FINAL REPORT

Project Title

Testing the effectiveness of bioretention at reducing the toxicity of urban stormwater to coho salmon

Lead Entity

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Abstract

Myriad efforts are underway to reduce the ecological impacts of polluted stormwater runoff throughout Puget Sound with Low Impact Development methods (LID) being just one example. While these activities represent an unprecedented societal investment, there is still little known about their effectiveness – specifically, whether toxic loadings are reduced to the extent that the biological integrity of "receiving waters" (aquatic habitats) is ensured. Common metrics for evaluating LID effectiveness have either been physical (e.g., % surface flow reduction) or chemical (e.g., % reduction in mass loading for specific metals or nutrients). Comparatively little research has focused on biological metrics. This is important because a LID approach might be successful from the standpoint of physical and chemical indicators, and yet fail to protect salmonids and other aquatic species from the harmful effects of toxics. In recent years, research by NOAA Fisheries, the U.S. Fish and Wildlife Service (USFWS), and Washington State University (WSU-Puyallup) has shown that degraded stormwater quality can significantly impact the health of fish and the biological communities that support productive aquatic ecosystems. Major findings include mortality in coho salmon (*Oncorhynchus kisutch*) adults returning to restored urban streams (pre-spawn mortality; PSM) and mortality and developmental abnormalities in coho embryos rearing in restored urban streams.

For the project, stormwater was collected from SR 520 near the NOAA Northwest Fisheries Science Center in Seattle, WA and transported to the Grovers Creek Salmon Hatchery (Poulsbo, WA). The water was treated on-site using bioretention columns and both adult and embryonic coho were exposed to either well water, untreated stormwater or treated stormwater. The objective was to test the biological effectiveness of bioretention for preventing PSM in coho spawners and developmental toxicity in coho embryos exposed to urban runoff. This project combined research expertise on the toxic impacts of stormwater (NOAA and USFWS), expertise in the design and implementation of LID methods at Washington State University's Stormwater Center, and ongoing salmon management activity at the Suquamish Tribe's Grovers Creek Salmon Hatchery. The objective was fulfilled and successfully demonstrated the effectiveness of a specific LID strategy (bioretention using rain barrels) at preventing toxic effects to two life stages of coho salmon. This new information on bioretention effectiveness is helping inform a broad diversity of Puget Sound stakeholders as they adaptively manage stormwater runoff in urbanizing watersheds.

Purpose of the project

The literature on bioretention shows it to be a highly effective means of reducing many pollutants in stormwater runoff, especially contaminants associated with particulate matter (Taylor 2013; Section 2.4.2). Our pilot work filtering highway stormwater runoff through bioretention columns at WSU (McIntyre et al. 2014; 60% sand:15%

compost:15% shredded bark:10% water treatment residuals) showed reductions in metals, PAHs, and conventional water quality parameters such as TSS and DOC on par with values summarized in Taylor (2013). Recent work has shown that toxicity of road runoff to developing fish is associated with the dissolved fraction rather than particulates (McIntyre et al. 2016). What is unknown is what level of reductions in pollutants is sufficient to protect aquatic wildlife in receiving waters. Review of the literature on LID notes a clear lack of ecological effectiveness studies for any LID techniques employed to control stormwater quantity and quality. Biological effectiveness adds a layer of assessment above and beyond performance metrics of flow control and contaminant reduction that is directly relevant to the ultimate goal of LID – to protect the resiliency of aquatic ecosystems.

Assessing the biological benefits of LID to receiving waters is mentioned only at the basin scale in the recent review by Taylor (2013; Section 3.1.3.1). In contrast, we believe it is imperative to incorporate biological impacts at smaller scales (e.g. as part of 'internal scale effectiveness studies': Section 3.1.1) in order to increase the likelihood of ecological success as we move towards larger and more comprehensive installations.

Project Description

Project Objectives

Coho salmon are important ecologically, culturally, and economically to the Puget Sound basin. They are vulnerable to urban stormwater runoff in Puget Sound because they spend a year rearing in lowland streams before migrating to the ocean, and return to these same streams as adults to spawn. The Puget Sound lowlands are also home to most of the human development in the Puget Sound basin. Coho embryos rearing in urban creek water suffer higher rates of mortality and cardiac abnormalities compared to coho embryos rearing in urban creek water that has been pre-filtered (J. Incardona, NOAA-NWFSC, unpublished results). Adult coho returning to urban streams to spawn suffer from high rates of prespawn mortality (PSM) (Scholz et al. 2011), which has been linked to land development and urban or urbanizing land uses (Feist et al. 2011). The high mortality rates (up to 90%) observed in basins with PSM are a serious threat to the resilience of coho populations in developing areas like the Puget Sound basin (Spromberg & Scholz 2011). In 2012 we experimentally determined that exposure to unfiltered highway runoff was sufficient to trigger PSM in adult coho returning to the Suquamish Tribe's Grovers Creek Salmon Hatchery in Poulsbo, WA (Spromberg et al. 2015).

More than a decade of research into coho pre-spawn mortality has linked mortality events with stormwater runoff from highly developed watersheds (Scholz et al. 2011, Feist et al. 2011). In clear agreement with Taylor (2013) on Traditional BMPs Retrofitting (Section 3.0), existing developments do not contain sufficient stormwater treatment measures to protect sensitive aquatic biota in receiving waters. The current

study was designed to test whether bioretention treatment of highway runoff can prevent mortality in exposed coho embryos and spawning adults.

Project Outcomes

The primary outcome of the study was knowledge about the effectiveness of LID methods at reducing the biological impact of stormwater runoff. Importantly, this was measured through direct effects on fish. In this case, by testing the effectiveness of bioretention treatment to prevent PSM in adult salmon and mortality and development defects in salmon embryos. By identifying ecologically effective LID methodologies the results directly targeted the desired outcome of adaptive design and implementation of LID technologies to reduce toxic loadings to Puget Sound and its surrounding watersheds. Finally, the project produced highly visible results from the standpoint of public education and engagement. Salmon declines have a direct bearing on the "why do we care?" questions that are at the core of ongoing societal efforts to reverse ongoing ecological decline in Puget Sound.

Secondarily, we helped expand the regional knowledge base of contaminants present in untreated runoff as well as the effectiveness of bioretention to remove these contaminants. Additionally, an evaluation on the impacts the BSM had on water quality and a comparison to current standards is discussed. This information will help permittees better understand how bioretention can help them meet their goals and has demonstrated that bioretention can be an effective means of protecting the health of aquatic wildlife in receiving waters.

Project Activities and Tasks

Task 1: Build & condition bioretention cells

Task 2: Baseline water quality testing

Task 3: Coho exposures & chemistry

Task 4: Data analysis & communications

Results

Task 1

Deliverable 1: Bioretention cell construction and preparation

In October 2013 the research team constructed a portable bioretention treatment system for pilot work treating runoff for adult salmon exposures at Grover's Creek Salmon Hatchery (Poulsbo, WA). The bioretention cells were built to current industry and regulatory standards (Western Washington Stormwater Manual). Four new 55-

gallon polyethylene drums were fitted with a slotted underdrain. The underdrain was constructed from a 2" PVC pipe capped on one end and the other end attached to a bulkhead fitting near the base of the drum (Figures 1, 3). Slots in the underdrain were cut following guidance in Section 6.1.2 of the 2012 Low Impact Development Technical Guidance Manual for Puget Sound (Publication No. PSP 2012-3). On the exterior of each drum, a 2" PVC ball valve was attached to the bulkhead fitting.

In September 2014, the bioretention system was emptied of treatment media used in 2013. The new drainage layer (12") was a Seattle Type 26 mixed gravel aggregate obtained from CalPortland in DuPont, WA (product #8495). The bioretention soil media (BSM) was a mixture of 60% sand and 40% Cedar Grove compost mixed in 2011 and stored at Washington State University in Puyallup (WSU-P). The BSM was tamped down every 6" to reduce settling during conditioning to a total depth of 24". The BSM was topped with 2" of bark mulch created by Barri Hermann at WSU-P (Crop & Soil Sciences). One sample of BSM was taken from the center of each drum during construction for analysis of metals. Chilled samples were taken to ARI Laboratories in Tukwila, WA for analysis.

After transporting the bioretention system to Grover's Creek Salmon Hatchery, the bioretention media were conditioned in preparation for use in the coho study. Over two days, a total of 660 L of well water was passed through each bioretention cell at a rate of 2 L/min, equivalent to 2 months of summer rainfall on a contributing area 20x that of the treatment area (i.e., the treatment area is 5% of the contributing area – within recommended guidelines for the use of bioretention for treatment of runoff). Influent and effluent well water samples were collected on October 15, 2014 and transported on ice to Am-Test Laboratories in Kirkland, WA for analysis of metals and conventional water chemistry. Samples for PAH analysis were preserved with 10% methylene chloride and transported on ice to NOAA-Northwest Fisheries Science Center (NWFSC) for analysis.

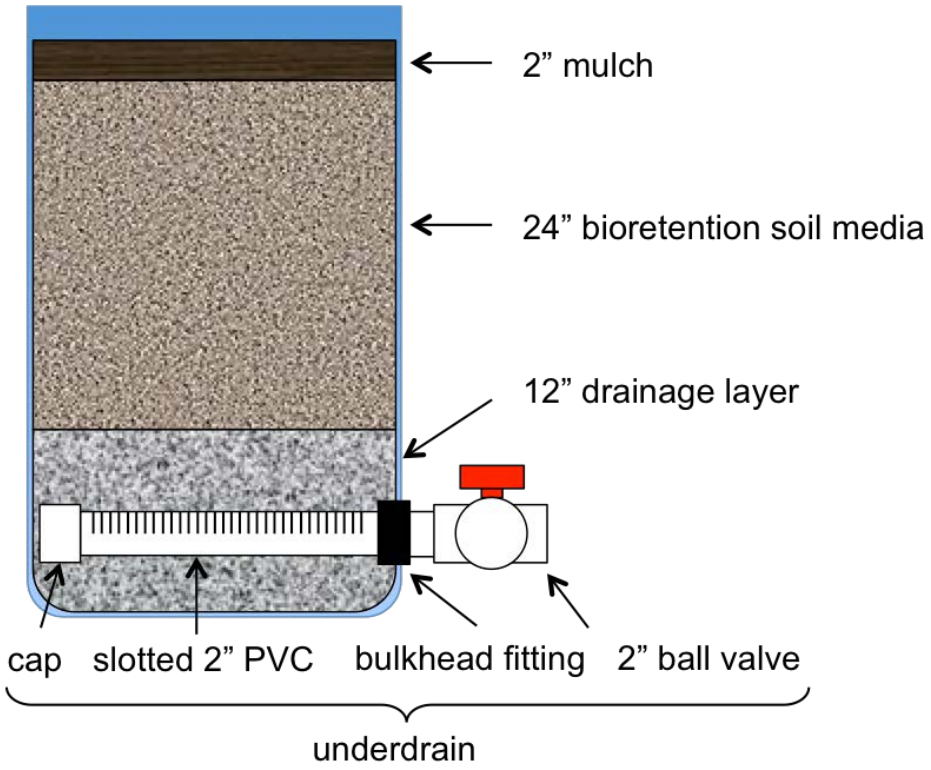


Figure 1. Diagram of bioretention unit using 55-gallon drum.

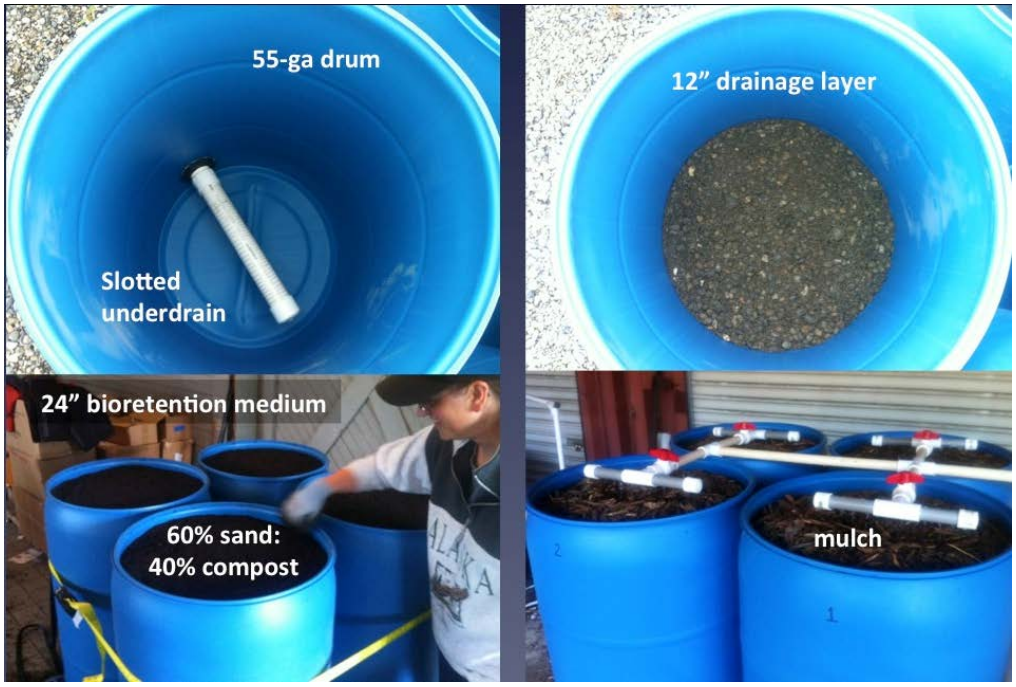


Figure 2. Images of the construction of the bioretention treatment system.

Figure 3. Image of the exterior of one treatment unit and effluent from conditioning.

Task 2

Deliverable 2.1: Metal concentrations of BSM used in bioretention cells

The most abundant metal in the BSM was Zn. Metal abundance was in the order Zn>Ni>Cr=Cu>Pb>As>Cd. Silver (Ag) was below the 0.2 mg/kg limit of quantitation in all BSM samples.

Table 1. Metal concentrations in the bioretention soil medium from each bioretention cell.

mg/kg dry	LOQ[a]	Cell 1	Cell 2	Cell 3	Cell 4	Mean	SE
As	0.2	2	2	1.9	2.2	2.0	0.1
Cd	0.09	0.11	0.12	0.1	0.1	0.11	0.0
Cr	2	28	28	26	25	27	1
Cu	0.5	21.4	21.1	21.5	22.3	21.6	0.3
Pb	0.09	6.97	7.17	6.6	7.5	7.06	0.19
Ni	0.5	35.9	38.2	38	33.8	36.5	1.0
Ag	0.2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.0
Zn	4	50	51	49	52	51	1

[a] LOQ = limit of quantitation

Deliverable 2.2: Water chemistry of effluent from conditioned cells

Following conditioning of bioretention cells, well water leached significant ($p < 0.01$) concentrations of most water chemistry parameters from the cells including bacteria, solids, organic matter, nitrogen, total P, and metals. There was a slight but significant loss of minerals (Ca, Mg), and total Ag from well water to the bioretention cells. The only parameter not significantly changed by passing through the bioretention cells was dissolved P ($p = 0.77$). Neither total nor dissolved Cd was above the detection limit for well water either before or after filtration through the conditioned bioretention cells.

Dissolved metals in the effluent from bioretention cell conditioning were in the order Zn>Cu>As>Ni>Cr, with undetectable levels of Cd, Pb, and Ag. Based on the concentrations native to the BSM (Table 1), we conclude that As in the BSM was relatively mobile and Cr was relatively immobile.

Table 2. Water chemistry of effluent from conditioned cells on Oct 15, 2014. Values are the mean and standard error of the mean of triplicate samples.

Category	Parameter	D.L.	Units	Well Water	Filtered Well Water
Microbiological	Fecal Coliform	5	CFU/100 mL	< D.L.	307 (63)
	E. coli	5	CFU/100 mL	< D.L.	287 (56)
Conventionals	pH	0.1	-	7.7 (0.1)	7.3 (0.0)
	TSS	1	mg/L	29 (1)	18 (6)
	SSC	0.2	mg/L	< D.L.	25.3 (0.3)
Demand	TOC	0.5	mg/L	0.5 (0)	32.7 (0.3)
	COD	10	mg/L	< D.L.	89 (6)
	DOC	0.5	mg/L	< D.L.	30.3 (1.2)
Minerals	Alkalinity	1	mg CaCO3/L	85 (1)	110 (0)
	Hardness	0.05	mg CaCO3/L	74.00 (1.00)	56.33 (0.33)
	Ca	0.05	mg/L	18.33 (0.33)	14.00 (0.00)
	Mg	0.01	mg/L	6.83 (0.03)	5.17 (0.07)
Nutrients	Ammonia	0.01	mg/L	0.29 (0.02)	1.47 (0.00)
	Total N	0.1	mg/L	0.5 (0.0)	4.7 (0.9)
	Nitrate	0.025	mg/L	< D.L.	2.893 (0.009)
	Ortho-P	0.005	mg/L	0.223 (0.006)	0.205 (0.059)
	Total P	0.005	mg/L	0.251 (0.002)	0.571 (0.014)
Total Metals	As	0.02	ug/L	0.25 (0.00)	7.18 (0.15)
	Cd	0.025	ug/L	< D.L.	< D.L.
	Cr	0.05	ug/L	0.21 (0.00)	2.57 (0.03)
	Cu	0.1	ug/L	1.6 (0.1)	15.0 (0.4)
	Pb	0.05	ug/L	0.06 (0.01)	0.7 (0.07)
	Ni	0.05	ug/L	0.49 (0.01)	8.58 (1.66)

	Ag	0.05	ug/L	0.15 (0.01)	< D.L.
	Zn	0.05	ug/L	4.70 (0.58)	36.53 (3.25)
Dissolved Metals	As	0.02	ug/L	0.14 (0.01)	6.72 (0.04)
	Cd	0.025	ug/L	< D.L.	< D.L.
	Cr	0.05	ug/L	< D.L.	2.07 (0.09)
	Cu	0.1	ug/L	0.8 (0.1)	12.6 (0.1)
	Pb	0.05	ug/L	0.05 (0.00)	< D.L.
	Ni	0.05	ug/L	0.11 (0.00)	4.82 (0.21)
	Ag	0.05	ug/L	< D.L.	< D.L.
	Zn	0.05	ug/L	1.55 (0.04)	23.37 (1.66)

Table 3. Polycyclic aromatic hydrocarbons (PAHs) in unfiltered well water and well water filtered with bioretention during cell conditioning.

Polycyclic aromatic hydrocarbons	Well Water (ug/L)	Filtered Well Water (ug/L)
Naphthalene	0.05 (0.00)*	0.05 (0.00)
C1-alkyl-napthalene	0.04 (0.00)	0.04 (0.00)
C2-alkyl-naphthalene	0.07 (0.00)	0.07 (0.00)
C3-alkyl-napthalene	0.13 (0.00)	0.13 (0.00)
C4-alkyl-napthalene	0.12 (0.02)	0.11 (0.01)
Acenaphthylene	0.01 (0.00)	0.01 (0.00)
Acenaphthene	0.01 (0.00)	0.01 (0.00)
Fluorene	0.03 (0.00)	0.02 (0.00)
C1-alkyl-fluorene	0.03 (0.01)	0.03 (0.00)
C2-alkyl-fluorene	0.06 (0.02)	0.04 (0.01)
C3-alkyl-fluorene	0.13 (0.00)	0.05 (0.02)
Dibenzothiophene	0.01 (0.00)	0.01 (0.00)
C1-alkyl-dibenzothiophene	0.06 (0.02)	0.04 (0.01)
C2-alkyl-dibenzothiophene	0.13 (0.00)	0.04 (0.02)
C3-alkyl-dibenzothiophene	0.06 (0.00)	0.03 (0.01
C4-alkyl-dibenzothiophene	0.01 (0.00)	0.01 (0.00)
Anthracene	0.01 (0.00)	0.01 (0.00)
Phenanthrene	0.14 (0.02)	0.10 (0.01)
C1-alkyl-phenanthrene	0.27 (0.11)	0.16 (0.07)
C2-alkyl-phenanthrene	0.25 (0.11)	0.12 (0.07)
C3-alkyl-phenanthrene	0.08 (0.04)	0.07 (0.04)
C4-alkyl-phenanthrene	0.03 (0.00)	< D.L.**
Pyrene	0.08 (0.03)	0.03 (0.02)
Fluoranthene	0.10 (0.04)	0.06 (0.03)
C1-alkyl-fluoranthene	0.01 (0.00)	0.02 (0.00)
C2-alkyl-fluoranthene	< D.L.	< D.L.
C3-alkyl-fluoranthene	< D.L.	< D.L.

C4-alkyl-fluoranthene	< D.L.	< D.L.
Chrysene	0.01 (0.00)	< D.L.
C1-alkyl-chrysene	< D.L.	< D.L.
C2-alkyl-chrysene	< D.L.	< D.L.
C3-alkyl-chrysene	< D.L.	< D.L.
C4-alkyl-phenanthrene	< D.L.	< D.L.
Benzo[*a*]anthracene	< D.L.	< D.L.
Benzo[*b*]fluoranthene	0.01 (0.00)	< D.L.
Benzo[*k*]fluoranthene	< D.L.	< D.L.
Benzo[*e*]pyrene	< D.L.	< D.L.
Benzo[*a*]pyrene	< D.L.	< D.L.
Perylene	< D.L.	< D.L.
Indeno[*1,2,3-cd*]pyrene	< D.L.	< D.L.
Dibenz[*a,h*]anthracene (and [*a,c*])	< D.L.	< D.L.
Benzo[*ghi*]perylene	< D.L.	< D.L.
Total PAHs	1.77 (0.56)	1.24 (0.34)

* Mean and standard error of triplicates

** D.L. = detection limit was 0.01 ug/L

Task 3

Deliverable 3.1: Effects of treated effluent on adult coho salmon

During the 2013 spawning season (Sep-Dec), we tested the ability of bioretention to prevent pre-spawn mortality in adult coho at the end of the run (November) for highway runoff during one 4-h and one 24-h exposure for two separate storms. During the 2014 spawning season (Oct-Dec), we completed three exposures, focusing on the early part of the run (October). All exposures were 24 h duration with an observation period at 4 h. Additionally, in 2014 an exposure was run comparing well water exposure with well water passed through the bioretention cells.

Healthy adult coho returning to the Suquamish Tribal Hatchery on Grovers Creek were randomly selected and placed in individual PVC holding tubes (Figure 4). Only fish exhibiting normal behavior and with no obvious signs of trauma, disease, or poor condition were included. Four fish per treatment were placed in 440L of experimental water. Each holding tube was equipped with a hose to pump water flow (4L/min) across the fish's head and each treatment tank was aerated to maintain dissolved oxygen at optimum levels for adult coho health during exposures.

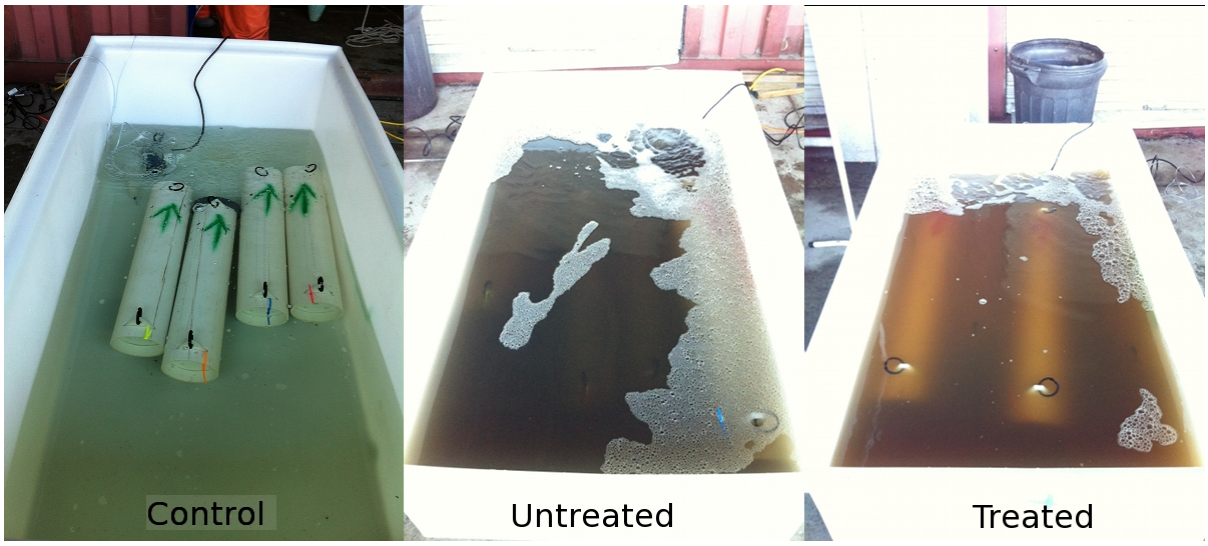


Figure 4. Experimental setup for adult coho exposures during 2013 and 2014 testing.

In both years, all of the coho exposed to the unfiltered runoff were dead at the end of the exposure period, whereas all of the coho exposed to the filtered runoff or to well water were still alive at the end of the exposure period (Table 4). All fish exposed to well water or filtered well water were alive and behaving normally at 24 h. During 2014, nearly all (11/12) coho exposed to unfiltered runoff were dead within 4 h of exposure. By the end of the 24 h trials, not only was there 0% mortality in the filtered runoff exposure, we did not observe any of the overt symptoms of 'pre-spawn mortality' that were observed in coho exposed to unfiltered runoff prior to death.

Table 4. Mortality of adult coho exposed to well water, or highway runoff that was unfiltered or filtered through the bioretention cells during 2013 or 2014. N = 4 spawners were used in each treatment for each trial.

Exposure Trial		Adult Coho Mortality		
Date	Duration (h)	Well Water	Unfiltered Runoff	Filtered Runoff
11/8/2013	4	0% (0/4)	100% (4/4)	0% (0/4)
11/18/2013	24	0% (0/4)	100% (4/4)	0% (0/4)
10/20/2014	24	0% (0/4)	100% (4/4)	0% (0/4)
10/22/2014	24	0% (0/4)	100% (4/4)	0% (0/4)
10/27/2014	24	0% (0/4)	100% (4/4)	0% (0/4)

Deliverable 3.2: Effects of treated effluent on coho embryo development

On November 13, 2014, eggs and milt were removed from ripe adult coho spawners returning to Grovers Creek Salmon Hatchery. Fertilization took place in paper cups. Approximately 90 eggs were placed in each cup and each cup fertilized by one male. Fertilized eggs were poured into mesh-bottomed cups (Figure 5) that were placed in the trays of heath stacks (Figure 5) used for the experimental rearing. Each tray held nine cups of eggs, with seven vertical trays per stack. Each stack was a separate exposure (Table 5). Each stack was supplied with flow-through 'control' water that was a mixture of well water and stream water. Water was distributed to the top tray of each stack and flowed down through each tray and out a common trough. On exposure days, water was switched from flow-through control water to recirculating water from a 114-L aluminum sump from which treatment water for each stack was pumped to the top tray using a submersible pump (Lifeguard Aquatics Quiet One 3000, 2-3 gal/min). The outflow from each stack returned to the sump for each stack. After 24-h of recirculating exposure waters, all stacks were switched back to flow-through control water. Temperature was measured in the top tray of each stack at 15-min intervals throughout development. Temperature across stacks averaged 9.9 °C, with short-term deviations ranging from 6.9 to 12.2 °C. Stacks were treated 1/week with formalin (Parasite-S, 37% formalin, 15 min at 167 ppm formalin) to prevent fungal buildup per standard hatchery protocol. Hatchery personnel stopped formalin treatment after 12/22/2014 - two weeks before the termination of the experiment.

Figure 5. Embryo cups on a tray in the heath stack (left). Heath stack showing sump filled with 100% unfiltered runoff (right).

Seven exposures were conducted during the experiment (Table 6) - one during gastrulation and the rest during organogenesis (Figure 6). Lack of precipitation during

early development prevented more exposures during cleavage and gastrulation. Three cups were sampled from each treatment on each sampling date: one cup from each of trays 1-3 for each stack. Although a higher number of replicates would have been preferred, 15 replicates (3 x 5 treatments) were the most our crew of 6-8 could process in a full field day. Sampling included dechorionating and inspecting 10 embryos from each cup. Each embryo was examined for developmental stage, and digitally photographed using a SMZ-800 stereomicroscope. Images were later analyzed (ImageJ software) for embryo length, eye size, and cardiovascular abnormalities.

Table 5. Treatments used in the episodic exposure study

Stack	Treatment	Trays/Sampling
1	Filtered 100%	3
2	Unfiltered 10%	3
3	Control	2-3
4	Unfiltered 50%	3
5	Unfiltered 100%	3

Table 6. Precipitation and developmental details for each sampling and exposure date during coho embryo development.

Date	Sampling / Exposure	Days Post Fertilization	Degree Days (°C)	Development Period
11/19/2014	Sampling	6	62	Cleavage
11/24/2014	Exposure	11	112	Gastrulation
12/1/2014	Sampling	18	182	Organogenesis
12/5/2014	Exposure	22	222	Organogenesis
12/8/2014	Sampling	25	250	Organogenesis
12/9/2014	Exposure	26	259	Organogenesis
12/12/2014	Exposure	29	291	Organogenesis
12/15/2014	Sampling	32	320	Organogenesis
12/17/2014	Exposure	34	348	Organogenesis
12/19/2014	Exposure	36	367	Organogenesis
12/22/2014	Sampling	39	388	Organogenesis
12/23/2014	Exposure	40	398	Organogenesis
12/29/2014	Sampling	46	455	Hatch
1/5/2015	Sampling	53	525	Hatch

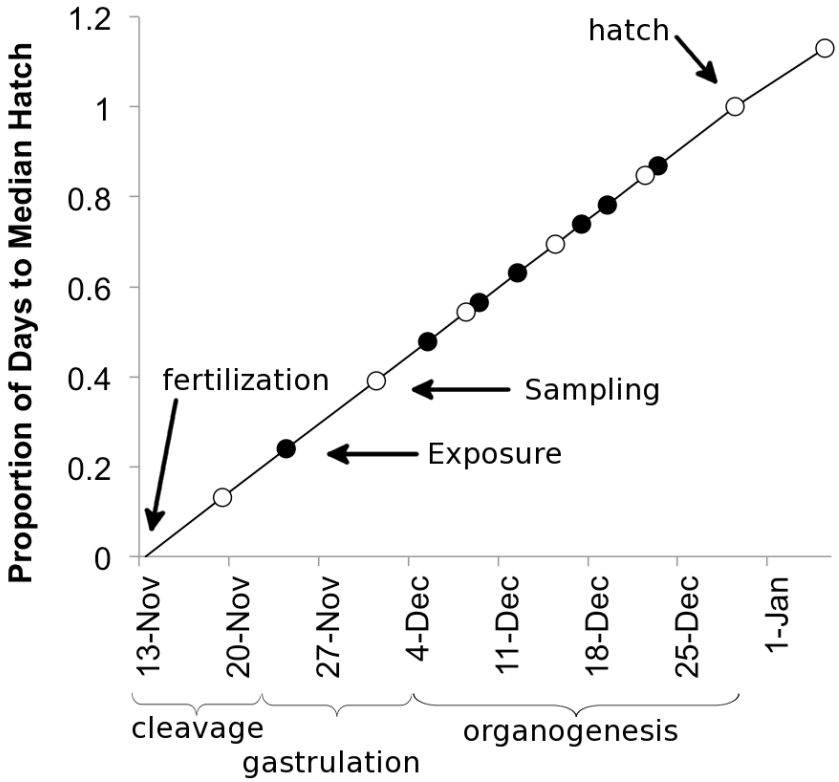


Figure 6. Developmental sequence of coho embryos during the study, including exposure (closed circles) and sampling events (open circles).

Fertilization success was generally high (>90%) across treatments, although individual cups sometimes contained high numbers of unfertilized embryos (4 cups had 24-51% unfertilized). This was likely due to low fertility of individual males – a disadvantage of the single-male cup approach. Embryo development followed expected timelines based on measured degree-days (Table 6).

Survival and hatch rates across treatments showed a similar trend with highest rates in filtered runoff, followed by 10% runoff, 50% runoff, control, and the lowest survival and hatch rates in the 100% runoff.

By the end of the experiment, embryos exposed to the 7 episodes of 100% unfiltered runoff were significantly smaller than controls (Figure 7, Appendix A), whereas embryos exposed to the 7 episodes of filtered runoff were not different than controls. Although eye size followed the same trend, there were no significant differences among treatments (Figure 8). Proportion of embryos with cardiovascular abnormalities similarly suggested more abnormalities in the 100% unfiltered treatment and a reduced effect in the filtered runoff treatment (Figure 9, Appendix A). One of the control replicates had anomalously high proportion of embryos with cardiovascular abnormalities (90% vs 20% for the other replicates), resulting in the high control

variability seen in Figure 9. Cardiovascular abnormalities included hemorrhage spots in the head, trunk, or tail. The score of abnormalities (sum of individual counts) increased with the concentration of runoff, but those differences were also not statistically significant.

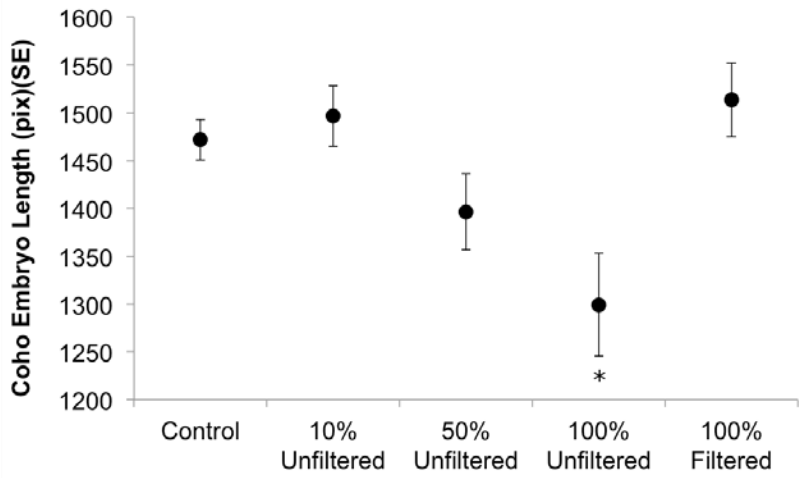


Figure 7. Embryo length following 7 episodic exposures to runoff. Embryos in the 100% unfiltered treatment were significantly smaller than controls ($F(4,14) = 4.992$, $p = 0.021$, Dunnett post-hoc $p = 0.042$).

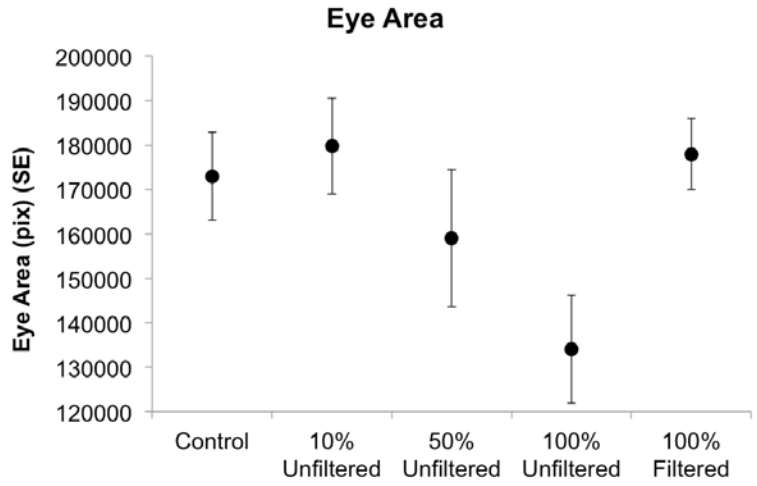


Figure 8. Eye area of embryos following 7 episodic exposures to runoff. There were no significant differences among treatments ($F(4,14) = 2.197$, $p = 0.150$).

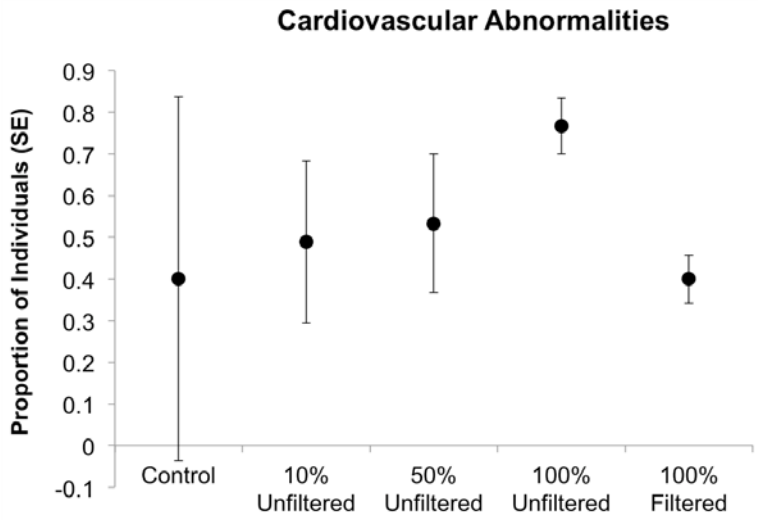


Figure 9. Proportion of embryos with cardiac abnormalities following 7 episodic exposures to runoff. Differences among treatments were not statistically significant ($F(4,14) = 2.235$, $p = 0.145$).

Deliverable 3.3: Chemistry of treated effluent

Unique water samples (n=1) from each treatment were analyzed for each storm for a wide variety of chemical and biological parameters (Table 7). Bioretention treatment significantly altered runoff chemistry. Nutrient concentrations in highway runoff were typically low (medians: Total N = 4.3 mg/L; Total P = 0.224 mg/L). Runoff water picked up nutrients as it passed through the bioretention cells, resulting in higher concentrations of nitrates and phosphates leaving than entering the bioretention cells (Table 7; Figure 10).

On a mass base of total metals, the bioretention cells had a net export of As (9.3 mg), Ni (0.4 mg), and Ag (0.5 mg), but a net retention of the other metals; 0.2 mg Cd, 12.9 mg Cr, 43.0 mg Pb, 121.7 mg Cu, and 625.1 mg Zn (Table 7).

PAH concentrations in influent runoff ranged from 5-31 µg/L, with a median concentration of 10 µg/L. In the bioretention-filtered effluent, PAH concentrations ranged from 0.1-1.6 µg/L with a median concentration of 0.8 µg/L; similar to control waters (Figure 11). The variability in PAH values was low, with a COV of <10% (mean COV = 3%). Therefore, error bars are not shown for the PAH values in Figure 11. In terms of pollutant mass, 19.4 mg of measured PAHs entered the bioretention cells during the 10 experimental filtrations whereas only 1.7 mg were released – a total reduction of 91%.

Table 7. Median (and range) of water chemistry values for influent untreated highway runoff and effluent treated runoff from the bioretention cells and net mass retained by bioretention soil media (BSM) across the 10 experimental storm events.

Category	Parameter	D.L.	Units	Untreated Runoff	Bioretention-Treated	Net Mass Retained	% Mass Retained
						10^6 CFU	
Microbiology	Fecal Coliform	5	CFU/100 mL	910 (150-3100)	567 (73-1200)	96	7
	E. coli	5	CFU/100 mL	730 (0-2200)	297 (0-1213)	831	47
						Net kg	
Conventional	pH	0.1	-	6.7 (5.6-7.3)	6.9 (6.5-7.1)	n.a.	n.a.
	TSS	1	mg/L	150 (93-500)	44 (28-52)	255	75
	SSC	0.2	mg/L	172 (93-510)	47 (41-701)	275	74
Demand	TOC	0.5	mg/L	31 (4-50)	40 (24-61)	(-38.3)	(-89)
	COD	10	mg/L	190 (24-1000)	103 (83-190)	70	24
	DOC	0.5	mg/L	5 (1-37)	34 (21-65)	(-59)	(-435)
Minerals	Alkalinity	1	mg CaCO3/L	31 (10-58)	79 (37-100)	(-102)	(-202)
	Hardness	0.05	mg CaCO3/L	39 (12-340)	68 (7-840)	(-180)	(-194)
	Ca	0.05	mg/L	12 (4-120)	17 (2-200)	(-38)	(-128)
	Mg	0.01	mg/L	3 (1-35)	5 (1-20)	(-8.2)	43
Nutrients	Ammonia	0.01	mg/L	0.70 (0-4.55)	0.75 (0.04-1.56)	(-0.2)	(-12)
	Total N	0.1	mg/L	4.3 (1.2-70.1)	5.2 (2.5-54.6)	(-1.3)	(-10)
	Nitrate	0.025	mg/L	0.35 (0.06-1.50)	11.45 (2.16-34.20)	(-15)	(-2141)
	Ortho-P	0.005	mg/L	0.004 (0-0.884)	0.767(0.011-1.287)	(-1.1)	(-262)
	Total P	0.005	mg/L	0.224 (0.094-0.438)	1.300 (0.278-2.063)	(-2.3)	(-594)
						Net mg	
Total Metals	As	0.02	ug/L	3.49 (1.23-15.00)	8.34 (2.73-10.90)	(-9.3)	(-152)
	Cd	0.025	ug/L	0.43 (0.14-2.64)	0.14 (0.09-3.59)	0.2	26
	Cr	0.05	ug/L	11.6 (7.6-23.0)	4.8 (3.0-6.5)	13	57
	Cu	0.1	ug/L	115.5 (46.5-184.0)	26.6 (18.4-60.5)	122	69
	Pb	0.05	ug/L	24.9 (12.7-67.1)	3.1 (1.40-5.4)	43	88
	Ni	0.05	ug/L	11.18 (3.90-20.00)	8.46 (5.54-18.60)	(-0.4)	(-2)
	Ag	0.05	ug/L	0.11 (0.05-0.52)	0.10 (0-1.58)	(-0.5)	(-110)
	Zn	0.05	ug/L	375.3 (140.7-1320.0)	46.0 (25.7-191.0)	625	85
Dissolved Metals	As	0.02	ug/L	1.4 (0.4-7.4)	7.1 (3.2-10.4)	(-11)	(-355)
	Cd	0.025	ug/L	0.14 (0.04-7.35)	0.12 (0.06-0.23)	0.2	41
	Cr	0.05	ug/L	3.82 (2.16-6.17)	2.86 (1.42-9.36)	(-0.3)	(-4)
	Cu	0.1	ug/L	20.1 (5.1-115.0)	25.8 (13.3-38.7)	0.5	1
	Pb	0.05	ug/L	3.53 (0.14-24.80)	1.91 (0.34-3.28)	3.9	64
	Ni	0.05	ug/L	3.25 (0.49-9.48)	6.35 (3.50-15.40)	(-7.1)	(-154)
	Ag	0.05	ug/L	0.00 (0-0.07)	0.04 (0-0.95)	(-0.4)	(-1471)
	Zn	0.05	ug/L	148.0 (44.0-876.0)	32.5 (17.8-57.2)	261	86
Total	PAHs	<0.01	ug/L	9.81 (4.93-30.76)	0.76 (0.13-1.62)	18	91

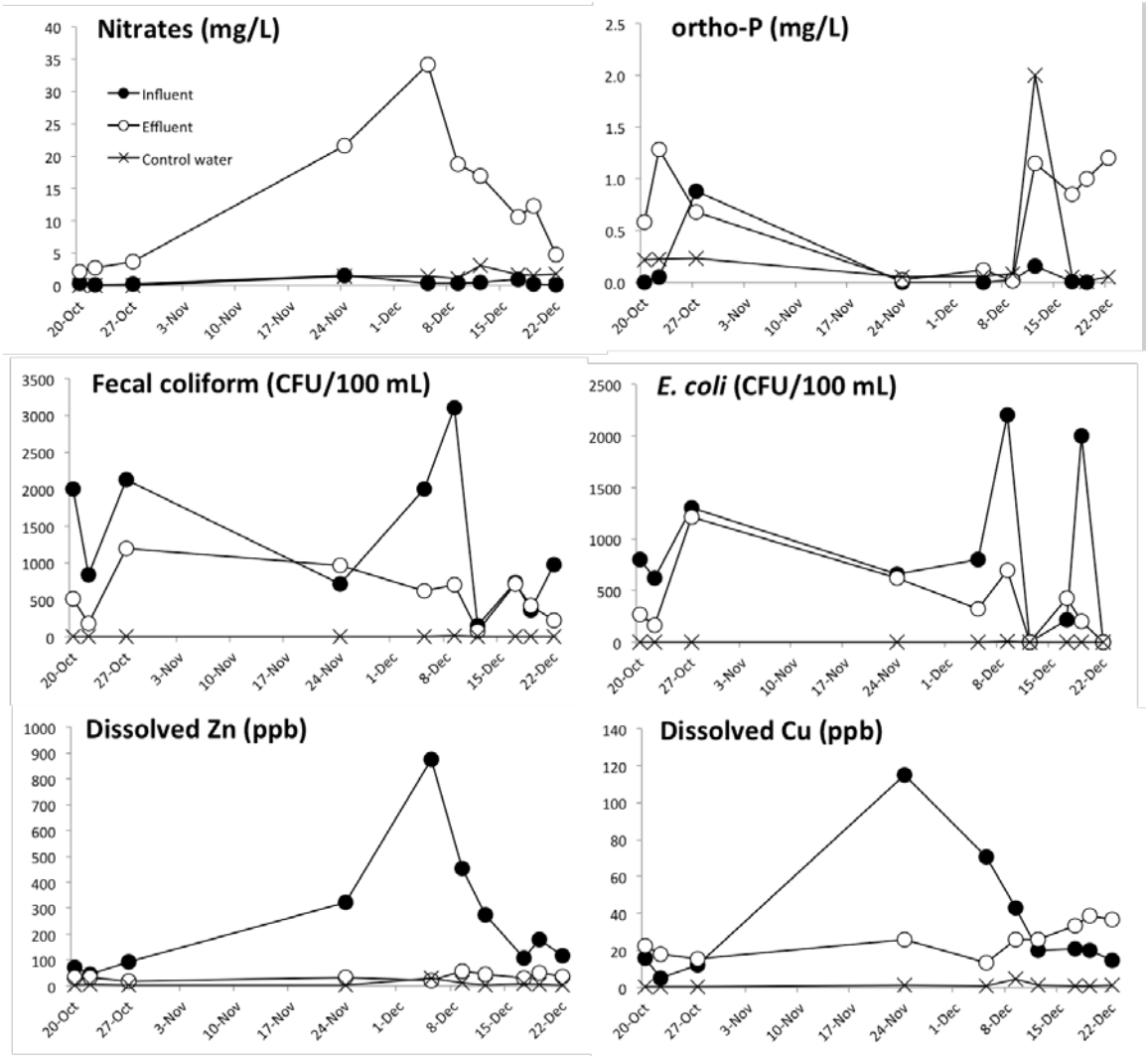


Figure 10. Nutrients, bacteria, and dissolved metals in unfiltered highway runoff, runoff filtered with bioretention, and control well water across 10 storm events during 2014.

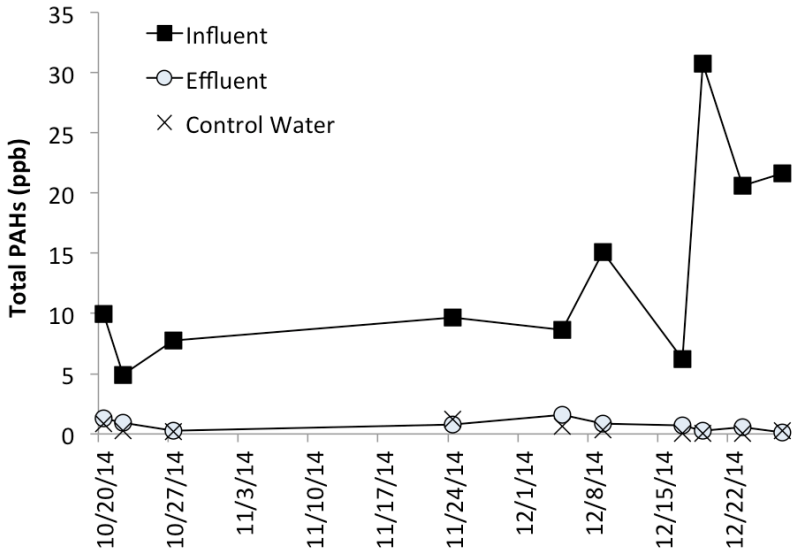


Figure 11. The sum of 42 parent and alkylated homologue PAHs in unfiltered highway runoff, runoff filtered by bioretention, or control well water for 10 storm events during 2014.

Task 4

Deliverable 4: Data analysis & communication

Bioretention Performance - Biology

Filtration through bioretention was able to completely prevent mortality in adult coho salmon exposed to highway runoff. This test was repeated three consecutive times with different adult spawners and runoff from different storm events during Oct 2014 – each time with identical results. The results of the adult portion of the study were recently published Open Access in the Journal of Applied Ecology (Spromberg et al. 2015).

Coho embryos were episodically exposed to runoff collected during seven additional storm events (Nov and Dec 2014). Embryos were raised until hatch at 53 days post fertilization. At the end of 53 days, embryos in the unfiltered runoff treatment were significantly smaller than embryos in the control well water. In contrast, embryos for which the seven storm events were filtered through bioretention were not smaller than controls. Embryos exposed to unfiltered runoff also had significantly lower hatch rate than embryos exposed to filtered runoff.

Bioretention Performance – Chemistry

The chemistry of runoff collected for the 10 storm events showed no temporal trend either before or after filtration. In terms of bioretention performance, we conclude that ten filtration events at 2 L/min treatment rate were not sufficient to exceed the performance capacity of the bioretention cells.

Discussion on Water Quality Standards

Concentrations of contaminants in stormwater runoff and bioretention effluent were compared to Washington State water quality standards for contaminants of concern for surface waters (WA DOE Publication 06-10-091). Aquatic life pH criteria of 6.5-8.5 were used (Chapter 173-201A-200 WAC). Dissolved metals concentrations were compared to standards for toxic substances (173-201A-240) - for the more sensitive 'salmonids present' when possible. Both acute and chronic criteria were used where provided. Most criteria were hardness and/or pH dependent as described in the relevant documents cited.

Concentrations were below standards in influent and effluent runoff for the contaminants ammonia, As, Cd, and Ni (Table 8). Influent samples (untreated urban stormwater runoff) exceeded acute and chronic water quality standards for Pb (10% of samples), Cu (100%) and Zn (100%). In two cases (20%) of untreated runoff, pH was lower than the water quality standard (Table 8).

In treated runoff (bioretention effluent), water quality improvements resulted in a 30% drop in the exceedance of Cu standards, and a 90% drop in exceedance of Zn standards. The two pH exceedances and the single Pb exceedance were removed by bioretention treatment. In contrast, bioretention treatment resulted in a single exceedance of the Ag standard. The Zn and Ag exceedances in bioretention-treated runoff were a result of the very low hardness of one effluent sample (Table 7). That one value (7 mg/L $CaCO_3$ on Dec 23, 2014) was 6x lower than the next lowest value (46 mg/L).

The toxicity of most metals varies with water chemistry conditions. Although hardness influences toxicity, the presence of dissolved organic matter (as dissolved organic carbon; DOC) tends to be the strongest determinant of toxicity. Higher concentrations of DOC afford greater protection by having a stronger affinity for most metals than do biological tissues. Current Washington State water quality standards for metals do not account for the protective presence of DOC. Therefore, although standards were exceeded for Cu, Pb, and Zn in many samples, we expect that this finding significantly overestimates the toxic potential of bioretention effluents.

Table 8. Percentage of untreated and bioretention-treated runoff that exceeded Washington State water quality standards. Each runoff type was 10 samples.

	Acute Standards		Chronic Standards	
Parameter	Untreated	Treated	Untreated	Treated
pH	20%	0%	n.a.[1]	n.a.
NH_3	-[2]	-	-	-
As	-	-	-	-

Cd	-	-	-	-
Cu	100%	70%	100%	70%
Pb	10%	0%	10%	0%
Ni	-	-	-	-
Ag	-	-	0%	10%
Zn	100%	10%	100%	10%

[1] No chronic pH standard

[2] '–' indicates no standards were exceeded for any sample

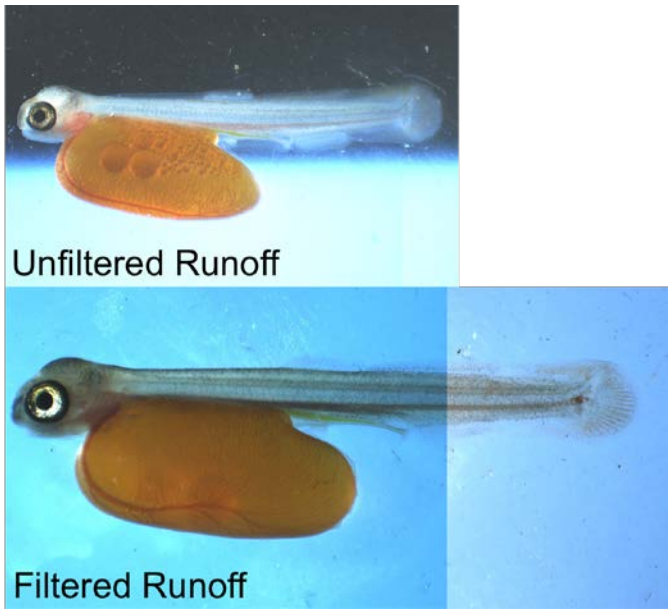
Summary/Conclusions

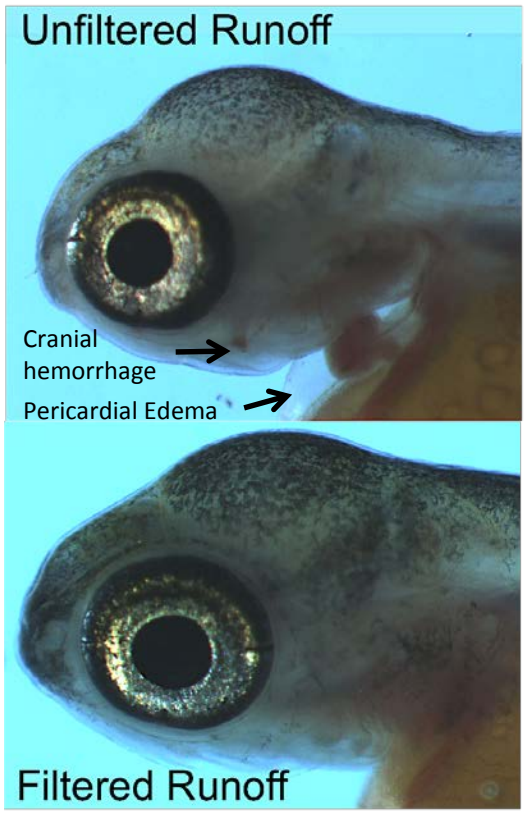
Bioretention soil media (BSM) used in these experiments (60% sand : 40% compost) contained bacteria, nutrients, and metals that were sometimes leached out during stormwater treatment. The most mobile metal was As – resulting in net export of 9.3 mg across the 10 treatment events. Although the BSM also contained measurable amounts of other metals, there was a net removal of Zn (625.1 mg), Cu (121.7 mg), Pb (43 mg), Cr (12.9 mg) and Cd (0.2 mg). Polycyclic aromatic hydrocarbons (PAHs) were always reduced by bioretention treatment, showing an overall 91% reduction in mass (18 mg).

Bioretention filtration of urban stormwater runoff prevented pre-spawn mortality in adult coho salmon during 24 hour exposures and eliminated toxic impacts to coho embryos developing in episodic exposure to runoff. Embryos were only exposed to seven 24-hr storm events during the 53-day experiment, amounting to about 13% of the developmental period. There were no rain events to expose embryos during the critical developmental windows of gastrulation and hatching, which may have contributed to the mild effects observed in this study.

Appendix A

Representative images of embryos in unfiltered runoff treatment or filtered runoff treatment at the end of the experiment, showing differences in length and cardiovascular abnormalities.




Unfiltered Runoff
Cranial hemorrhage
Pericardial Edema
Filtered Runoff

Citations:

Feist, B. E., E. R. Buhle, P. Arnold, J. W. David & N. L. Scholz (2011). Landscape ecotoxicology of coho salmon prespawn mortality in urban streams. *PLoS1,* **6**(8): 1.

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McIntyre, J. K., R. C. Edmunds, M. G. Redig, E. M. Mudrock, J. W. Davis, J. P. Incardona, J. D. Stark & N. L. Scholz (2016). Confirmation of stormwater bioretention treatment effectiveness using molecular indicators of cardiovascular toxicity in developing fish. *Environmental Science & Technology*. *doi: 10.1021/acs.est.5b04786*

Scholz, N. L., M. S. Myers, S. G. McCarthy, J. S. Labenia, J. K. McIntyre, G. M. Ylitalo, L. D. Rhodes, C. A. Laetz, C. M. Stehr, B. L. French, B. McMillan, D. Wilson, L. Reed, K. D. Lynch, S. Damm, J. W. Davis & T. K. Collier (2011). Recurrent die-offs of adult coho salmon returning to spawn in Puget Sound lowland urban streams. *PLoS1,* **6**(12): 1.

Spromberg, J. A. & N. L. Scholz (2011). Estimating the future decline of wild coho salmon populations due to early spawner die-offs in urbanizing watersheds of the Pacific Northwest. *Integrated Environmental Assessment and Managment*. *doi: 10.1002/ieam.219*

Spromberg, J. A.; Baldwin, D. H.; Damm, S.; McIntyre, J. K.; Huff, M.; Sloan, C. A.; Anulacion, B. F.; Davis, J. W.; Scholz, N. L., Widespread coho salmon spawner mortality in western U.S. urban watersheds: Lethal stormwater impacts are reversed by soil bioinfiltration. *J. Appl. Ecol.* **2015,** *doi: 10.1111/1365-2664.12534*.

Taylor, W.J. (2013). White paper for stormwater management program effectiveness literature review - Low impact development techniques. Prepared for Association of Washington Cities and Washington State Department of Ecology. April 2013.