# **Deliverable 4.4: Progress Report 4**

		Water Quality		Toxicology		Stormwater		
Water Year	Event	Basic	Full	Zfish	Coho	Collection Date	Treatment Dates	
6	31	х				2/16/21	2/17/21-2/18/21	
	32	х				1/24/21	1/25/21-1/26/21	
	33	х				1/31/21	2/1/21-2/2/21	
	34	х				2/6/21	2/8/21-2/9/21	
	35	х				2/15/21	2/16/21-2/17/21	
	36	х	х	*	x	3/14/21	3/15/21-3/16/21	
7	37	х				3/24/21	3/25/21-3/26/21	
	38	х				4/7/21	4/8/21-4/9/21	
	39	х				5/18/21	5/18/21-5/19/21	
	40	х				5/23/20	5/24/21-5/25/21	

### Overview of Work Period: 2/16/21 - 5/25/21

\*Zebrafish molecular assays have not yet been completed for Event 36.

#### **Report Summary**

#### Work Progress Status

Project Tasks	% Completion
1. QAPP development	100
2. Prepare experimental columns	100
3. Condition experimental columns	100
4. Bioretention performance throughout accelerated aging	70
5. Outreach and communication	0

### Discussions/decisions made since last report period

N/A

### **Summary of Events**

Summary of Full Water Chemistry

Event 36 (Post WY6)

- Dissolved zinc concentrations were significantly higher in effluent from the shallower than the deeper BSM treatment depths.
- Copper concentrations were significantly higher in effluent from the 6" treatment depths compared with the 12" depths.
- A net export of nitrates was observed for all treatment depths, with significantly higher effluent concentrations from the deeper than the shallower BSM treatment depths.

• Orthophosphate was not detected in effluent from the 6" treatment depths. Orthophosphate concentrations were significantly higher in effluent from the deeper than the shallower treatment depths.

Summary of Toxicology: Event 36 (Post WY6)

- Untreated highway runoff was acutely lethal to juvenile coho salmon (100% mortality).
- Treatment of runoff with bioretention completely prevented juvenile coho mortality for all BSM depths.

# **Full Water Chemistry**

Full water chemistry analysis was conducted for Event 36 (End of WY6). Samples for water chemistry were collected and analyzed as previously (Deliverable 4.1: Progress Report 1).

## Event 36 (End of WY6)

### Metals

**Table 1.** Average concentrations of dissolved and total metals in  $\mu g/L$  (standard error) for influent waters (clean water and influent stormwater runoff) and triplicate effluent waters from each of the three treatment depths plus the clean water control for Water Year 6. One-half of the value of the detection limit was substituted for the value of non-detects in calculating means; used when the compound was detected in at least one replicate for the treatment. Values following '<' are equal to the detection limit.

Compound	Clean Water	Influent	6"	12"	18"	18" CWC
Dissolved As	<0.05	0.4	<0.05	<0.05	<0.05	<0.05
Dissolved Cd	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Dissolved Cu	<0.05	21.3	10 (0.2)	7.2 (0.9)	8.3 (0.3)	2.17 (0.03)
Dissolved Pb	<0.079	<0.079	<0.079	<0.079	<0.079	<0.079
Dissolved Ni	<0.2	1.2	<0.2	<0.2	<0.2	<0.2
Dissolved Zn	<0.19	19.2	2.8 (0.06) <sup>a</sup>	2.5 (0.2) <sup>ab</sup>	2 (0.1) <sup>b</sup>	<0.19
As	<0.05	0.9	<0.05	<0.05	<0.05	<0.05
Cd	<0.05	0.3	<0.05	<0.05	<0.05	<0.05
Cu	<0.2	58.4	12 (0.2)ª	8.6 (0.9) <sup>b</sup>	10 (0.5) <sup>ab</sup>	5.1 (0.2)
Pb	<0.079	3.5	<0.079	<0.079	<0.079	<0.079
Ni	<0.2	4	<0.2	0.4 (0.3)	<0.2	<0.2
Zn	<0.19	151	5.7 (0.2)	5.3 (0.2)	4.9 (0.2)	<0.19

Note: Treatments with different superscript group labels (a, b, c) are significantly different at 🛛 = 0.05 (Kruskal-Wallis with post-hoc Dunn Test).

#### **Nutrient & Conventional Water Chemistry**

**Table 2.** Average water chemistry values (standard error) for influent waters (clean water and influent stormwater runoff) and triplicate effluent waters from each of the three treatment depths plus the clean water control for Water Year 6. One-half of the value of the detection limit was substituted for the value of non-detects in calculating means; used when the compound was detected in at least one replicate for the treatment. Values following '<' are equal to the detection limit. n.m. = not measured for this event.

Compound	Units	Clean Water	Influent	6"	12"	18"	18" CWC
Conventional							
Dissolved Organic	mg/L	<0.08	27.4	18.6 (0.3)	16 (1)	15.5 (0.7)	1.67 (0.07)
Carbon							
Total Suspended	mg/L	0.8	57.2	1 (0.2)	0.4 (0.2)	0.5 (0.1)	0.8 (0.1)
Solids							
Turbidity	NTU	0.09	58.7	1.5 (0.2)	0.7 (0.2)	0.8 (0.1)	2.9 (0.7)
Conductivity	μS/cm	1515	3450	3500 (0)	3513 (7)	3530 (20)	1528 (0.6)
рН	n.a.	7.724	7.647	7.44 (0.03)	7.28 (0.02)	7.201 (0.003)	7.33 (0.02)
Alkalinity	mg/L as CaCO₃	54.5	67.2	59 (0.3) <sup>a</sup>	56.6 (0.4) <sup>ab</sup>	54.1 (0.4) <sup>b</sup>	35.2 (0.3)
Temperature	°F	n.m.	37.2	39.6 (0.7)	37.07 (0.07)	37.3 (0.1)	37.5 (0.3)
Dissolved Calcium	mg/L	8.41	56.5	40 (1)	34 (2)	32 (1)	9.6 (0.1)
Dissolved	mg/L	22.6	1.55	1.18 (0.04)	1.11 (0.07)	1.07 (0.04)	22.1 (0.3)
Magnesium							
Dissolved Sodium	mg/L	228	583	600 (10)	578 (4)	574 (3)	226 (2)
Nutrients							
Nitrate/Nitrite	mg/L	0.12	1.15	1.79 (0.05)ª	1.97 (0.05) <sup>ab</sup>	2.14 (0.04) <sup>b</sup>	0.37 (0.02)
Orthophosphate,	mg/L	<0.01	<0.01	<0.01ª	0.017 (0.003) <sup>ab</sup>	0.02 (0) <sup>b</sup>	0.143 (0.003)
as P							
Microbiology							
Fecal Coliform by	CFU/100 mL	10	200	40 (30)	20 (6)	20 (6)	20 (10)
MF							

Note: Treatments with different superscript group labels (a, b, c) are significantly different at 🛛 = 0.05 (Kruskal-Wallis with post-hoc Dunn Test).

#### PAHs

**Table 3.** Average polycyclic aromatic hydrocarbon (PAH) concentrations in  $\mu$ g/L (standard error) for influent waters (clean water and influent stormwater runoff) and triplicate effluent waters from each of the three treatment depths plus the clean water control for Water Year 6. One-half of the value of the detection limit was substituted for the value of non-detects in calculating means; used when the compound was detected in at least one replicate for the treatment. Values following '<' are equal to the detection limit.

PAHs	Clean Water	Influent	6"	12"	18"	18" CWC
1-Methylnaphthalene	0.003	0.02	0.021 (0.002)	0.019 (0.003)	0.017 (7e-04)	0.002 (3e-04)
2-Chloronaphthalene	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2-Methylnaphthalene	0.003	0.015	0.005 (0.003)	0.007 (0.005)	0.003 (6e-04)	0.002 (3e-04)
Naphthalene	0.007	0.046	0.01 (0.002)	0.006 (3e-04)	0.006 (7e-04)	0.007 (7e-04)
Acenaphthene	<0.003	<0.003	0.003 (0.002)	<0.003	<0.003	<0.003
Acenaphthylene	<0.002	0.004	<0.002	<0.002	<0.002	<0.002
Anthracene	<0.001	0.007	<0.001	<0.001	<0.001	<0.001
Carbazole	<0.001	0.008	0.001 (8e-04)	<0.001	0.001 (2e-04)	<0.001
Dibenzofuran	0.002	0.007	0.002 (0.001)	0.003 (3e-04)	0.003 (3e-04)	0.002 (6e-04)
Fluorene	<0.002	0.021	<0.002	<0.002	0.012 (6e-04)	<0.002
Phenanthrene	0.002	0.062	0.001 (5e-04)	0.002 (0)	0.002 (0)	0.001 (5e-04)
Benz[a]anthracene	<8e-04	0.012	<8e-04	<8e-04	<8e-04	<8e-04
Chrysene	<9e-04	0.037	<9e-04	<9e-04	<9e-04	<9e-04
Fluoranthene	<0.002	0.084	<0.002	<0.002	<0.002	<0.002
Pyrene	<0.001	0.117	<0.001	<0.001	<0.001	<0.001
Benzo(a)pyrene	<0.002	0.016	<0.002	<0.002	<0.002	<0.002
Benzo(b)fluoranthene	<5e-04	0.033	<5e-04	<5e-04	<5e-04	<5e-04
Benzo(j)fluoranthene	<0.002	0.009	<0.002	<0.002	<0.002	<0.002
Benzo(k)fluoranthene	< 0.003	0.013	<0.003	<0.003	<0.003	<0.003
Dibenzo(a,h)anthracene	<0.001	0.002	<0.001	<0.001	<0.001	<0.001
Perylene	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006
Benzo(ghi)perylene	<0.001	0.085	<0.001	<0.001	<0.001	<0.001
Indeno(1,2,3-cd)pyrene	<0.001	0.025	<0.001	<0.001	<0.001	<0.001
Total PAHs	0.033	0.628	0.058 (0.005)	0.053 (0.006)	0.058 (0.001)	0.03 (0.001)
Sum High Molecular Weight	0.011	0.436	0.011 (0)	0.011 (0)	0.011 (0)	0.011 (0)
(HMW)						
Sum Low Molecular Weight (LMW)	0.022	0.192	0.047 (0.005)	0.042 (0.006)	0.048 (0.001)	0.019 (0.001)

### Basic Water Chemistry: Events 31-35; 37-40

Basic chemistry events were used to age the experimental columns. Influent water (stormwater runoff and clean lab water) and effluent waters (filtered through experimental columns) were not sub-sampled for chemistry or toxicology during these events. Temperature, pH, conductivity, and turbidity of influent and effluents waters were recorded (Table 4). Differences among the five treatment depths for concentrations of each conventional parameter in effluent were assessed by a Kruskal-Wallis test, followed by a post-hoc Dunn's test.

Table 4. Mean (standard error) of conventional parameters in influent waters (clean water and stormwater runoff; SW) and triplicate effluent waters from each of the three treatment depths plus the clean water control (CWC).

Compound	Clean Water	Influent	6"	12"	18"	18" CWC				
Event 31										
Conductivity	1537	79.2	87 (1)	86.6 (0.4)	87 (1)	1568 (2)				
рН	7.62	7.495	7.52 (0.04)	7.427 (0.008)	7.42 (0.03)	7.37 (0.03)				
Temperature	NA	40.2	41.9 (0.5)	38.4 (0.3)	39 (0.3)	38.9 (0.4)				
Turbidity	0.05	58.6	6 (0.2)	4.5 (0.3)	5.1 (0.9)	2 (0.1)				
			Event 3	32						
Conductivity	1500	10620	10443 (10)ª	10317 (9) <sup>ab</sup>	10280 (10) <sup>b</sup>	1522 (1)				
рН	7.602	7.552	7.27 (0.04) <sup>a</sup>	7.15 (0.03) <sup>ab</sup>	7.01 (0.02) <sup>b</sup>	7.359 (0.006)				
Temperature	NA	63.8	66.1 (0.7) <sup>a</sup>	63.4 (0.2) <sup>b</sup>	63.7 (0.2) <sup>ab</sup>	62.9 (0.3)				
Turbidity	0.02	47.4	0.57 (0.05)	0.6 (0.1)	0.46 (0.04)	5.8 (0.2)				
			Event 3	3						
Conductivity	1494	134.1	454 (5)	627 (10)	780 (10)	1519 (3)				
рН	8.102	7.712	8.25 (0.05) <sup>a</sup>	7.98 (0.02) <sup>ab</sup>	7.888 (0.004) <sup>b</sup>	7.368 (0.004)				
Temperature	NA	53.2	56 (0.7)ª	53 (0.3) <sup>ab</sup>	53.3 (0.2) <sup>b</sup>	53.6 (0.2)				
Turbidity	0.08	30.5	5.9 (0.2)	6.1 (0.6)	6 (0.6)	3.18 (0.03)				
			Event 3	34						
Conductivity	1545	176.7	186 (1) <sup>a</sup>	193.6 (0.4) <sup>ab</sup>	200.7 (0.3) <sup>b</sup>	1567 (2)				
рН	7.804	7.935	8 (0.02)	8.027 (0.005)	8.03 (0.01)	7.41 (0.004)				
Temperature	NA	45.3	48 (0.7)ª	44.9 (0.2) <sup>b</sup>	45.1 (0) <sup>ab</sup>	45.2 (0.2)				
Turbidity	0.02	34.7	7.1 (0.1)	7.1 (0.7)	6.7 (0.8)	1.8 (0.2)				
			Event 3	5						
Conductivity	1549	5110	4990 (20) <sup>a</sup>	4960 (10) <sup>ab</sup>	4877 (9) <sup>b</sup>	1557.7 (0.3)				
рН	7.663	7.61	7.4 (0.04)	7.32 (0.03)	7.13 (0.02)	7.402 (0.005)				
Temperature	NA	35.7	40.1 (0.5)	34.9 (0.4)	35.8 (0.4)	35 (0.3)				
Turbidity	0.07	45.7	1.3 (0.4)	0.6 (0.1)	0.4 (0.1)	1 (0.1)				

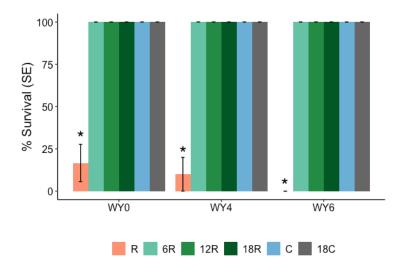
			Event 3	7		
Conductivity	1514	105.6	164.2 (0.7) <sup>a</sup>	195 (2) <sup>ab</sup>	229 (10) <sup>b</sup>	1520 (2)
рН	7.645	7.583	7.83 (0.03) <sup>a</sup>	7.737 (0.003) <sup>b</sup>	7.765 (0.008) <sup>ab</sup>	7.3 (0.02)
Temperature	NA	37.7	42.4 (0.9) <sup>a</sup>	37.6 (0.6) <sup>b</sup>	39 (0.7) <sup>ab</sup>	38.7 (0.3)
Turbidity	0.01	56.4	8.4 (0.5)	8 (1)	7 (0.5)	2.4 (0.4)
			Event 3	8		
Conductivity	1481	332	333 (2)ª	335 (1) <sup>ab</sup>	345.7 (0.7) <sup>b</sup>	1495 (2)
рН	7.665	7.134	7.33 (0.05) <sup>ab</sup>	4 (1) <sup>a</sup>	7.47 (0.01) <sup>b</sup>	7.36 (0.04)
Temperature	NA	46.9	48.1 (0.6)	44.97 (0.07)	44.8 (0.2)	44.8 (0.4)
Turbidity	0.03	40.8	4 (0.7)	3.4 (0.6)	2.9 (0.2)	2.6 (0.4)
			Event 3	9		
Conductivity	1481	173.8	200 (2)	203 (3)	214 (3)	1547 (3)
рН	7.482	7.551	7.4 (0.04)	7.26 (0.02)	7.25 (0.01)	7.28 (0.03)
Temperature	NA	NA	55.9 (0.7)	53.1 (0.3)	53.3 (0.2)	53.2 (0.3)
Turbidity	0.01	35.7	3.43 (0.08)	3.6 (0.6)	4.5 (0.4)	5.1 (0.3)
			Event 4	0		
Conductivity	1484	176.7	175 (2)ª	172 (0.3) <sup>ab</sup>	169 (2) <sup>b</sup>	1526 (3)
рН	7.621	7.65	7.45 (0.04)	7.255 (0.009)	7.27 (0.03)	7.33 (0.02)
Temperature	NA	43.3	46 (0.9)	42.8 (0.4)	43.3 (0.3)	43.3 (0.1)
Turbidity	0.14	47.8	3.2 (0.4)	2.5 (0.5)	6 (2)	2.05 (0.06)

Note: Treatments with different superscript group labels (a, b, c) are significantly different at 🛛 = 0.05 (Kruskal-Wallis with post-hoc Dunn Test).

# Toxicology – Event 36 (End of WY6)

#### **Toxicity Testing – Salmon**

Toxicity testing using juvenile coho salmon was generally conducted as previously (Deliverable 4.1: Progress Report 1). Untreated highway runoff was acutely lethal to juvenile coho salmon. At the end of the exposure (24 h), 100% mortality was observed. Treatment of runoff through any of the BSM treatments completely prevented mortality.



**Figure 1.** Survival of juvenile coho salmon following 24-h exposures to influent and effluent waters for three sampling Events: 1 (WYO), 24 (post-WY4), and 36 (post-WY6). Coho were exposed to influent stormwater (SW), control water (C), and bioretention-treated runoff pooled across triplicates (Event 1 and post-WY6) or duplicates (post-WY4) of each bioretention treatment depth (6", 12", 18", and 18" clean water control (CWC)). \* indicates an exposure that significantly affected survival relative to controls.

## Saturated Hydraulic Conductivity

Saturated hydraulic conductivity (K<sub>sat</sub>) of each bioretention treatment was measured following Event 36 (Post-WY6) using the falling head method (Klute and Dirksen 1986).

**Table 5.** Average (standard deviation) saturated hydraulic conductivity (Ksat) for each treatment for each test. 6R, 12R, and 18R refer to the 6", 12", and 18" treatment depths; 18C refers to the 18" clean water control columns.

Treatment	Ksat (cm/hr)							
	Pre-WY1	Post-WY1	Post-WY2	Post-WY3	Post-WY4	Post-WY5	Post-WY6	
6R	423 (127)	391 (50)	415 (31)	345 (48)	125 (140)	416 (87)	174 (36)	
12R	525 (150)	280 (94)*	279 (65)*	235 (67)*	149 (89)*	362 (8)*	146 (37)	
18R	391 (27)	200 (70)	243 (56)	194 (8)	141 (55)	324 (81)	95 (15)*	
18C	431 (94)	164 (4)*	204 (30)*	208 (31)*	216 (96)*	368 (72)	268 (115)	
Average	442 (107)	259 (106)	285 (93)	245 (72)	158 (92)	368 (68)	171 (85)	

\*Average of two replicates because of leaking from one replicate.