimate based on analytical results. Used based on laboratory method reporting limit; MQOs for analytical results have not been met.

Estimated values may be used for evaluation purposes, while rejected values will not be used.

The following guidelines will be applied when evaluating holding times for parameters with holding times that are less than 7 days:

- Data from samples that exceed the specified maximum post-filtration holding times by less than 24 hours will be considered estimates (J)

- Data from samples that exceed the maximum post-filtration holding times by more than 24 hours will be rejected values (REJ)

Reporting Limits

Both raw values and reporting limits will be presented in each laboratory report. If the proposed reporting limits are not met by the laboratory, the laboratory will be requested to reanalyze the samples and/or revise the method, if time permits. Proposed reporting limits for this project are summarized in Tables 3 and 4.

Duplicates

Duplicate results exceeding the MQOs for this project (see Quality Objectives section) will be recorded in the raw data tables, and noted in the quality assurance worksheets and associated values will be flagged as estimates (J). If the objectives are severely exceeded (e.g., more than twice the objective), then associated values will be rejected (REJ).

Matrix Spikes

Matrix spike results exceeding the MQOs for this project (see Quality Objectives section) will be noted in the quality assurance worksheets, and associated values will be flagged as estimates (J). However, if the percent recovery exceeds the MQOs and a value is less than the reporting limit, the result will not be flagged as an estimate. Non-detected values will be rejected (REJ) if the percent recovery is less than 30 percent.

Control Standards

Control standard results exceeding the MQOs for this project (see Quality Objectives section) will be noted in the quality assurance worksheets and associated values will be flagged as estimates (J). If the objectives are severely exceeded (e.g., more than twice the objective), then associated values will be rejected (REJ).

Field Blanks

One field blank for each water quality parameter will be analyzed with every sample batch. Field blanks will consist of commercially available distilled water introduced in the field into a randomly selected glass carboy that is handled in the field as if it were a stormwater sample container. This test will assure that glass carboy, stir bar, and sample dispensation processes are not a source of contamination. Water quality data values (except for bacteria) within 2-times the blank detected value will be qualified as “J.” Bacteria field blanks will be run and used for qualitative QC interpretation, however no quantitative limit will be set for differences between blanks and samples.

Data Quality Assessment

The subsection below describes the process for determining whether the data meet project objectives once the data results are compiled. Data analysis procedures that will be used to meet these objectives are then summarized in the following subsection.

Data Usability Assessment

Based on the results from the processes described in the Data Verification section, the Quality Assurance Coordinator will prepare annual Data Quality Assurance Memoranda to summarize quality control results, identify when data quality objectives were not met, and discuss the resulting limitations, if any, on the use or interpretation of the data. Specific QA information that will be noted in each data validation memorandum is as follows:

- Changes in the monitoring and quality assurance plan
- Results of performance and/or system audits
- Significant quality assurance problems and recommended solutions
- Data quality assessment results in terms of precision, bias, representativeness, completeness, comparability, and reporting limits
- Discussion of whether the quality assurance objectives were met, and the resulting impact (if any) on decision-making
- Limitations on use of the measurement data

These Data Quality Assurance Memoranda will establish the usability of data and will be included as an appendix to data reports (see Audits and Reports section) that are prepared for each water year.

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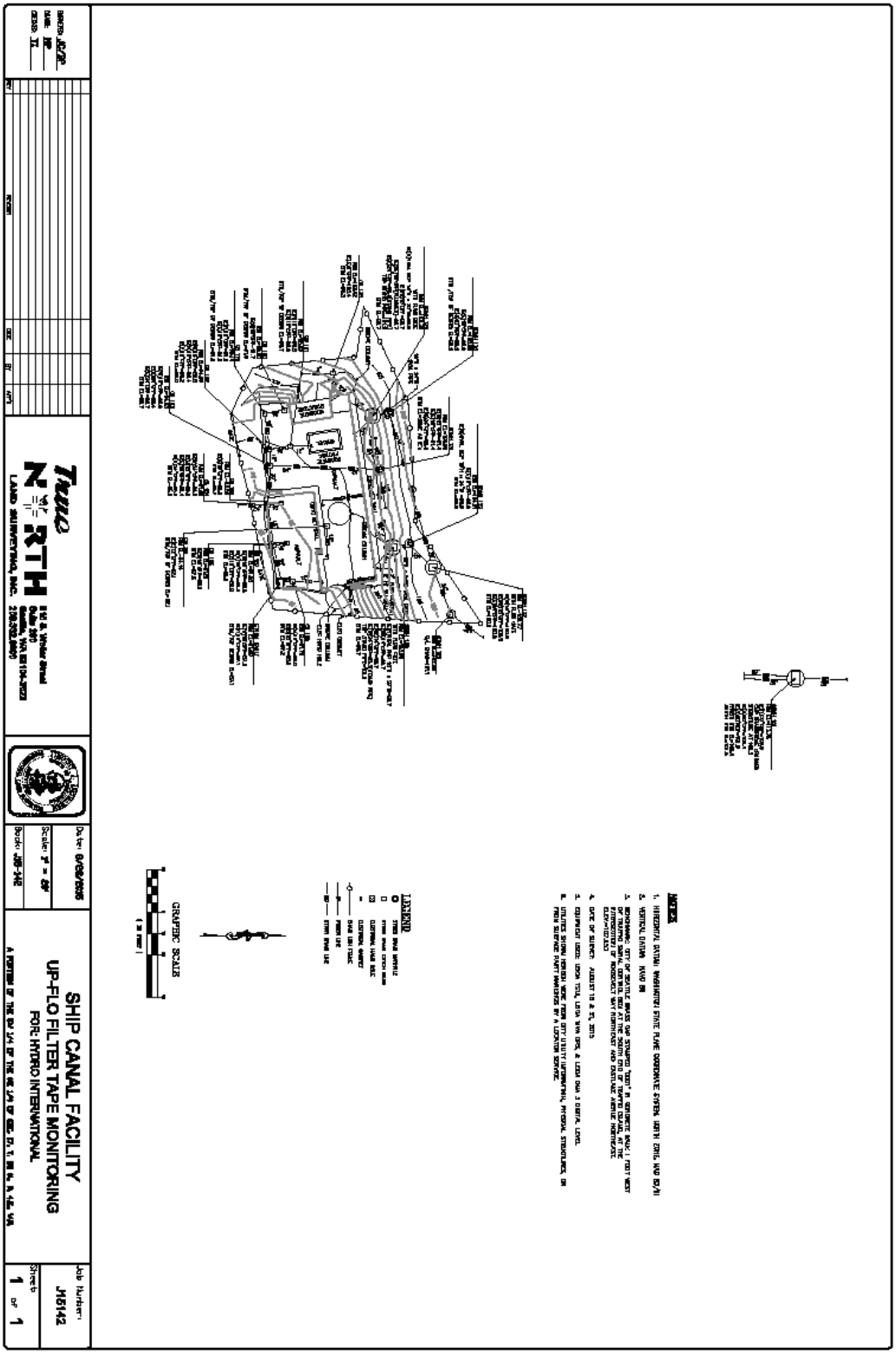
Appendix A


SHIP CANAL FACILITY
UP-FLO FILTER TAPE MONITORING
FOR: HYDRO INTERNATIONAL
J16142
1 of 1

Appendix B

Date

Time

Personnel

Weather

Mesocosm ID	Rim Height from Soil Surface		Mesocosm ID	Rim Height from Soil Surface	
	Start Time			Start Time	
	Stop Time			Stop Time	
Notes			Notes		
Mesocosm ID	Rim Height from Soil Surface		Mesocosm ID	Rim Height from Soil Surface	
	Start Time			Start Time	
	Stop Time			Stop Time	
Notes			Notes		
Mesocosm ID	Rim Height from Soil Surface		Mesocosm ID	Rim Height from Soil Surface	
	Start Time			Start Time	
	Stop Time			Stop Time	
Notes			Notes		
Mesocosm ID	Rim Height from Soil Surface		Mesocosm ID	Rim Height from Soil Surface	
	Start Time			Start Time	
	Stop Time			Stop Time	
Notes			Notes		
Mesocosm ID	Rim Height from Soil Surface		Mesocosm ID	Rim Height from Soil Surface	
	Start Time			Start Time	
	Stop Time			Stop Time	
Notes			Notes		

Appendix C

Date							
Time							
Personnel							
Weather							
Site Inspection							Notes
Pump		Power		Flow		Tubing	
Flow Logger		Power		Logging		Download (after water sampling)	
Dist. Pipes		Flow on all 12 outlets		Cracks/Leaks		Fatigue	

Cell ID	Influent bottle connected and placed on ice (time)	Effluent bottle connected and placed on ice (time)	1 minute	5 Minutes		20 Minutes		5 Minutes		20 Minutes		5 Minutes		20 Minutes	
			Calibration (measure influent volume to check pumping rate)	**Influent ON**	**Influent OFF**	**Efflent ON**	**Effluent OFF**	**Influent ON**	**Influent OFF**	**Effluent ON**	**Effluent OFF**	**Influent ON**	**Influent OFF**	**Effluent ON**	**Effluent OFF**

Appendix D

Date					
Time					
Personnel					
Weather					
Mesocosm ID	# Samples Collected for Composite		Mesocosm ID	# Samples Collected for Composite	
	Sample Depth			Sample Depth	
	Sample Duplicated?			Sample Duplicated?	
Unusual Conditions?:			Unusual Conditions?:		
Notes:			Notes:		
Mesocosm ID	# Samples Collected for Composite		Mesocosm ID	# Samples Collected for Composite	
	Sample Depth			Sample Depth	
	Sample Duplicated?			Sample Duplicated?	
Unusual Conditions?:			Unusual Conditions?:		
Notes:			Notes:		
Mesocosm ID	# Samples Collected for Composite		Mesocosm ID	# Samples Collected for Composite	
	Sample Depth			Sample Depth	
	Sample Duplicated?			Sample Duplicated?	
Unusual Conditions?:			Unusual Conditions?:		
Notes:			Notes:		
Mesocosm ID	# Samples Collected for Composite		Mesocosm ID	# Samples Collected for Composite	
	Sample Depth			Sample Depth	
	Sample Duplicated?			Sample Duplicated?	
Unusual Conditions?:			Unusual Conditions?:		
Notes:			Notes:		
Mesocosm ID	# Samples Collected for Composite		Mesocosm ID	# Samples Collected for Composite	
	Sample Depth			Sample Depth	
	Sample Duplicated?			Sample Duplicated?	
Unusual Conditions?:			Unusual Conditions?:		
Notes:			Notes:		
Mesocosm ID	# Samples Collected for Composite		Mesocosm ID	# Samples Collected for Composite	
	Sample Depth			Sample Depth	
	Sample Duplicated?			Sample Duplicated?	
Unusual Conditions?:			Unusual Conditions?:		
Notes:			Notes:		

Appendix E

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

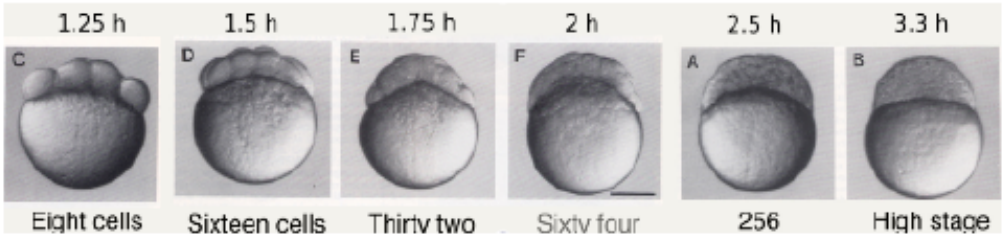
DAY 0

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.



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.

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.

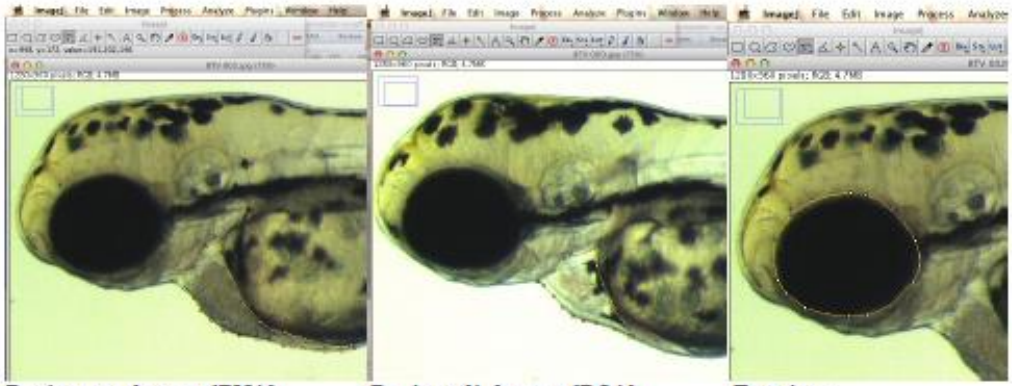
IMAGE ANALYSIS

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

From stills:

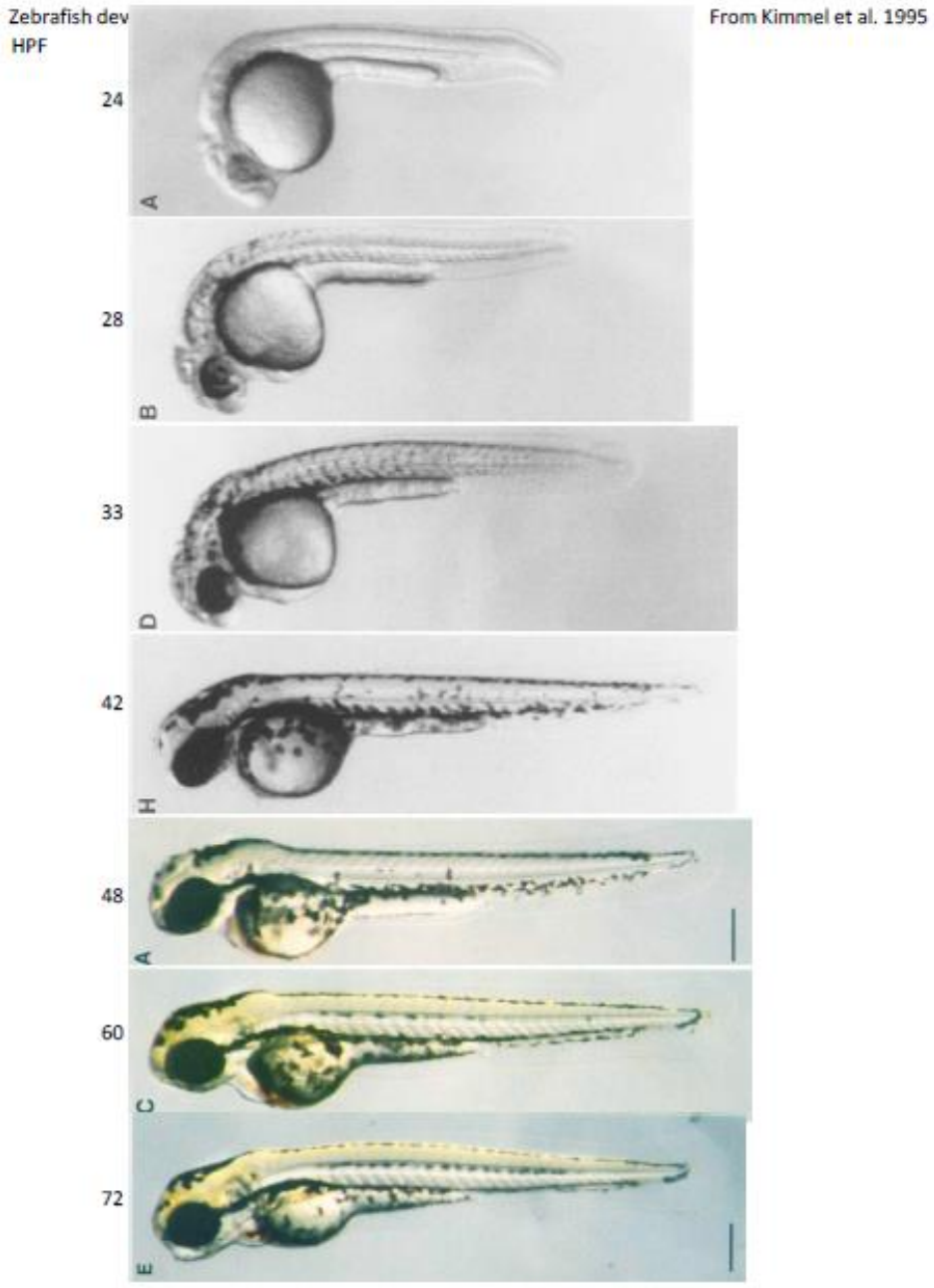
Using Image J, open the first image file in the length folder. Choose the segmented line tool by right clicking on the line tool. Click on the end of the embryo tail, follow the notochord to the ear, and finish the line at the fish head by crossing through the middle of the eye. Double-click to end line. 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.

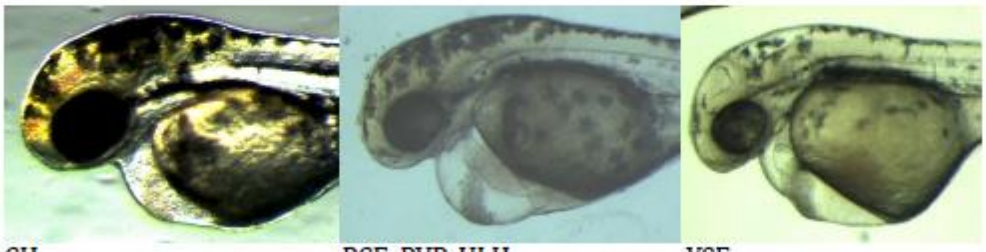
Regurgitation (R). Score = 1 if mild atrial regurgitation. Score = 2 if severe regurgitation (very little net forward motion). Score = 3 if circulatory stasis.

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.