Persistent Organic Pollutant Sources and Pathways to Juvenile Steelhead Trout in the Nisqually River

Sandra M. O'Neill[1], Andrea J. Carey[1], and William Hobbs[2]

[1]Washington Department of Fish and Wildlife

[2]Washington Department of Ecology

Final Contract Report Submittted to the Nisqually Tribe

April 2020

WDFW Report Number FPT 20-12

Author and Contact Information

Sandra M. O'Neill[1]
sandra.oneill@dfw.wa.gov
(360) 480-3359

Andrea J. Carey[1]
andrea.carey@dfw.wa.gov
(360) 480-3443

William Hobbs[2]
whob461@ECY.WA.GOV
(360) 407-7512

[1]Washington Department of Fish and Wildlife
Marine Resources Division
600 Capital Way N
Olympia, WA 98501-1051

[2]Washington Department of Ecology
Environmental Assessment Program
300 Desmond Dr SE
Lacey, WA 98503

Any use of product or firm names in this publication is for descriptive purposes only and does not imply endorsement by authors or the Washington Department of Fish and Wildlife or Washington Department of Ecology.

Table of Contents

List of Tables

List of Figures

Acronymns, Abbreviations, and Units

Acronyms and abbreviations used frequently in this report are listed below; those used infrequently are excluded.

CBR	Critical body residue
DOC	Dissolved organic carbon
Ecology	Washington Department of Ecology
EPA	Environmental Protection Agency
ESA	Endangered Species Act
EST	Environmental Sampling Technologies
JBLM	Joint Base Lewis-McChord
MDL	Method detection limit
MQL	Method quantitation limit
PBDE	Polybrominated diphenyl ether
PCA	Principal component analysis
POP	Persistent organic pollutant
PRCs	Performance reference compounds
QA/QC	Quality assurance/quality control
RPD	Relative percent difference
RSD	Relative standard deviation
SPMD	Semipermeable membrane device
TBiOS	Toxics focused Biological Observation System
TOC	Total organic carbon
TSS	Total suspended solids
USGS	U.S. Geological Survey
WDFW	Washington Department of Fish and Wildlife
WWTP	Wastewater treatment plant

Units of Measurement

mg/L	milligrams per liter
NTU	Nephelometric Turbidity Units
ng/g	nanograms per gram (parts per billion)
pg/g	picogram per gram (parts per trillion)
pg/L	picogram per gram per liter (parts per quadrillion)
ppt	parts per trillion
rm	river mile
ww	wet weight

ABSTRACT/SUMMARY

A 2014 study on contaminant exposure in outmigrating steelhead trout (*Oncorhynchus mykiss*) from in-river and the estuary habitats of Skagit, Green/Duwamish and Nisqually rivers and their associated nearshore marine habitats documented that polybrominated diphenyl ethers (PBDEs) were highest in the Nisqually River system. Moreover, PBDEs concentrations in steelhead trout were above critical body resides (CBRs) for increased disease susceptibility throughout the Nisqually river system: 33% of fish in-river at the smolt traps, 33% of fish caught in the estuary and 50% of fish in the associated marine basin. Subsequent sampling of steelhead trout at the Nisqually River smolt trap in 2015 also confirmed that approximately one third of the fish had PBDE at levels known to increase disease susceptibility in salmonids. PBDEs were only detected in a portion of the samples, and PBDE contaminant exposure was hypothesized to be limited to a subset of the watershed. **The purpose of this study was to conduct a source assessment study to identify and prioritize potential sources of PBDE to the Nisqually River so that corrective actions may be implemented.** Specific objectives were to 1) conduct a synoptic survey to assess the spatial distribution of PBDEs in the main stem Nisqually River and its tributaries, and 2) to identify and characterize potential sources of PBDEs to the Nisqually River system, based on the results of the synoptic survey. PBDE concentrations were measured in water samples (via semi-permeable Membrane SPMDs) and in biofilms (i.e., algae and microbial biomass).

The results of the 2017 PBDE source assessment suggested that PBDEs were primarily entering the Nisqually River system via the three major tributaries of the Nisqually River: the Mashel River, Muck Creek, and Ohop Creek, **however, limited spatial sampling within the tributaries restricted our ability to definitively conclude the major sources**. Generally, PBDEs were measured in low concentrations in both water and biofilm samples with the exception sites in the three major tributaries. Elevated PBDEs concentrations were only measured in water samples at the upper Mashel River site whereas the highest total PBDE concentrations were measured in biofilm samples from Muck Creek, followed by the upper Mashel River site, and then Ohop Creek. Biofilms were shown to bioconcentrate PBDEs approximately 1000 times and generally well correlated at higher concentrations. At the upper Mashel River site, the biofilm PBDE congeners had a different pattern of PBDE congeners than all other sites and had an enriched signal of nitrogen stable isotope, $\delta^{15}N$. The combination of elevated PBDE levels, a different pattern of PBDE congeners and an enrichment of $\delta^{15}N$ strongly suggested exposure to a wastewater source. The outfall for the Eatonville WWTP outfall is located slightly upstream from the site, suggesting that that it is potentially the source of PBDEs in this tributary. The elevated concentrations return to near background concentrations just five miles downstream where the Mashel River meets the mainstem Nisqually River, suggesting that the PBDEs were diluted as they flow away from the source, possibly by input from the Little Mashel River that enters downstream of the Eatonville outfall but upstream of the confluence with the Nisqually River, or from groundwater sources. **Additional sampling would be necessary to document the extent of the Mashel River that is affected by PBDE inputs from the Eatonville wastewater treatment plant outfall and how this relates to Steelhead rearing habitat**.

The pattern of PBDE congeners measured in Muck and Ohop creeks were distinct from those in the upper Mashel. River site. Heavier PBDE congeners were detected in both Muck Creek (i.e., 203, -206, -

207, -208 and -209) and, Ohop Creek (i.e., 203, -206, and -208), however, there is some uncertainty regarding the true concentration due to laboratory blank contamination. Although, Muck and Ohop creeks biofilms have a heavier PBDE congener pattern, the source of these PBDEs was not likely from a wastewater source as their nitrogen stable isotopes, $\delta^{15}N$, which are often altered when exposed to wastewater, are not altered compared to other biofilm samples, and there are no waterwater treatment plant discharges directly to these creeks. **Additional sampling would be necessary to document the source PBDEs to Muck and Ohop creeks.**

INTRODUCTION

Steelhead trout (*Oncorhynchus mykiss*) are the official fish of Washington State, however, Puget Sound populations have been on the Endangered Species Act's (ESA) Threatened list since 2007. Currently, the Puget Sound steelhead population in Washington State is less than 10% of its' historic size, and is facing possible extinction (Salish Sea Marine Survival Project, 2018). Poor marine survival of juvenile steelhead is a contributing factor leading to their decline, with parasites, predation, disease and contaminant exposure identified as possible causes of early mortality.

Numerous laboratory and field assessments have demonstrated the adverse effects of contaminants on the health of salmonids. Toxic contaminant exposure can directly impact the health of juvenile salmon by impairing growth, and metabolism (Varanasi et al., 1993; Meador et al., 2006), altering hormone levels (Arkoosh et al., 2010, and 2013), and disrupting reproductive development (Peck et al., 2011). Toxic contaminants can also impair immune function of salmon either alone (Arkoosh et al., 1994, 2010, 2015, and 2018), or in conjunction with other stressors (Jacobson et al., 2003), thereby increasing their susceptibility to naturally occurring infectious diseases, potentially leading to population level effects (Arkoosh et al., 1998; Loge et al., 2005; Spromberg and Meador, 2005). Impairment of immune response is particularly important for endangered and threatened salmonid species and populations because a properly functioning immune system is vital for both individual survival and population productivity (Segner et al., 2012).

As part of a 2014 multi-agency effort to assess juvenile steelhead trout survival in Puget Sound, the Washington Department of Fish and Wildlife's (WDFW's) Toxics-focused Biological Observing System (TBiOS) unit conducted a toxic contaminant study of out-migrating steelhead in several river systems that included the Nisqually River (Puget Sound Steelhead Marine Survival Workgroup 2015; Chen et al., 2018). The TBiOS unit found that one third of the steelhead samples collected from the Nisqually River in-river and estuary habitats in 2014 had elevated lipid normalized (as well as wet wet concentrations) of polybrominated diphenyl ethers (PBDEs) at critical body residue (CBR) concentrations known to adversely impact the immune function of juvenile Chinook salmon (Chen et al. 2018). PBDEs are a group of 209 chemical compounds (congeners) manufactured as flame retardants. The CBR adverse effects concentrations for increased disease susceptibility were based on laboratory studies (Arkoosh et al., 2010, 2013, 2018). In contrast, steelhead samples from other river systems (Skagit and Green/Duwamish rivers), had lipid normalized PBDEs concentrations below the CBR for increased disease susceptibility (Chen et al. 2018). In 2015, TBiOS repeated contaminant analyses on individual steelhead from Nisqually in-river habitats (collected at the smolt trap) that confirmed the 2014 findings, approximately one third of the steelhead whole-body samples had lipids normalized PBDEs at CBR concentrations for increased disease susceptibility in salmonids[1] (WDFW, unpublished data).

[1] There is some uncertainty with using lipid weight to normalize and model concentrations of PBDEs in fish (Bethune et al. 2005). For the 2015 data, if PBDE wet weights instead of lipid weights were used to model CBR concentrations, none of the 2015 data exceeded the CBR.

Based on the results of the 2014 and 2015 studies, we hypothesized that PBDE contaminant exposure is geographically limited within the in-river habitat because only a portion of the steelhead samples at the smolt trap in both years had PBDE concentrations above CBR for increased disease susceptibility. Uniform contaminant exposure among individual steelhead collected at the trap would have indicated widespread PBDE contaminant sources throughout the in-river habitat.

Understanding the sources of PBDE exposure for steelhead trout originating from the Nisqually River is necessary to identify and prioritize corrective management actions that may increase their survival. In 2014, the Nisqually Steelhead Recovery Team reported that the number of adult Steelhead returning to spawn in the mainstem had decreased to 300 or less in four of the previous 10 years. Klungle et al. (2018) noted that the Nisqually River has one of more productive steelhead populations in the species range, with larger numbers of smolts leaving the river, suggesting decreased marine survival is probably the main contributor to the low numbers of returning adults. In an effort to protect the Nisqually steelhead, co-managers eliminated tribal and sport-harvest in the river almost 30 years ago. Additionally, numerous restoration projects have taken place over the years in the Nisqually River and its tributaries to improve habitat and increase steelhead numbers throughout the watershed (Nisqually Steelhead Recovery Team 2014). Identifying the area in the river where Steelhead are exposed to PBDEs would be another beneficial step in preserving the health of this species in the Nisqually River.

The purpose of this study was to conduct a source assessment to identify and prioritize potential sources of PBDEs to the Nisqually River. WDFW's TBiOS team, in collaboration with the Department of Ecology (Ecology), used co-located SPMD and biofilm samples to collect three types of data, 1) total PBDE concentrations, 2) detailed PBDE congener composition (i.e. patterns) and 3) stable isotopes, across diverse ecological sites throughout the Nisqually River watershed. Specific objectives were to 1) conduct a synoptic survey to assess the spatial distribution of PBDEs in the mainstem Nisqually River, the Centralia Canal, and its tributaries, and 2) to identify and characterize potential sources of PBDEs to the Nisqually River system, based on the results of the synoptic survey and using patterns of PBDE congeners in the samples. Additionally, naturally occurring stable isotopes of carbon and nitrogen were analyzed in biofilms to provide potential insights into ecological processes associated with flow of energy in the system Boecklen et al., 2011; Peterson and Fry, 1987; Thompson et al., 2005).

METHODS

Species of concern

Steelhead trout are the anadromous, or sea-going, form of freshwater rainbow trout, with a complex life history. In general, steelhead are iteroparous and spawn in the spring, unlike Pacific salmon that are semelparous and spawn in the fall. After spending as many as four years at sea, adult winter steelhead enter the Nisqually River between December and May, where they wait in pools near their spawning habitat until they spawn from April until June. Peak fry emergence occurs during mid-July. Juveniles actively rear in the rivers and creeks of the Nisqually River and its tributaries for one to three years (Nisqually Steelhead Recovery Team 2014) however, the exact rearing locations are unknown. Typically, smolts migrate to saltwater between mid-April to mid-June (Nisqually Steelhead Recovery Team 2014).

Study Area

Watershed Description

Originating from the Nisqually glacier on the southern slope of Mount Rainier, the Nisqually River flows west-northwest for approximately 78 miles and enters Puget Sound approximately 8 miles northeast of Olympia, Washington. Overall, the watershed encompasses approximately 761 square miles. Steelhead can only access the lower 42.5 miles of the mainstem, downstream of La Grande Dam. In addition to the mainstem, several tributaries enter the Nisqually River below La Grande Dam, and provide potential steelhead habitat. Collectively, steelhead utilize over 100 river miles of the Nisqually River and its tributaries for spawning and juvenile rearing habitat. Furthermore, Nisqually Steelhead Recovery Team (2014) designated the mainstem Nisqually River below LaGrande Dam and most of the major tributaries as core summer salmonid habitat because they provide ideal rearing conditions. Chinook salmon, pink salmon, and steelhead make up the larger portion of spawning salmonids in the mainstem while coho salmon, chum salmon and cutthroat trout contribute to a lesser degree (Pierce County 2012).

Our sampling area included the mainstem below La Grande Dam, and the largest three tributaries of the nine tributaries important to historical Steelhead production, Muck Creek, Ohop Creek, and the Mashel River. Muck Creek and its tributaries is the largest tributary system by area in the Nisqually watershed, with a total drainage of 93 square miles, and contributes over 40 miles of potential Steelhead habitat (Pierce County 2005; Nisqually Steelhead Recovery Team 2014). It drains prairie lands and some areas of the river can have little to no flow in the summer (Nisqually Steelhead Recovery Team 2014). The lower portion of the creek runs through southeastern section of the 87,000-acre Joint Base Lewis-McChord (JBLM) facility and lands, a military complex consisting of a training and mobilization center for the armed services. Chum salmon primarily utilize the creek but steelhead and coho have also been viewed there, though in much smaller numbers (Pierce County 2005).

The Mashel River is the second largest Nisqually tributary by watershed area with a total drainage area over 80 square miles (Pierce County 2012; Nisqually Steelhead Recovery Team 2014). The summer flow of this river is maintained by snowmelt. The town of Eatonville, located along the Mashel River, not only draws its water from the river but it also discharges secondary-treated wastewater from a wastewater treatment plant (WWTP) downstream of the town. Primarily, the river flows through forested land (Nisqually Steelhead Recovery Team 2014). Coho salmon, fall Chinook salmon, pink salmon and steelhead trout are all known to utilize the river system (Pierce County 2012). The Mashel River supports approximately 30% of the steelhead production in the Nisqually River system, based on the average proportion of the total Nisqually escapement distributed in the Mashel River from 2013 through 2019 by WDFW (Sayre Hodgson, Nisqually Tribe, personal communication).

Ohop Creek is the third largest Nisqually River tributary by watershed area, with a total drainage area of 40 square miles (Pierce County 2012; Nisqually Steelhead Recovery Team 2014). Snowmelt maintains the flow of the creek in the summer. Historically, the main land use was for agriculture but recently it has switched to a more residential use (Nisqually Steelhead Recovery Team 2014). Primarily, fall Chinook salmon, coho salmon, and pink salmon are found in Ohop Creek while steelhead trout are presumed to utilize it but their presence is not well known (Pierce County 2012).

Potential PBDE Sources

This study investigated whether the inputs of PBDEs to the steelhead habitat area of the Nisqually river-system were from one of the three main tributaries (i.e., Muck Creek, Ohop Creek, and Mashel River), the Centralia Canal, the mainstem, or a combination of these inputs. During the study's planning phase, WDFW and Nisqually Tribal staff identified potential sources of PBDEs within the Nisqually River watershed based on historical and current land uses (Table 1, normal text). Additional potential sources were identified after the study was initiated (Table 1, text in italics). Specific major potential sources included three wastewater treatment plant (WWTP) outfalls, a major stormwater outfall, and surface stormwater runoff from a former (i.e. legacy) dump used by Weyerhaeuser, the Fort Lewis facility/lands, and the University of Washington's (UW) Charles L. Pack Experimental Forest research facility/lands (UW Pack Forest) are noted in Figure 1. Additionally, diffuse runoff from agricultural, residential and forested lands within the Ohop Creek and Mashel River watersheds could potentially input PBDEs to the Nisqually river-system, although we did not explicitly identify sources.

Table 1. Specific potential sources of PBDEs to the Nisqually River watershed.

River or Tributary Receiving Water	Potential PBDE source	Location of Potential Inputs
Muck Creek	Fort Lewis facilities/lands	associated watershed flows to Muck Creek
Centralia Canal	City of Yelm WWTP outfall 002	discharges to Centralia Canal
Nisqually River	City of Yelm WWTP outfall 003	discharges to mainstem Nisqually
Nisqually River	*City of Yelm application of reclaimed water (from WWTP outfall 001for irrigation and other uses*	*reclaimed water is distributed throughout the City of Yelm potentially*
Ohop Creek	Eatonville stormwater outfall	discharges to a tributary of Ohop Creek (Lynch Creek)
Mashel River	City of Eatonville WWTP outfall	discharges to Mashel River, just below Eatonville
Mashel River	Legacy dump (used by Weyerhaeuser)	associated watershed flows to Mashel River
Mashel River	UW Pack Forest - occasional application of sewage sludge as an experimental fertilizer	associated watershed flows to Mashel River
Mashel River	*Fire Mountain Farms application of sewage biosolids as fertilizer*	*Little Mashel, downstream of the Eatonville WWTP outfall*
Nisqually River	Atmospheric deposition to upper watershed	flows to Alder Lake, above LaGrande Dam

Sampling Site Selection

To identify potential inputs of PBDEs to steelhead habitat, we measured PBDEs in co-located water and stream biofilm samples (described in the next section) collected from the 11 sites strategically located within the mainstem Nisqually River below La Grande Dam, and three of its major tributaries, Muck Creek,

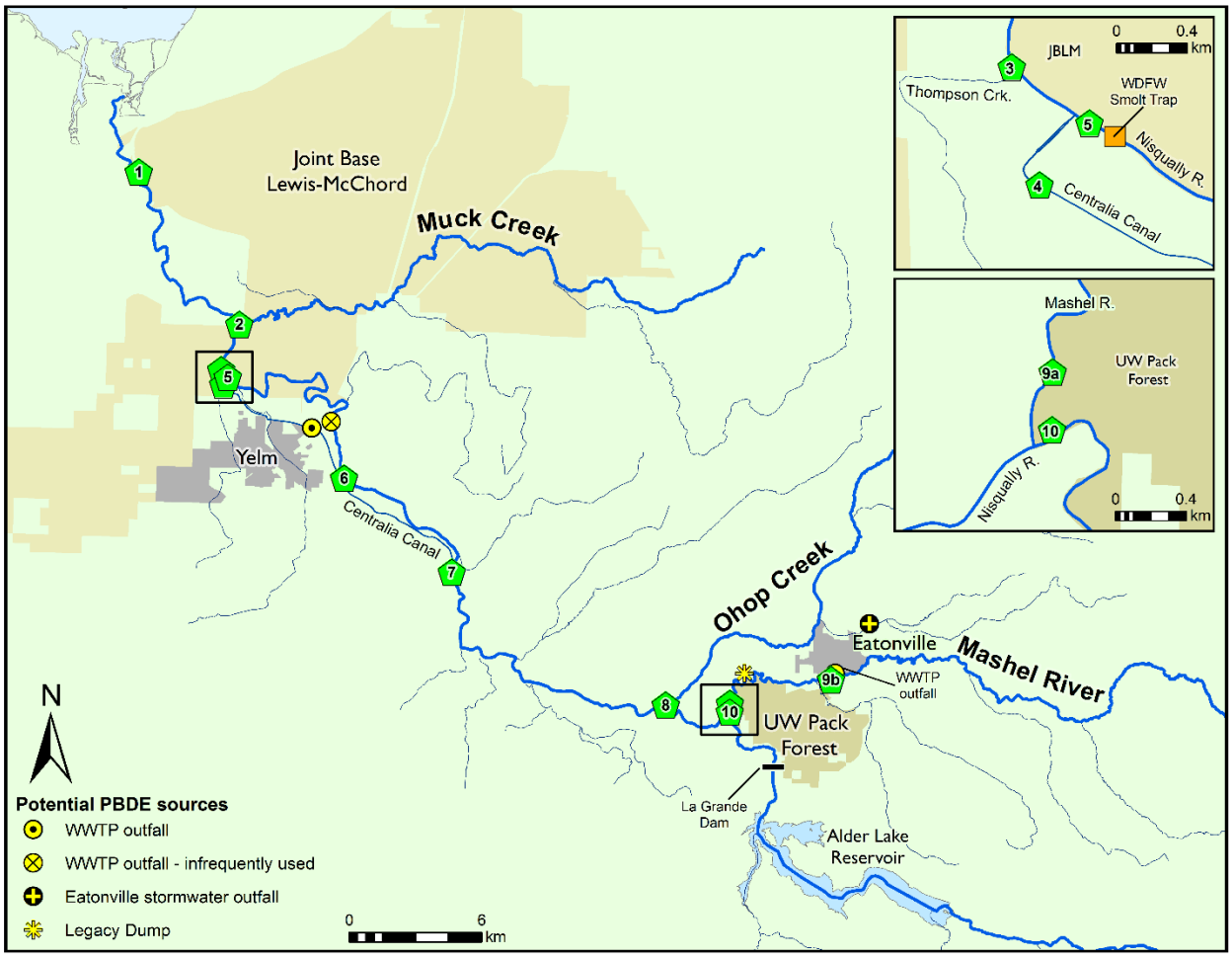

Figure 1 Map of the Nisqually River watershed noting the mainstem and major tributaries (colored dark blue), major WWTP and stormwater outfalls, a legacy dumpsite, the major land use facilities/land (i.e., Fort Lewis and UW Pack Forest) and the locations where passive water samplers were deployed and biofilm was collected to measure PBDEs in water.

Ohop Creek and the Mashel in the summer of 2017. The sampling locations included six sites along the mainstem Nisqually River, one site on Centralia Canal before the confluence with the Nisqually River, one each in Muck Creek and Ohop Creek, and two in the Mashel River (Figure 1 and Table 2). Collectively, these sites encompass an area of known potential inputs of PBDEs to the system, noted in Table 1.

The Muck Creek sampling site was located just upstream of the confluence with the mainstem Nisqually River (site 2) and was selected to assess potential PBDE inputs from surface stormwater runoff from the JBLM facility/lands, although we are not aware of any known PBDE sources (Figure 1 and Table 1).

Table 2. Passive sampler location information and the number of biofilm samples collected at each site (NA = none available)

Site Number in Fig. 1	Site ID	Site Name	Approx. River Mile[a]	Approx. Trib. Mile[a]	Site Latitude	Site Longitude	Number of SPMDs deployed	Number of biofilm samples collected
1	11NR3.75	lower Nisqually River	3.75	-	47.05765	-122.690706	2[c]	1
2	11MC0.25	Muck Creek	10.5	0.25	46.99675	-122.6282333	1	1
3	11NR12.25	Nisqually River – Thompson Creek	12.25	-	46.977972	-122.638528	1	1
4	11CC0.25	Centralia Canal	12.5	0.25[b]	46.971992	-122.636864	1	NA
5	11NR12.5	Nisqually River – smolt trap	12.5	-	46.974981	-122.634567	1	1
6	11NR21.6	Nisqually River – McKenna	21.6	-	46.935175	-122.563536	1	1
7	11NR26.25	Nisqually River – diversion dam	26.25	-	46.898011	-122.498017	1	NA
8	11OC0.1	Ohop Creek	37.5	0.1	46.846017	-122.36885	1	1
9a	11MR0.5	lower Mashel River	39.5	0.5	46.847322	-122.330669	2[d]	2
9b	11MR4.9	upper Mashel River site	39.5	4.9	46.857911	-122.270903	2[c]	1
10	11NR39.75	upper Nisqually River - background	39.75	-	46.843797	-122.330592	1	1

[a]Washington Department of Fisheries 1975

[b]the Centralia Canal is not considered a tributary of the Nisqually so the distance from the mainstem to the site was approximated using the measuring device in ArcGIS

[c]two SPMD canisters were deployed at these locations due to concerns about potential loss due to vandalism, however, as both were retrieved, only one was analyzed for PBDEs

[d]one of the two SPMD canisters deployed at this location was a field duplicate

The sample site on Ohop Creek site, located just upstream of the confluence of the mainstem Nisqually River (site 8), was selected to assess potential PBDE inputs to the mainstem Nisqually River from the Ohop Creek. It receives stormwater from the City of Eatonville via an outfall that is located on one of its tributaries, Lynch Creek. Additionally, the Ohop Creek watershed consists of mixed uses (agriculture, residential properties, and forests) that may contribute inputs of diffuse stormwater surface runoff of PBDEs (Figure 1 and Table 2).

Within the Mashel River, the furthest upstream of the two sampling sites was selected to assess inputs from the City of Eatonville's WWTP, and was located just downstream of the outfall (site 9b; Figure 1and Table 1). The second sampling site was located just upstream of the confluence of the Mashel River and the mainstem Nisqually River (site 9a; Figure 1 and Table 2). We selected this site to assess multiple potential inputs from the Mashel River (i.e., Eatonville WWTP outfall, stormwater runoff from legacy dump (Weyerhaeuser/City of Eatonville), the UW Pack Forest, Fire Mountain Farms, and other unknown inputs on the Mashel River. Surface water runoff from the legacy dump could contain PBDEs leaching from previously dumped furniture and electronics. The UW Pack Forest encompasses 4,300 acres of working forestland and has occasionally used sewage sludge as an experimental fertilizer, a possible source of PBDEs. Fire Mountain Farms has a permit to apply biosolids from a sewage treatment facility as fertilizer, excluding sensitive area (Midway Creek and wetlands located wet of the hay fields (Fire Mountain Farms Inc. Biosolids Management Permit BT9902, Version 6/2/17)

In addition to sampling the waters of the three major tributaries, we sampled waters in the Centralia Canal, a 9-mile long man-made canal that runs alongside the Nisqually River and provides electricity to the town of Centralia. A diversion dam (at rm 26) diverts water into the canal. Fish cannot access the canal because screens in place at the diversion dam deflect them back into the mainstem Nisqually River. The city of Yelm WWTP distributes some of its secondary treated wastewater (from outfall 001) as reclaimed water throughout the city but it also has two effluent outfalls, one within the Centralia Canal (the primary outfall -outfall 002, approximately 6 miles downstream from the diversion dam), and one within the Nisqually River mainstem (the standby outfall 003). The Nisqually River outfall is used, only when the outfall within the Centralia Canal has to be shut off for canal maintenance or an emergency (City of Yelm Wastewater Treatment and Water Reclamation Facility 2005). Our sampling site in the Centralia Canal was located downstream of the WWTP outfall but immediately upstream of the confluence of the Canal with the mainstem (site 4). We selected this site to assess inputs from the Yelm-Centralia Canal WWTP outfall that discharges within the canal (Figure 1 and Table 2). A hydroelectric powerhouse, located at approximately river mile 12.5 on the Nisqually River subsequently discharges water from the canal to the mainstem. Thus, the Centralia Canal serves as a potential PBDE source to the mainstem Nisqually River, but not as rearing habitat by salmonids.

Within the mainstem Nisqually River the six sampling sites were located between river mile 3.75 and 39.75 to assess various potential PBDE sources. The furthest upstream of the six sampling sites was located downstream of the La Grande Dam (site 10, upper Nisqually River) and was selected to assess if the upper mainstem was a PBDE source, primarily through atmospheric deposition of PBDEs in snowmelt and precipitation from Mt. Rainier and the surrounding area. Data from this site was used to

establish PBDE background levels for the Nisqually River and its tributaries since no known PBDE sources are located upstream of the site besides possible air deposition. Traveling downstream, another site was located just upstream of the diversion dam for the Centralia Canal at river mile 26.25 (site 7, Diversion Dam), selected to aid in identifying the level of PBDEs in the mainstem, prior to potential PBDE inputs from WWTPs located further downstream (i.e., City of Yelm and Yelm WWTP). Continuing downstream, we selected a site upstream of the city of Yelm in McKenna at river mile 21.6 (site 6, McKenna) to assess any inputs between that location and the Centralia Canal divergence but before the Yelm WWTP outfall. Another sampling site on the mainstem was located at river mile 12.5, just upstream of the Centralia Canal powerhouse where the canal empties into the mainstem and near salmonid smolt trap (site 5, Smolt Trap). We used this site to assess any input from Yelm's WWTP outfall and other cumulative upstream inputs. The second to last downstream sampling site on the mainstem, located below the confluence of Thompson Creek at river mile 12.25 (site 3, Thompson Creek). We selected this site to assess any inputs from the City of Yelm, the WWTP outfall located within the Centralia Canal and any runoff from Yelm's water reclamation program (effluent from outfall 001 that is distributed throughout the city for irrigation, as well as any other sources. The further downstream site on the Nisqually River (site 1, lower Nisqually River)), located after the confluence with Muck Creek but upstream of the saltwater influence, was selected to assess cumulative PBDE inputs throughout the river-system (Figure 1 and Table 2).

Based on Puget Sound loading studies (Ecology and King County, 2011) WWTPs are a more likely source of PBDEs than stormwater outfalls or surface stormwater runoff. Accordingly, sampling for this project took place during the low flow period at the end of the summer (i.e. September) when inputs from WWTPs are concentrated in the river water, improving the chances to detect PBDEs.

Sample Collection and Analyses

We sampled water and biofilm samples for the presence of PBDEs, following general methods described by Hobbs et al. (2014) for sampling organic contaminants in the same matrices. The concentrations of PBDEs in the ambient water were passively assessed using semipermeable membrane devices (SPMDs) deployed for approximately one month. Additionally, we collected water grab samples from all 11 SPMDs sites on the day of deployment, mid-study (14 days post deployment), and on the day of retrieval and analyzed for conventional water quality parameters: total organic carbon (TOC), dissolved organic carbon (DOC), total suspended solids (TSS), and turbidity. Biofilm samples, an aggregation of periphyton, microbes and fine sediments, were scraped from rock adjacent to where the SPMDs were deployed, at the time the SPMDs were retrieved.

Water Samples

SPMDs

SPMDs are composed of a thin-walled, lay-flat polyethylene tube (91.4 cm x 2.5 cm x 70-95 um thickness) filled with 1 ml of triolein, a neutral lipid compound (Figure 2). The goal of any passive sampling device is to emulate natural biological uptake by allowing chemicals to diffuse through the membrane and concentrate over time (typically a 28-day deployment). After deployment, the membranes are removed, extracted, and analyzed for the contaminant of interest. In this study, 14

SPMDs were deployed at 11 sites on August 28 and 29th, 2017 and retrieved 27 – 29 days later on September 25 and 26th, 2017.

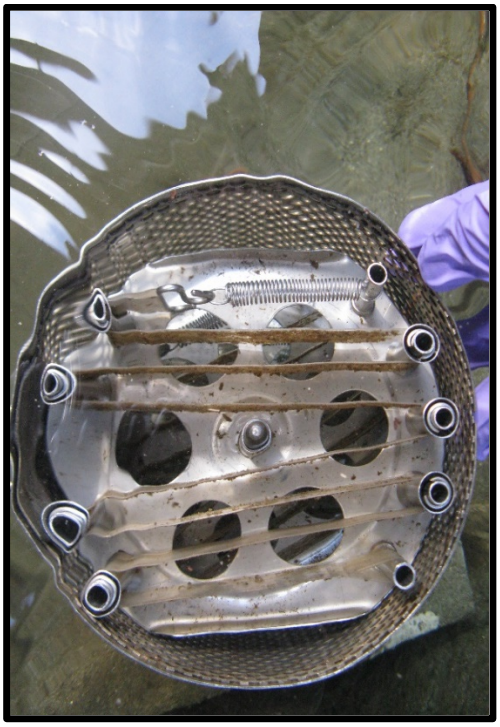

Figure 2. An SPMD canister showing the upper membrane. Some biofouling on the membrane is evident.

SPMDs were deployed in stainless steel canisters and spindle devices provided by Environmental Sampling Technologies (EST). Each canister contained five membranes preloaded onto spindles by EST, and shipped in solvent-rinsed metal transport-cans under argon gas. Prior to deployment, performance reference compounds (PRCs) were spiked into the membranes in order to assess biofouling and the non-equilibrium uptake of the compounds of interest (Huckins et al., 2006). The use of PRCs is essentially an *in situ,* site-specific calibration technique based on the observation that the rate of residue loss is proportional to the rate of residue uptake. Native congeners, or performance reference compounds (PRCs), BDE-10 (CAS 51930-04-2), BDE-37 (CAS 147217-81-0), BDE-126 (CAS 366791-32-4) and the isotopically labelled congener, BDE-138L were injected into the triolein oil at a concentration of 2.5 ng/SPMD prior to manufacturing the SPMD to measure the rate of uptake of PBDEs by the SPMDs.

A StowAway® TidbiT™ temperature logger was attached to each canister to continuously monitor the water temperature during deployment. A second temperature logger was attached nearby to monitor air temperature. The data collected from the temperature loggers was used to confirm that the SPMD remained submerged during the entire exposure period. With one exception, all SPMDs were shown to remain submerged during the period of deployment (Appendix Figure A1). The SPMDs at the Muck Creek site (11MC0.25) were out of the water for approximately one day around September 21, 2017.

SPMDs were exposed to air during deployment and retrieval for approximately 30 seconds. Upon retrieval, SPMDs were stored at -20 ° and shipped on ice directly to SGS AXYS Analytical for analysis. SPMDs were extracted by dialysis in dichloromethane (DCM) and analyzed by high-resolution mass spectrometry using EPA Method 1614, AXYS method MLA-033.

We calculated dissolved PBDE congener concentrations from the mass extracted and measured from the SPMDs using the most recent U.S. Geological Survey (USGS) model (Alvarez, 2010). This model is based on the octanol-water partition coefficient (MacKay et al., 1997), the physical properties of the SPMD, water temperature, and length of deployment. Total PBDE concentrations were estimated based on the formula (Meadows et al. 1998):

$$C_{tot} = C_w\ (1 + [TOC]\left({}^{K_{oc}}/_{M_w}\right))$$

Where C_{tot} is the total contaminant concentration (pg L^{-1}), C_w is the dissolved concentration estimated in water (pg L^{-1}) from the USGS model, TOC is the total organic carbon concentration (mg L^{-1}), K_{oc} is the organic carbon-water partition coefficient (median value from MacKay et al., 1997), and M_w is the mass of water (10^6 mg L^{-1}).

Surface water grab samples

Water grab samples were collected to measure the total and dissolved organic carbon (TOC/DOC), total suspended solids (TSS), and turbidity at each site during the SPMD exposure time (Appendix Table A1). These parameters were used as ancillary data to help understand relationships between suspended matter and the PBDE contaminants. To get an integrated measure of conditions, we collected water grab samples three times over the duration of the SPMD exposure, at deployment, midway through the study, and at retrieval. Grab samples were collected using Ecology standard operating procedures (Joy, 2006). Water samples were shipped on ice to Ecology's Manchester Environmental Laboratory for analysis.

Biofilm

We collected ten biofilm samples from nine of the sites, including a replicate sample collected at one the lower Mashel River site (11MR0.5). Due to lack of biofilm in the area, samples were not collected at two sites, Centralia Canal (11CC0.25) and at the Diversion Dam site on the mainstem (11NR26.25; Table 2). Biofilm was scraped from rocks using stainless steel blades (Figure 3) and collected in a stainless bowl for weighing in the field to confirm that sufficient biomass was retrieved. Samples were transferred from the bowl to a certified cleaned glass jar. Biofilms were analyzed using the same analytical methods as the SPMDs, as well as organic carbon and nitrogen abundance and stable isotope ratios, and lipid content. Carbon and nitrogen isotopes were analyzed using a ThermoFinnigan MAT 253 / Costech EA and had an instrument precision of 0.11 ‰ for $\delta^{15}N$ and 0.06 ‰ for $\delta^{13}C$ during the analytical runs.

Figure 3. Example of a biofilm being scraped from a rock

Quality Assurance/Quality Control (QA/QC)

Measures of QA/QC in the field and laboratory for this project included: duplicate samples from the field and lab (precision); an assessment of laboratory recoveries and field -reference compound recoveries (bias); an assessment of instrument detection limits (sensitivity); an assessment of background contamination in the SPMDs (sensitivity).

Field duplicates for the conventional parameters of TOC/DOC, TSS and turbidity were all within 20% relative percent difference (RPD), with the exception of one sample from the Centralia Canal site (11CC0.25) for turbidity where duplicate results were 24 and 15 Nephelometric Turbidity Units (NTUs). This highlights the potential variability of this parameter. SPMD replicates for total PBDEs had good precision, as described by a RPD of 16% for total PBDEs. The RPD for total PBDE concentrations in biofilm replicates were much higher (71%), however the sample site (lower Mashel River, 11MR0.5) contained low PBDE concentrations (relative to upstream background) which tends to elevate the calculated RPD. In other studies, at sites where toxics were measured in biofilms at concentrations well above the site background, the RPD has been observed to range from 6-13% (Hobbs et al., 2019). Laboratory duplicates for all samples were less than 20% RPD.

Laboratory recoveries of labelled matrix spikes for the SPMDs were all within the method QC limits, as per EPA 1614, with the exception of some low recoveries for BDE-209L. This might introduce a slight bias towards higher concentrations for the isotope dilution calculations; however, the project laboratory

and field blanks provided the necessary QC to prevent a false identification. Laboratory recoveries for the biofilm samples were all within the method QC acceptance criteria. Laboratory recoveries for all matrix and blank spikes for the conventional parameters met the method QC limits. The recovery of the field PRCs showed measurable loss during deployment, with recoveries ranging from 41-68% for BDE-10, 74-92% for BDE-37 and 77-96% for BDE-126.

Laboratory detection limits for all parameters met the sensitivity necessary for the project. All PBDE data were censored against the laboratory method blank specific to the sample batch and media. Censoring occurred at a threshold of five times the method blank – i.e., if an individual PBDE congener was measured at a concentration that was less than five times the concentration of that congener in the blank samples, the result was considered not detected. For the heavier PBDE compounds (e.g. BDE-209) this often resulted in non-detected results, especially for the SPMD samples. Lastly, to explicitly incorporate the level of PBDE compounds present in the SPMD sample, media detection and quantitation limits were calculated based on the relative standard deviation (RSD) of the field and equipment blanks combined, where the detection limit is the mean + 3 times the RSD and the quantitation limit is the mean + 10 times the RSD (Alvarez, 2010). Based on this approach, the mean (± RSD) blank background was 3.0 ± 0.26 pg/L, therefore the method detection limit (MDL) is 5.4 pg/L and the method quantitation limit (MQL) is 11 pg/L for the SPMD samples.

Data Analysis

To illustrate variation in PBDE congener patterns among sample locations, the proportion of PBDE congeners in SPMD and biofilm samples (i.e., PBDE fingerprints) were compared using principal component analyses (PCA), with the stats package in R (R Core Team 2017). Similar PBDE fingerprints among groups were inferred to indicate a consistent source, whereas dissimilar PBDE patterns would suggest inputs associated with different sources. To compare PBDE fingerprints within a sample matrix (i.e., SPMDs, and biofilms), congeners not detected in any of the samples were excluded from the PCA analyses, and for congeners that were detected in only a subset of the samples, detection limits MDL or average of the non-detected censored value) were used for non-detected concentrations. For SPMD samples, 27 primary (and coeluting) congeners of the total 37 primary (and coeluting) PBDE congeners were included in the PCA analyses. Ten congeners (BDE-30, -32, -105, -119, -128, -203, -206, -207, -208, and -209) were excluded from the analysis because they were never detected in the SPMD samples. For the 27 congeners included in the PCA, the detection limit was used for undetectable congeners (67 of 324 SPMD sample x congener combinations, 20.7 % of the data set). For biofilm samples, 26 primary (and coeluting) congeners of the total 40 primary (and coeluting) PBDE congeners were included in the PCA analyses. Fourteen congeners (BDE-7, -8 + 11, -10, -12 + 13, -30, -32, -35, -77, -105, -116, -126, -128, -155, and -181) were excluded from the analysis because they were never detected in the biofilm samples. For the 26 congeners included in the PCA, the detection limit (or the average censored detection limit) was used for undetectable congeners (132 of 260 sample x congener combinations, 50.8% of the data set).

RESULTS AND DISCUSSION

Sampling Timeframe

Sampling periods for water (August 28 to September 26, 2017) and biofilms (September 25 and 26, 2017) were optimized to increase the likelihood of measuring PBDEs inputs from wastewater. Water and biofilm samples were collected during the low flow (or dry) period based on annual river flow data (Figure 4). Sampling during the low flow season is optimized for measuring PBDE inputs from WWTP as effluent is less likely to be diluted by stormwater and meltwater events events. Minimum river flow data were recorded in August in the Nisqually River mainstem and September and August in Ohop Creek and Mashel River, respectively (U.S. Geological Survey 2017). Flow data was not available for Muck Creek.

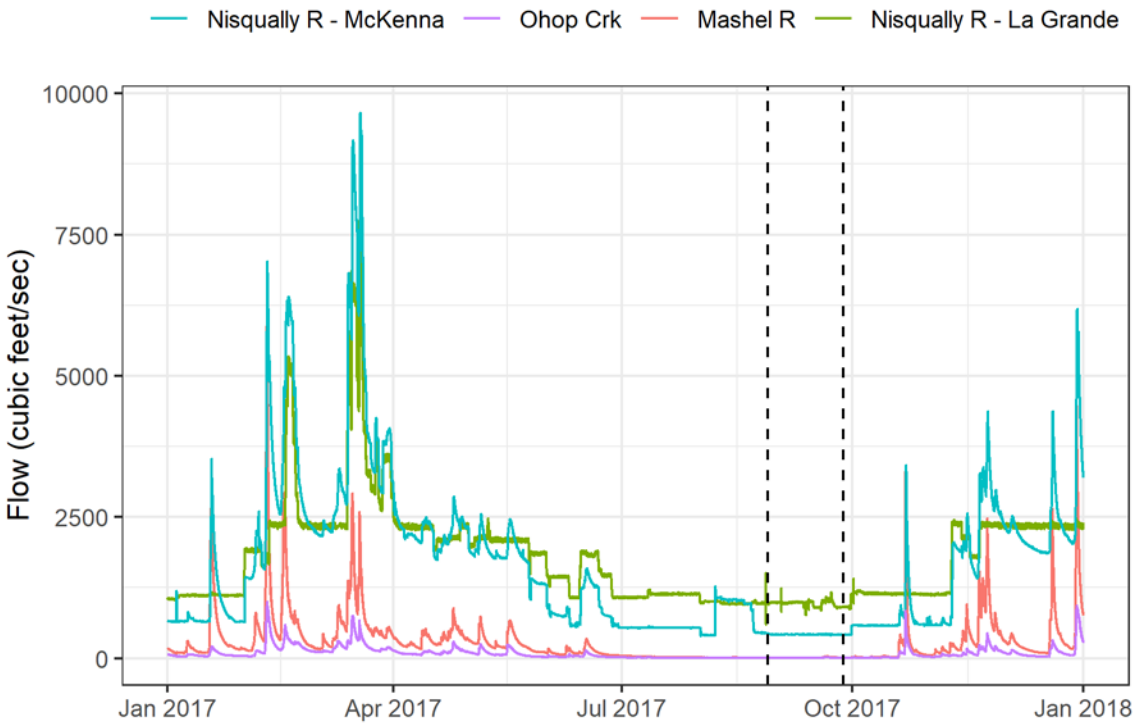

Figure 4. Stream flow (cubic feet per second) data collected from four USGS stream gages (U.S. Geological Survey 2017) in the mainstem Nisqually River (McKenna - 12089500 and La Grande, WA - 12086500), Ohop Creek - 12088000, and the Mashel River - 12087000. The area between the two dotted lines signifies the SPMD exposure time for this study.

Additionally, monthly effluent volumes from the two WWTP outfalls that discharge to the Nisqually River and Mashel River confirm that we sampled at times when the WWTPs were discharging (Table 3). For the one-month exposure period, the average flow of effluent from Eatonville's WWTP outfall 001was 0.178 million gallons per day (MGD), which is below their 2017 average monthly flow of 0.259 MGD. A higher average effluent volume 0.209 MGD was discharged from the city of Yelm's WWTP on the mainstem (outfall 001), however the majority of the effluent was distributed throughout the city as class A reclaimed water for use in constructed wetlands, infiltration basins, and irrigation projects (City of Yelm Wastewater Treatment and Water Reclamation Facility 2005), potentially creating a more

diffuse source. During the study time, the average flow of effluent from the City of Yelm's outfall 002 to the Centralia Canal was 0.183 MGD, below the 2017 average monthly flow of 0.305 MGD, similar to the pattern observed for the Eatonville outfall (Table 3; Washington Department of Ecology PARIS database, Discharge Monitoring Reports). The City of Yelm's outfall 003 outfall that discharges to the Nisqually River mainstem, did not discharge during our study period.

Table 3. Wastewater treatment plant (WWTP) effluent flow data for the time of the study, August 28 to September 26, 2017, and the mean monthly effluent for all of 2017 (Washington Department of Ecology PARIS database, Discharge Monitoring Reports). MGD = millions of gallons per day

				Aug. 28 to Sept. 26, 2017		2017
WWTP	Permit No.	Outfall No.	Outfall discharge location	Range of effluent flow (MGD)	Mean effluent flow (MGD)	Mean monthly effluent flow (MGD)
Yelm WWTP & Water Reclamation Facility	WA0040762	001[a]	Water reclamation	0.062 – 0.321	0.209	0.0944
		002	Centralia Canal	0.029 – 0.312	0.183	0.305
		003[b]	Nisqually River	0.00 - 0.00	0.000	0.055
Eatonville WWTP	WA0037231	001	Mashel River	0.145 – 0.271	0.178	0.259

[a] Effluent from this outfall is distributed throughout the city of Yelm as Class A reclaimed water. Uses include constructed wetlands, infiltration basins, and irrigation projects (City of Yelm Wastewater Treatment and Water Reclamation Facility 2005).
[b] This outfall did not discharge any effluent during the study time period

Water Samples

Conventional Parameters

Lipophilic and hydrophobic compounds like PBDEs bind preferentially to carbon-rich particulates, potentially underestimating PBDE concentration measured by the SPMD, that accumulate PBDE compounds that are largely dissolved. However, the concentrations of particulate carbon and dissolved carbon measured was very low over the SPMD deployment (Appendix, Table A1). Based on ancillary parameters that represent carbon content (TOC and DOC) and suspended material (TSS and turbidity) in the water column, ~99% of the PBDEs in the water column were in the dissolved or colloidal form. We therefore considered the results from the SPMDs to represent total-PBDEs.

SPMDs - PBDE Concentration and Patterns

With the exception of the upper Mashel River site, total PBDEs measured in the water were low, ranging from 3.7 to 8.5 pg/L (0.0037 to 0.0085 parts per trillion (ppt)). In contrast, the total PBDE concentration in water from the upper Mashel River site (11MR4.9) was 63 pg/L (0.063 ppt; Table 4, Figure 5).

Within the Nisqually River mainstem and Centralia Canal, total PBDEs measured in water were lowest (3.7 pg/L or 0.0037 ppt) at the most upstream Nisqually River site (11NR39.75). We assumed the PBDE concentration measured at this site represented a "background" concentration for the Nisqually River

Table 4. Concentrations of Total PBDEs (TPBDEs; pg/L and parts per trillion (ppt)) detected in the water using semipermeable membrane devices (SPMDs) at 11 sites in the Nisqually River mainstem, the Centralia Canal, and three major tributaries to the Nisqually River, Muck Creek, Ohop Creek, Mashel River.

Site Number	Site ID	Site Name	Deployment Time (days)	Exposure (seconds)	TPBDEs (pg/L)	TPBDEs (ppt)
1	11NR3.75	lower Nisqually River	27	35	8.0	0.0080
2	11MC0.25	Muck Creek	28	35	7.1	0.0071
3	11NR12.25	Nisqually River – Thompson Creek	29		4.7	0.0047
4	11CC0.25	Centralia Canal	29	60	6.2	0.0062
5	11NR12.5	Nisqually River – smolt trap	29	30	7.5	0.0075
6	11NR21.6	Nisqually River – McKenna	29	25	3.8	0.0038
7	11NR26.25	Nisqually River – diversion dam	29	30	7.4	0.0074
8	11OC0.1	Ohop Creek	27	30	4.5	0.0045
9a	11MR0.5	lower Mashel River	28	35	7.9	0.0079
9a	11MR0.5[a]	lower Mashel River[a]	28	30	8.5	0.0085
9b	11MR4.9	upper Mashel River site	28	30	63.3	0.063
10	11NR39.75	upper Nisqually River: (background)	27	30	3.7	0.0037

[a]Field duplicate

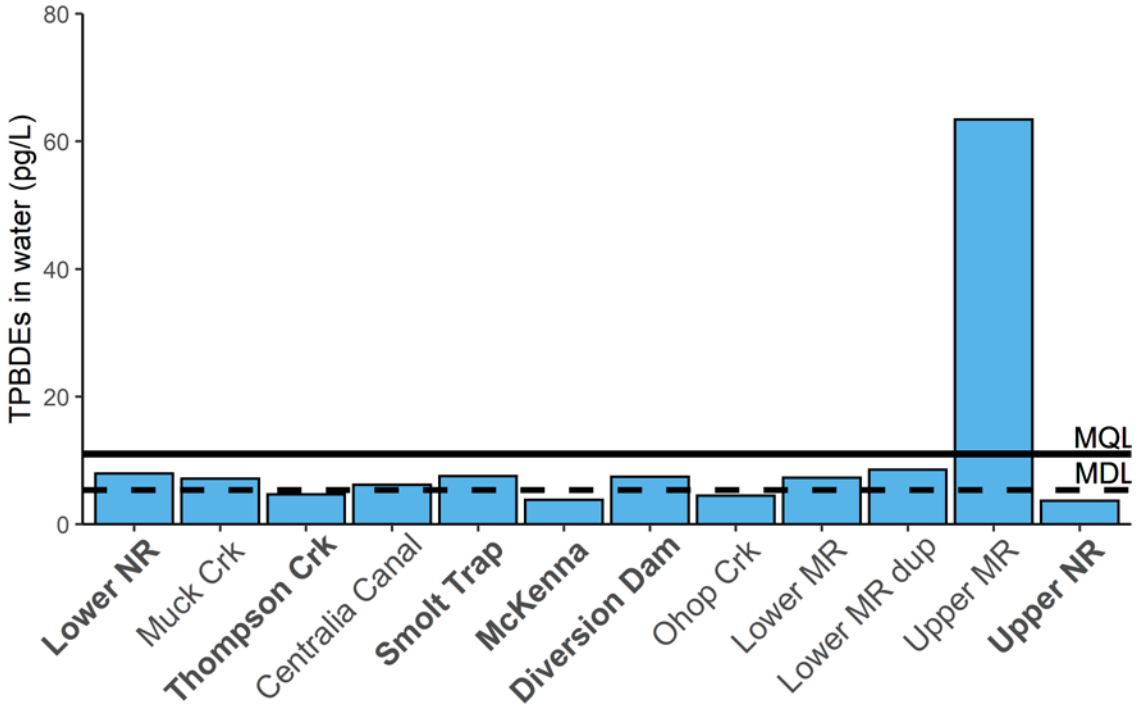

Figure 5. Modeled total PBDEs (TPBDEs) measured in water (pg/L). The dotted line, the calculated SPMD MDL (5.4 pg/L), and the solid line, the SPMD MQL (11 pg/L), illustrate how the concentrations of PBDEs measured in the water compare with the calculated detection limits based on the equipment blanks. Sites located on the mainstem Nisqually River are in bold. NR = Nisqually River, Crk = Creek, MR = Mashel River, dup = duplicate

watershed associated with atmospheric inputs, because it was located upstream of all other known potential PBDE sources (Table 1). Downstream of the most upstream site (11NR39.75), total PBDE concentrations measured in water from the mainstem ranged from 4.7 to 8.0 pg/L (0.0047 to 0.0080 ppt), averaging 6.3 pg/L (0.0063 ppt). The higher average PBDE concentrations in water from the downstream sites suggests there were further inputs of PBDEs to the Nisqually River, indirectly via the Mashel River, Ohop Creek or Muck Creek tributaries, or directly to the mainstem. However, the sensitivity of the method to detect PBDE sources was limited for concentration below the conservative blank MQL of 11 pg/L (0.0011 ppt), unless the pattern of detected congeners was distinct.

Among the Nisqually River tributaries, the highest total PBDE concentrations in water were measured in the Mashel River, 63.3 pg/L (0.0633 ppt) at the upstream site (11MR4.9) and 7.9 (0.0079 ppt) and 8.5 pg/L (0.0085 ppt) at the lower site (11MR0.5), located approximately five miles downstream (Table 4 and Figure 5). These results indicate an input of PBDEs within the vicinity of the SPMD exposure site on the upper Mashel River site, but those PBDEs are diluted in the river by the time the water arrives at the lower reaches of the Mashel River, prior to the confluence with the Nisqually River mainstem (Table 4 and Figure 5). These data suggest that other potential sources of PBDEs to the Mashel River, including, a former (i.e. legacy) dump used by Weyerhaeuser, the University of Washington's (UW) Charles L. Pack Experimental Forest research facility/lands (UW Pack Forest) and Fire Mountain Farms are not major sources of PBDE inputs. The Little Mashel River provides input to the Mashel River, between our upper and lower sample sites on the Mashel River, potentially diluting the upstream signal, and it is also likely that groundwater inputs play a role in dilution. It is uncertain whether degradation or transformation of the PBDE congeners is occurring between the sample sites.

In addition to having a higher PBDE concentration, when compared with all other locations, the water from the upper Mashel River site had slightly different pattern or fingerprint based on the proportions of individual PBDE congeners, as evident by its position along principal component 1 of the PCA (Figure 6; PCA). suggesting a different PBDE source at this sampling location. Most of the variation in the PBDE fingerprint was explained by PC1 (50.5%), with water from the upper Mashel River site (9b) having a distinct pattern compared to other samples (Figure 6).

A detailed comparison of the proportion of PBDE congeners in water from the upper Mashel River site and the background site on the upper Nisqually River, shows higher proportions of BDE-17, -28, -47, -49 and -71 in the upper Mashel location (Figure 7). For example, the proportion of BDE-47 was 44% of the total PBDE concentration in the upper Mashel River site, but only 33%% at the upper Nisqually River background site. Other prominent congeners that were present in the upper Mashel River site include PBDEs-99 and -100, though slightly higher proportions of those two congeners were measured in the background site (Figure 7). We did not positively identify the presence of heavier congeners BDE-203, -206, -207, -208, and -209 in the any SPMDs samples, because these congeners were also detected in the lab blank at similar concentrations to those detected in the SPMDS. Accordingly, we censored all data for BDE-203, -206, -207, -208, and -209 in SPMDs as non-detected values.

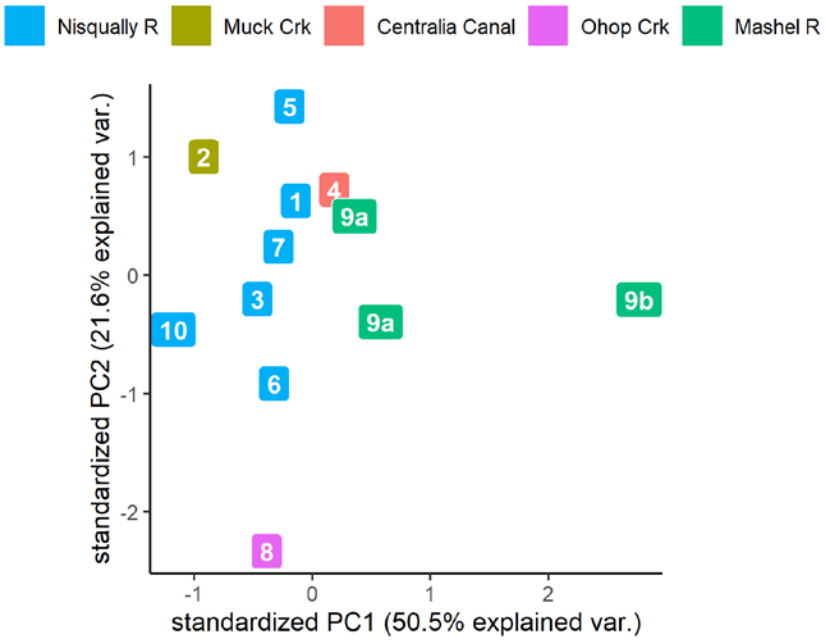

Figure 6. Principle component analysis of the 27 PBDE congeners measured in water (SPMDs) only. Numbers correspond to the site numbers in Table 2 and Figure 1

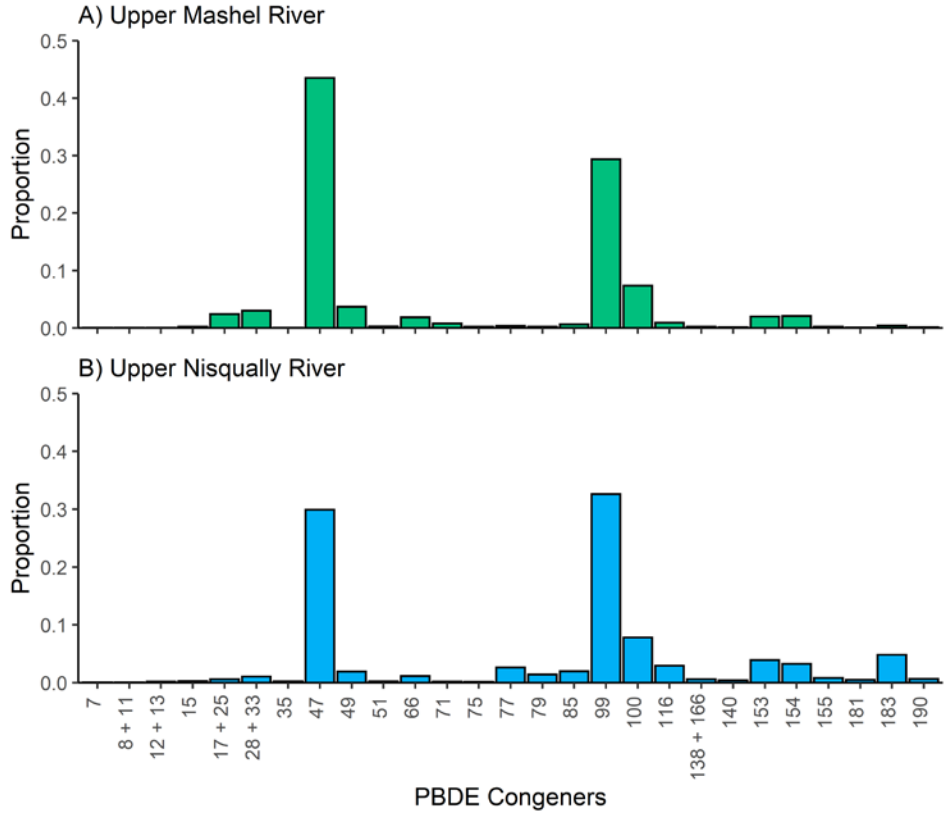

Figure 7. Comparison of the proportions of the 27 PBDE congeners (and coeluters) measured in water from A) the upper Mashel River site (11MR4.9) compared to B) the upper Nisqually River (NR) background site (11NR39.75).

Biofilm Samples

PBDE Concentrations and Patterns in Biofilms

The results of the 2017 PBDE source assessment suggest that PBDEs are primarily entering the Nisqually River system via the three major tributaries of the Nisqually River: the Mashel River, Muck Creek, and Ohop Creek. The summed total PBDE concentrations measured in all biofilm samples ranged from 26 to 880 pg/g (equivalent to ppt; Table 5), almost 1000 times higher than the concentrations detected in water samples via SPMDs (0.0037 to 0.063 ppt or 3.7 to 63 pg/L; Table 4). Overall, the highest PBDE concentrations were detected in biofilm collected from the tributaries of the Nisqually River: 880 pg/g in Muck Creek, followed by 290 pg/g in the upper Mashel River site, then 97 pg/g in Ohop Creek (Table 5, Figure 8A). For reference, the concentration found in the upper Nisqually River (background) was 34.8 pg/g.

The higher PBDE concentration in Muck Creek, and to a lesser extent Ohop Creek, were driven mostly by the heavier octa-, nona- and deca-PBDE congeners**,** (BDE-203, -206, -207, -208, and -209 in Muck Creek and BDE-203, -206, and -208 in Ohop Creek,) as evident by comparison of total PBDE concentrations that excludes these five congeners (Figure 8B). This is in contrast to the SPMDs from the Muck and Ohop Creek sites, where the presence of the heavier PBDE congeners were not positively identified due to laboratory and equipment contamination.

Table 5. Concentrations of Total PBDEs (TPBDEs; pg/g) measured in biofilm at nine of the 11 sites in the Nisqually River (NR) and its tributaries, Muck Creek (MC), Ohop Creek (OC), Mashel River (MR). Biofilm samples were not collected (NC) in the Centralia Canal… In addition, the percent lipids and stable isotopes of δ^{15}Nitrogen (δ^{15}N) and δ^{13}Carbon (δ^{13}C) measured in biofilm. NC = not collected, OC = organic carbon

Site Number	Site ID	Site Name	TPBDEs (pg/g)	Lipids (%)	OC (%)	δ^{15}N	δ^{13}C
1	11NR3.75	Lower Nisqually River	58	0.10	1.3	6.39	-20.7
2	11MC0.25	Muck Creek	880	0.15	15	5.54	-24.2
3	11NR12.25	Nisqually River – Thompson Creek	31	0.074	1.1	3.42	-29.9
4	11CC0.25	Centralia Canal	NC				
5	11NR12.5	Nisqually River – Smolt Trap	39	0.13	8.7	5.43	-15.3
6	11NR21.6	Nisqually River – McKenna	26	0.075	7.3	5.91	-24.6
7	11NR26.25	Nisqually River – Diversion Dam	NC				
8	11OC0.1	Ohop Creek	97	0.066	5.0	5.44	-31.0
9a	11MR0.5	Lower Mashel River	40	0.096	14	6.94	-15.8
9a	11MR0.5[a]	Lower Mashel River[a]	51.5	0.65	14	7.14	-15.7
9b	11MR4.9	Upper Mashel River site	290	0.11	12	10.8	-15.7
10	11NR39.75	Upper Nisqually River – Background	35	0.05	2.3	1.00	-24.2

[a]Field duplicate

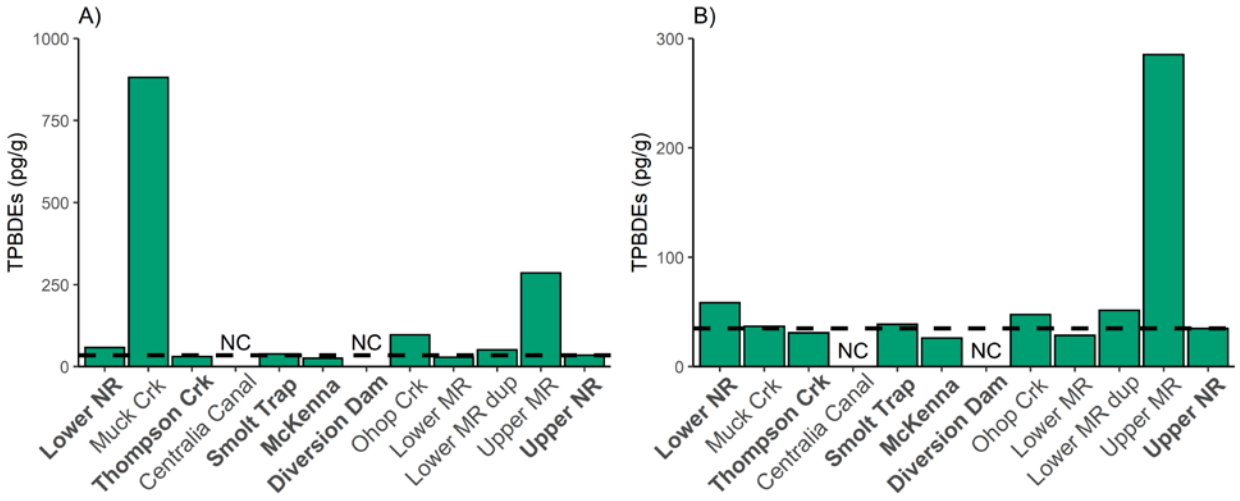

Figure 8. Total PBDEs measured in biofilm (pg/g) from all sites, based on A) all detected congeners and B) with 203, 206, 207, 208 and 2009 excluded. Sites located on the mainstem Nisqually River are bolded. The dashed line represents the background PBDE concentration measured at 11NR39.75 (Upper NR). NC = not collected.

A comparison of the proportion of PBDE congeners in biofilms from the upper Mashel River site, Muck Creek and Ohop Creek site clearly shows Muck Creek, and to a lesser extent Ohop Creek, had a heavier PBDE pattern (Figure 9), likely associated with inputs of heavier PBDE sources to these creeks. Proportions of heavier congeners were substantially higher at Muck reek (i.e., BDE-203, -206, -207, -208, and -209) and to a lesser extent at Ohop Creek(i.e., BDE-203, -206, and -208) compared to the Mashel River, whereas higher proportion of BDE-47, -99 and -100 were measured at the upper Mashel River site upper site (Figure 9).

The distinctness of the PBDE congener pattern or fingerprints between Muck, and Ohop creeks and the upper Mashel River site biofilm samples is further evident from the PCA analyses of congener proportions amongst all biofilm samples (Figure 10). Most of the variation in the PBDE fingerprint was explained by PC1 (54.5%), with samples in closer proximity to each other along PC1 axis indicative of a more similar fingerprint. The position along the PC1 axis is positively weighted (i.e., has a higher positive number) mostly by BDE congeners 207 and 209, followed by 203, 206 and 208. Muck Creek, and to a lesser extent and Ohop Creek, on PC1, is far to the right of the plot, whereas the upper Mashel River site is far to the left, and all other samples are intermediate between them. Ohop Creek is futher separated from the other locations by having higher PC2 scores, positively weighted by BDE congeners 183 and 153 followed by congeners 203 and 206.

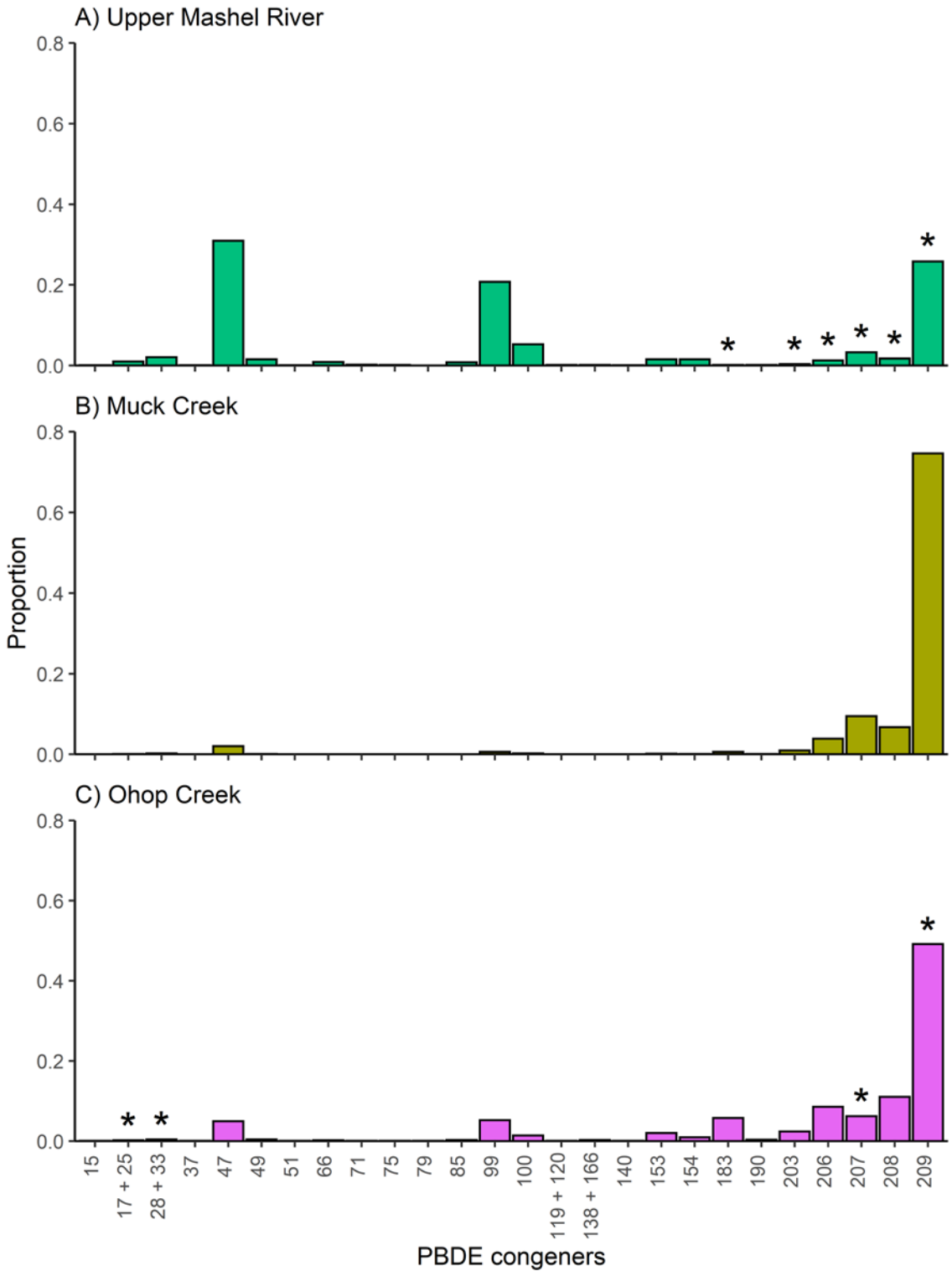

Figure 9. Proportions of 26 PBDE primary (+ coeluting) congeners) measured in biofilm collected from the A) upper Mashel River site, B) Muck Creek, and C) Ohop Creek. Note that LOQ values (or average censored values) were used for non-detected congeners. The asterisks mark the congeners where the average censored value was used.

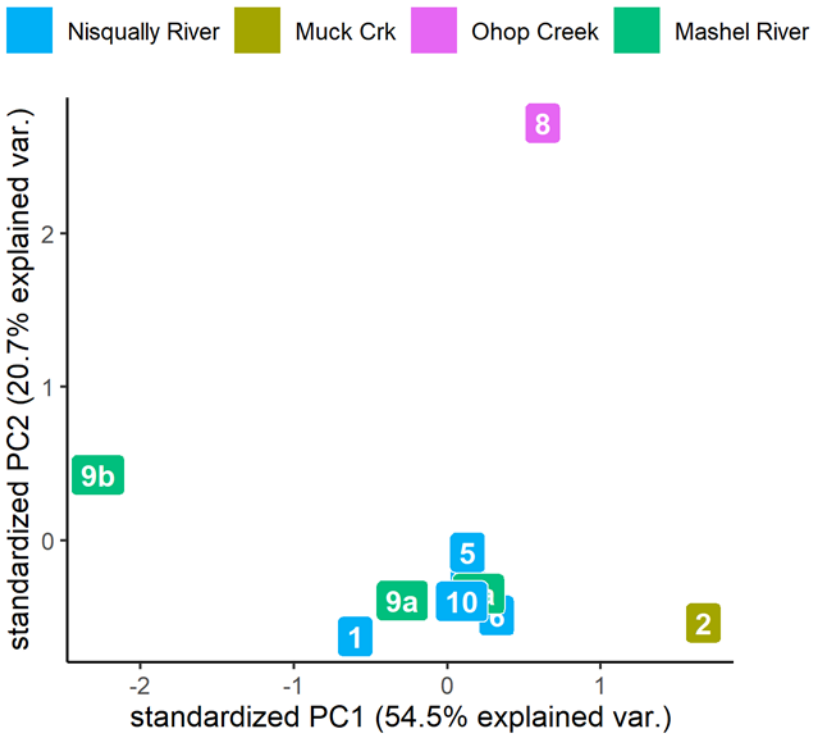

Figure 10 Principle component analysis of the 26 PBDE congeners measured in biofilm samples. Numbers correspond to the site numbers in Table 2 and Figure 1.

Comparison of PBDE Biofilms and SPMDs

In general, results from multiple studies have shown that biofilms are a reliable sampler of organic compounds like PBDEs and PCBs, and there is a strong linear relationship between concentrations in biofilms and water (as measured by SPMDs; Hobbs et al., 2019). PBDE concentrations measured in biofilm samples in this study showed a bioconcentration of approximately 1000 times relative to water samples. There is good agreement between the total PBDE concentrations measured in the biofilm and the water samples from the same site, with the exception of the Muck and Ohop Creek sites where heavier congeners were measured in the biofilm and not the SPMD samples. Excluding octa-, nona- and deca-, congeners from the total PBDEs concentration, the sites with higher concentration measured in water via SPMDs also had the higher concentrations measured in biofilms (Figure 11a). When these heavier congeners are included in the total PBDE calculation, there is less agreement between water and biofilm samples (Figure 11b).

At the upper Mashel River site, where we measured the highest PBDE concentrations in water, the distribution of congener common to common to both the water and biofilm samples are near identical to each other (Figure 12). This provides two lines of evidence that there were indeed dissolved PBDEs in the water near this site and these compounds were bound to the biofilms, the base of the food web in this aquatic system, without any transformation.

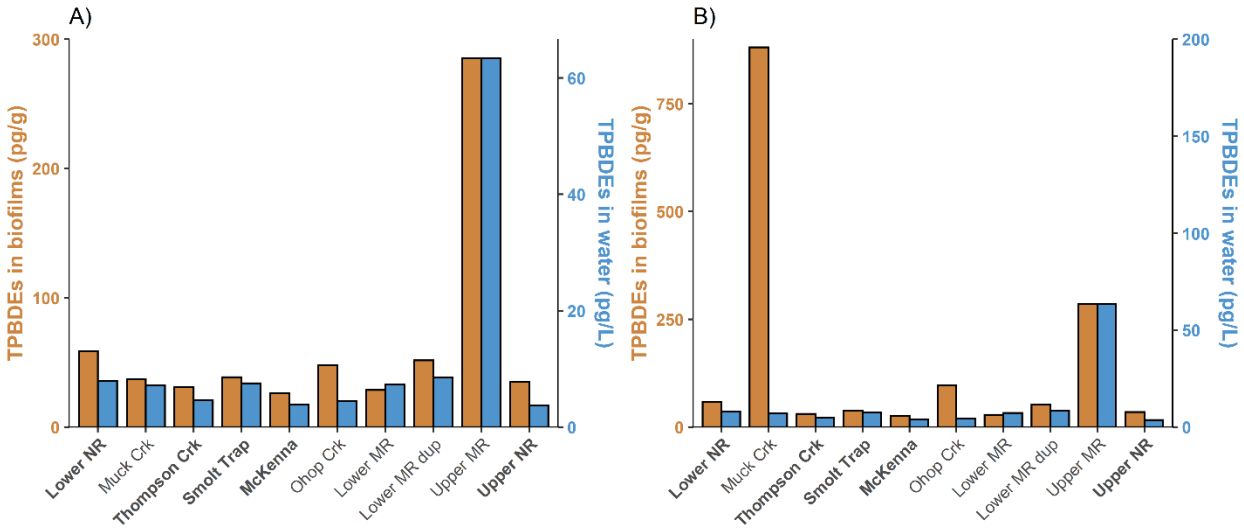

Figure 11. TPBDEs measured in biofilm (pg/g ww) and water (SPMDs; pg/L) with A) BDE-203, -206, -207, -208 and -209 excluded and B) those same congeners included in the TPBDEs calculation. Sites located on the mainstem Nisqually River are noted in bold text.

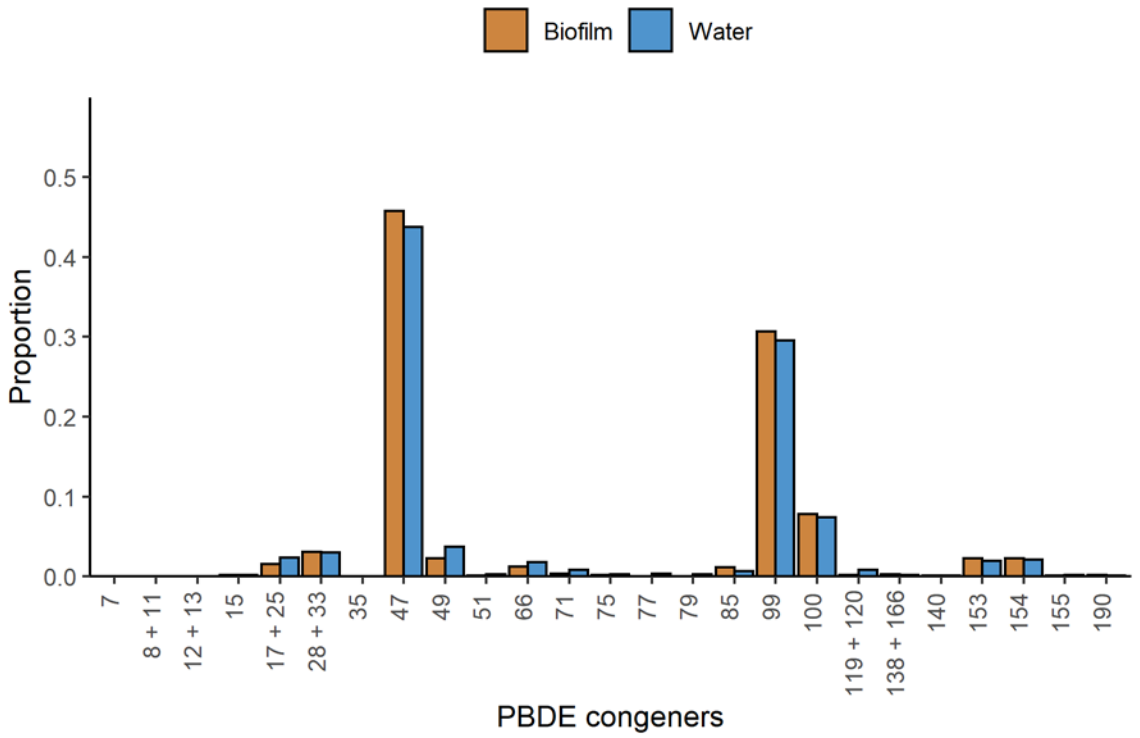

Figure 12. The proportions of PBDE congeners (+ coeluters) measured in biofilm and water (SPMDs) at the upper Mashel River site (11MR4.9). Congeners BDE-30, -32, -105, -116, -128, -181, -183, and -203-209 were excluded from this analysis because they were not detected in either sample. Note that LOQ values (or average censored values) were used for non-detected congeners.

Stable Isotopes of Biofilms

Heavier (enriched) nitrogen isotopes were observed in biofilm samples collected from the upper Mashel, site, suggesting possible exposure to WWTP effluent. The $\delta^{15}N$ in biofilm samples ranged from a low of

1.0‰ at the upper Nisqually River background site (11NR39.75) to a high of 10.8‰ at the upper Mashel River site (11MR4.9), with all other sites ranging between 3.42‰ and 7.14 ‰ (Table 4, Figure 13).

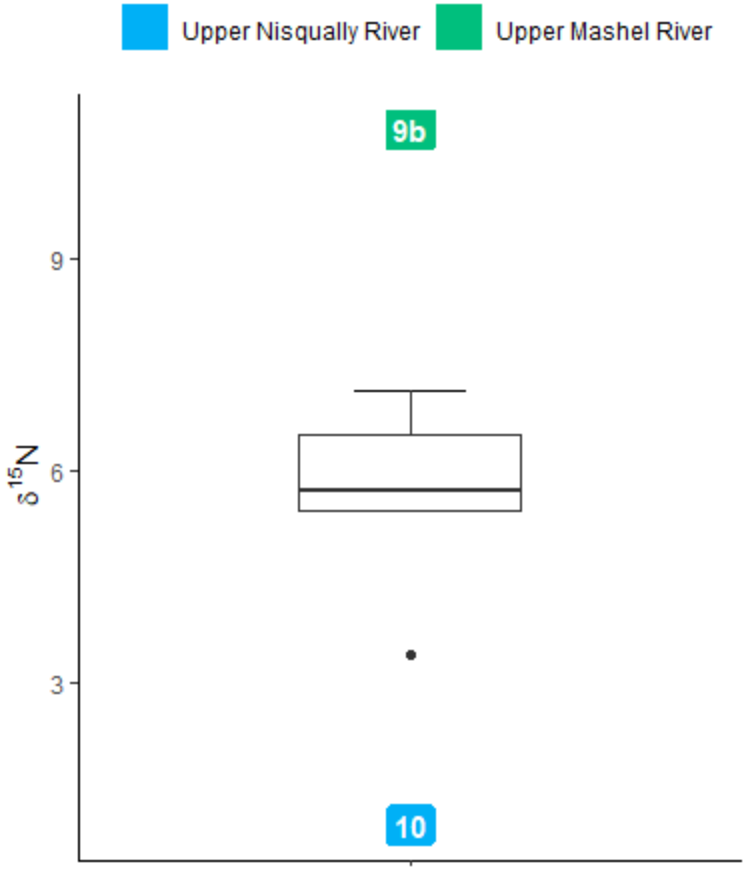

Figure 13. A boxplot showing the $\delta^{15}N$ measured in biofilm from nine sites. The middle bold line represents the median while the upper and lower portion of the box represents the 25th and 75th percentile values for all data. The upper whisker represents the largest observation and we considered any data beyond the whiskers as outliers. The upper Nisqually River-background (10; 11NR39.75) and the upper Mashel River site (9b; 11MR4.9) sites had the lowest and highest values of $\delta^{15}N$ measured in biofilm, respectively, and were outliers compared to all other samples.

Overall, $\delta^{15}N$ varied little over a wide range of $\delta^{13}C$ (Figure 14), with the exception of the depleted signal at the upper Nisqually River background site, and the enriched signal at the upper Mashel River site (Figure 14). The depleted $\delta^{15}N$ signal observed at the upper Nisqually River compared to all other sites, indicates, a difference nitrogen source at this location, likely because of input of atmospheric deposition, released from the melting glacier at the head of the river. The accepted standard for $\delta^{15}N$ is atmospheric dinitrogen gas (i.e., N_2), with a $\delta^{15}N$ of 0‰. Likewise, the enrichment of $\delta^{15}N$ at the upper Mashel River site, suggests a different source of nitrogen at the base of the food web, compared to all other sites, possibly a wastewater source (Leavitt et al., 2006).

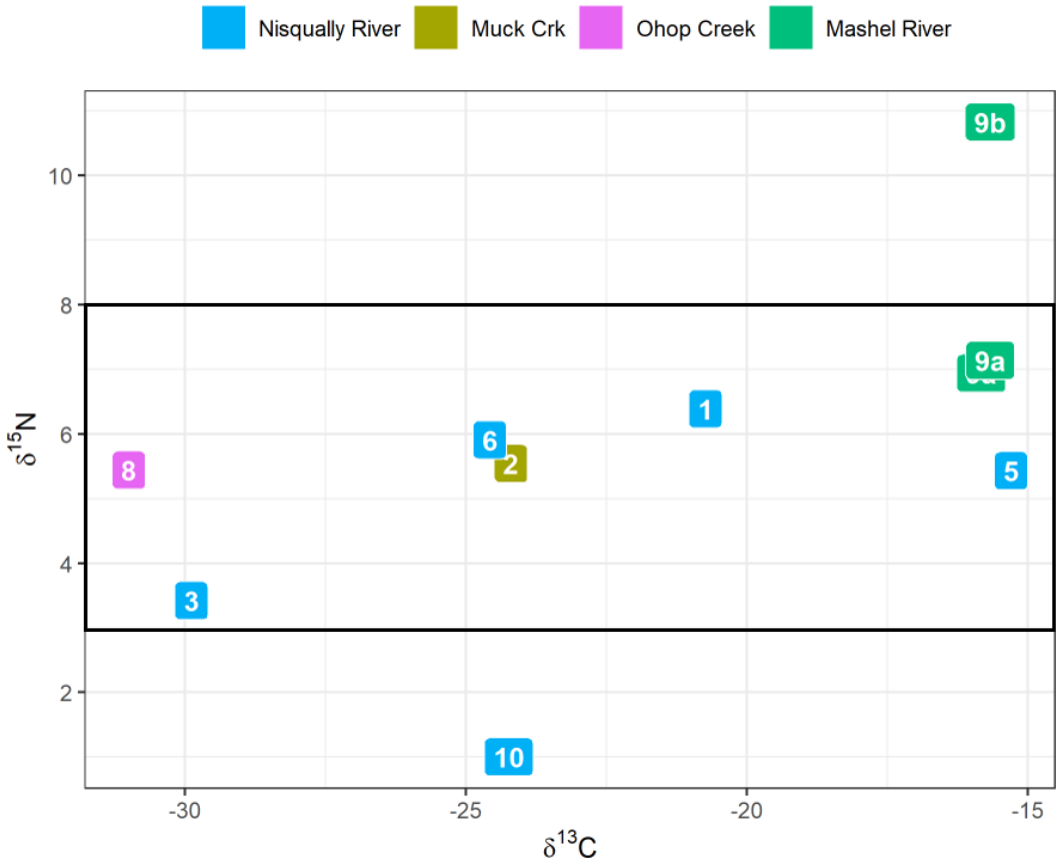

Figure 14. Comparison of $\delta^{15}N$ and $\delta^{13}C$ measured in biofilm samples. Numbers correspond to site numbers listed in Table 5 and colors reflect which river or tributary the biofilm were collected. The black box denotes sites with little variation in $\delta^{15}N$ over a wide range of $\delta^{13}C$.

Comparison of Stable Isotopes in Biofilm and Salmon

Steelhead feeding in the vicinity of the upper Mashel River site could potentially incorporate the enriched $\delta^{15}N$ signal at this site, resulting in enriched $\delta^{15}N$ signatures in those steelhead that resided there. However, there was no obvious pattern of $\delta^{15}N$ enrichment in any of the steelhead collected at the trap in 2014 and 2015 that overlapped with the enriched $\delta^{15}N$ in the biofilm sample from the upper Mashel River site (Figure 15), suggesting there was not a strong isotopic gradients of nitrogen that could be related to the residency of the juvenile steelhead sampled in 2014 and 2015.

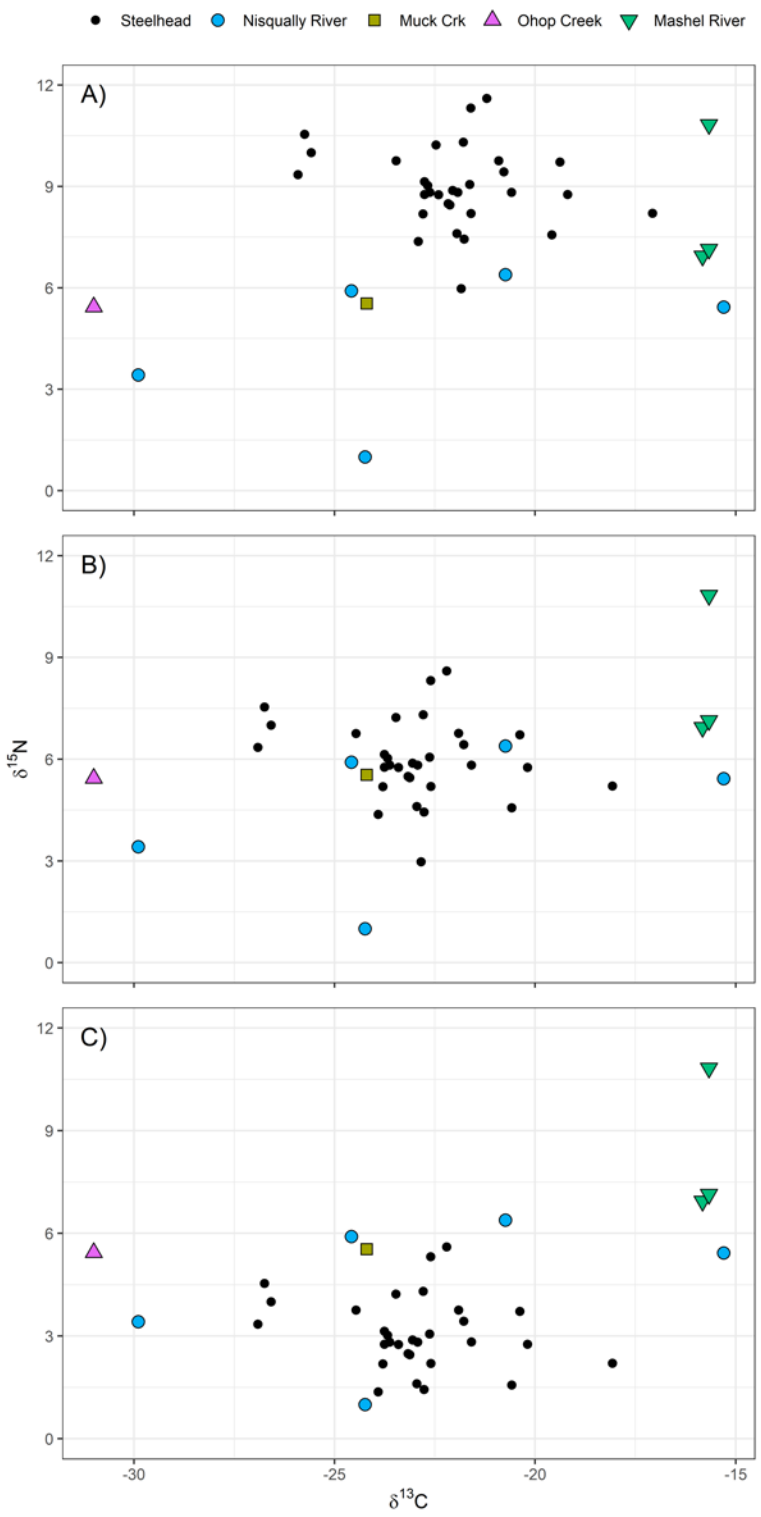

Figure 15. Stable isotopes of δ15N and δ13C measured in biofilm from this study and from steelhead trout collected in the 2014 and (Chen et al. 2018) and in 2015 (WDFW unpublished data) from the smolt trap located at approximately river mile 12.5 on the mainstem of the Nisqually River. Stable isotopes of steelhead are shown without a correction for any trophic enrichment factor (TEF; A), with an average TEF of 3‰ for nitrogen, and 1‰ for carbon representing 1 tropic level (B) and with an average TEF of 6‰ for nitrogen and 1‰ for carbon representing 2 trophic levels (C).

Sources of PBDEs

Mashel River

Two lines of evidence suggest that the upper Mashel River is a likely location for PBDE exposure to juvenile Steelhead in the Nisqually River and its tributaries. Given the increased PBDEs measured in both water and biofilm, in addition to the enriched $\delta^{15}N$ signal at this location, a possible source of PBDEs near the upper Mashel River site is the Eatonville WWTP. Biofilm has been shown to accurately reflect $\delta^{15}N$ in streams and rivers (Pastor et al., 2013; Hobbs et al., 2016). Thus, the enrichment of $\delta^{15}N$ in biofilm collected from the upper Mashel River site (11MR4.9), immediately downstream of the WWTP is consistent with exposure to inorganic nitrogen (nitrate; NO_4 and ammonium; NH_4) in WWTP effluent (Leavitt et al., 2006; Bunting et al., 2007; Hobbs et al., 2016). The effluent outfall is located on the Mashel River, less than a half mile upstream of where the SPMD was positioned (river mile 5.3). Wastewater treatment plants are known to be a major pathway loading PBDEs to aquatic systems (Osterberg and Pelletier 2015). PBDEs are found in numerous clothing items, furniture, electronics, building materials, etc., all of which gets into household dust which in turn gets washed down the drain and concentrates at WWTPs. Currently, in Washington State, there are no regulations for monitoring PBDEs at WWTPs or in their effluent.

As the water and biofilm samples show a decrease in PBDEs from the upper site to the lower site in the Mashel River, it is unlikely that there are additional sources of PBDEs between those two locations. However, because our study was conducted during the driest time of the year with minimal rainfall, stormwater runoff containing leached PBDEs from previously dumped furniture and electronics at the Weyerhauser legacy dump and from biosolid application in the UW Pack Forest and Fire Mountain Farms likely would not have contributed measurable quantities of PBDEs to the Mashel River during out study. Additional wet season sampling with SPMDs would more definitely rule out this potential source.

During the study, a river flow gage located midway between the upper and lower Mashel sampling sites (USGS gage number 12087000), measured flows that ranged from a low of approximately 6.2 cubic feet (cf)/second on September 16, 2017 to a high flow of approximately 39 cf/second on September 21, 2017 (U.S. Geological Survey, 2017). Though the flow increased during the study for a brief period, it was a relatively small occurrence compared to other rainfall events throughout the year (Table 3). It is unlikely that this high flow event on September 21 would have diluted any PBDEs in the Mashel River at the time and caused us to underestimate the amount of PBDEs present in the upper and lower Mashel River site during the dry season.

Muck Creek

Within Muck Creek, wastewater is likely not a source because there are no know waste water outfall in the creek and because of the presence of the heavier PBDE congeners (BDE-203, -206, -207, -208 and -209) and the lack of $\delta^{15}N$ in that system. Typically, the heavier congeners measured in the biofilm there are used in textiles, electronic equipment, and building and construction materials compatiable with the commercial deca-BDE mixture (Ecology 2006). A possible source could include groundwater or runoff from the nearby JBLM containing deca-BDE.

Ohop Creek

The amount of PBDEs in water from Ohop Creek did not indicate a source in the system, but biofilm had slightly higher levels of PBDEs and a slightly different pattern of the congener proportions, more similar to Muck Creek. Heavier PBDE congeners and the absence of $\delta^{15}N$ indicate a source other than wastewater in the creek. Stormwater runoff from Eatonville through the outfall on Lynch Creek could possibly be a source, but is unlikely given the low precipitation during the study period. Additionally, the stormwater outfall is located more than 6 rm upstream from our sampling site so the PBDEs would probably be considerably diluted by the time they reach our site, just prior to the confluence with the mainstem Nisqually River.

City of Yelm WWTP Outfalls

An initial potential PBDE source to the Nisqually River was the Yelm WWTP outfall located within the Centralia Canal (Table 3). Our sampling suggests this is an unlikely PBDE source, however unlike the Eatonville WWTP outfall, which was sampled immediately below the outfall, we sampled about three miles downstream from the Centralia Canal WWTP outfall. Our sample location does adequately characterize possible PBDE inputs to the Nisqually River from the Centralia Canal, which is not juvenile salmonid rearing habitat. At this site, we were only able to measure PBDEs in the water using the SPMDs due to a lack of biofilm in the canal. The PBDE concentrations were not elevated in the water from the sampling site downstream of the Centralia Canal WWTP outfall.

Additionally, it is unlikely that the Yelm WWTP outfall within the Nisqually River mainstem (outfall 003, Table 3) or the reclaimed water distributed throughout Yelm(outfall 001, Table 3) contributed any PBDEs to the river system during the study. According to the discharge monitoring reports, no flow was measured from that outfall 003 from August 28th to September 26th, 2017 (Table 3), and reclaimed water (from outfall 001, with an average discharge of 0.209 MGD) was spread throughout the town of Yelm. However, it is difficult to assess these outfalls as potential sources because one did not discharge at all during the study and the other results in a diffuse application all over the town. Our only indication that these are not sources of PBDEs to the Nisqually River system at the time, is that all sites downstream of outfall 003 and the city of Yelm had low levels of PBDEs in both water and biofilm samples.

CONCLUSIONS

The results of the 2017 PBDE source assessment suggest that PBDEs are primarily entering the Nisqually River system via the three major tributaries of the Nisqually River: the Mashel River, Muck Creek, and Ohop Creek, **however, limited spatial sampling within the tributaries restricted our ability to definitively conclude the major sources**.

Generally, PBDEs were measured in low concentrations in both water and biofilm samples with the exception sites in the three major tributaries. Elevated PBDEs concentrations were only measured in water samples at the upper Mashel River site , whereas the highest total PBDE concentrations were measured in biofilm samples from Muck Creek, followed by the upper Mashel River site, and then Ohop

Creek. Biofilms were shown to bioconcentrate PBDEs approximately 1000 times and generally well correlated at higher concentrations.

In addition to elevated PBDE concentrations in the biofilms, the pattern of PBDE congeners and stable isotopes provide insights in the potential sources of PBDEs within the Nisqually River system. At the upper Mashel River site, in addition to having an elevated PBDE concentration, the biofilm had a different pattern of PBDE congeners than all other sites. Also, the biofilm from that region of the Mashel River had an enriched signal of the stable isotope, $\delta^{15}N$. The combination of elevated PBDE levels, a different pattern of PBDE congeners and an enrichment of $\delta^{15}N$ strongly suggests exposure to a wastewater source. The outfall for the Eatonville WWTP outfall is located slightly upstream from the site, suggesting that that it is potentially the source of PBDEs in this tributary. Interestingly, the elevated concentrations return to near background concentrations just five miles downstream where the Mashel River meets the mainstem Nisqually River, suggesting that the PBDEs are diluted as they flow away from the source, possibly by input from the Little Mashel River that enters downstream of the Eatonville outfall but upstream of the confluence with the Nisqually River or other groundwater inputs. **Additional sampling would be necessary to document the extent of the Mashel River that is affected by PBDE inputs from the Eatonville wastewater treatment plant outfall and how this relates to Steelhead rearing habitat**.

The pattern of PBDE congeners measured in Muck and Ohop creeks were distinct from those in the upper Mashel River site. Heavier PBDE congeners were detected in both Muck Creek (i.e., BDE-203, -206, -207, -208 and -209) and Ohop Creek (i.e., BDE-203, -206, and -208), however, if these congeners are excluded from the sum of detected PBDEs, the highest concentrations were then measured in the upper Mashel River site, followed by Muck Creek, and then the lower Mashel and Ohop Creek. Although, Muck and Ohop creeks biofilms have a heavier PBDE congener pattern, the source of these PBDEs was not likely from a wastewater source as their nitrogen stable isotopes, $\delta^{15}N$, which are often altered when exposed to wastewater, are not altered compared to other biofilm samples and there are no WWTP discharges directly to these creeks. **Additional sampling would be necessary to document the source PBDEs to Muck and Ohop creeks.**

RECOMMENDATIONS

Additional studies are needed to further delineate the distribution of PBDEs in the Nisqually River system and to understand the exposure and uptake of PBDEs by steelhead trout, potentially impacting their early marine survival. Based on the results of this study, future studies should focus on the three major tributaries of the Nisqually River, (in order of importance): the Mashel River, Muck Creek and Ohop Creek. These tributaries contain the majority of steelhead habitat in the system.

Specific objectives for futher study should include: (1) further delineation of the locations of PBDE inputs to the Mashel River, (2) further confirmation of the presence of PBDEs in Ohop and Muck creeks, and (3) measurement and description of the uptake of PBDEs in aquatic insects that are prey for juvenile steelhead trout. Given that biofilms are now considered an effective tool to tracking sources of toxic chemicals in rivers and streams (Hobbs et al., 2019), the next study should focus on using biofilms as the main matrix to assess PBDEs.

Other possible future studies include:

- sampling during the wet season to better evaluate non-point sources in runoff (e.g. runoff from UW pack forest (biosolids) or the legacy Weyerhauser dump),and
- analyses of PBDEs in resident fish (sculpins, whitefish).

Wet season sampling would require SPMDs since biofilm is likely scoured off the rocks during high flow events.

ACKNOWLEDGEMENTS

Funding for this study was provided by the Nisqually Indian Tribe (WDFW contract number 17-09390). Sayre Hodgson, Nisqually Tribe, provided information and the potential PBDE sources in the Nisqually, identified field sampling sites, assisted with all phases of the field work, and reviewed draft versions of the report. Laurie Niewolny, formerly a WDFW staff member, now employed by the Department of Ecology, assisted with the study's sampling design. Robert Fisk, WDFW, and Melisa McCall from Ecology assisted with fieldwork. Access to sampling sites was provided by Matt Klungle, WDFW (smolt trap site), the Centralia Canal operators (diversion dam sites dam, and Nisqually Land Trust (McKenna site).

LITERATURE CITED

Alvarez, D.A. 2010. Guidelines for the Use of the Semipermeable Membrane Device (SPMDs) and the Polar Organic Chemical Integrative Sampler (POCIS) in Environmental Monitoring Studies. Techniques and Methods 1-D4; U.S. Geological Survey: 28.

Arkoosh, M.R., E. Clemons, M. Myers, and E. Casillas. 1994. Suppression of b-cell mediated immunity in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) after exposure to either a polycyclic aromatic hydrocarbon or to polychlorinated biphenyls. Immunopharmacology Immunotoxicology 16(2): 293-314.

Arkoosh, M.R., E. Casillas, P. Huffman, E. Clemons, J. Evered, J.E. Stein, and U. Varanasi. 1998. Increased susceptibility of juvenile Chinook salmon from a contaminated estuary to *Vibrio anguillarum*. Transactions of the American Fisheries Society 127(3): 360-374.

Arkoosh, M.R., D. Boylen, J. Dietrich, B.F. Anulacion, G. Ylitalo, C.F. Bravo, L.L. Johnson, F.J. Loge, and T.K. Collier. 2010. Disease susceptibility of salmon exposed to polybrominated diphenyl ethers (PBDEs). Aquatic Toxicology 98(1): 51-59.

Arkoosh, M.R., J. Dietrich, G.M. Ylitalo, L.L. Johnson, and S.M. O'Neill. 2013. Polybrominated diphenyl ethers (PBDEs) and Chinook salmon health. U.S. Department of Commerce, National Oceanic and Atmospheric Association, National Marine Fisheries Service, Northwest Fisheries Science Center, Newport, OR. 49 pp. plus Appendices.

Arkoosh, M.R., A.L. Van Gaest, S.A. Strickland, A.B. Krupkin, and J.P. Dietrich. 2015. Dietary exposure to individual polybrominated diphenyl ether (PBDE) congeners, BDE-47 and BDE-99, alters innate immunity and disease susceptibility in juvenile Chinook salmon. Environmental Science and Technology 49(11): 6974-6981.

Arkoosh, M.R., A.L. Van Gaest, S.A. Strickland, G.P. Hutchinson, A.B. Krupkin, M.B.R. Hicks, and J.P. Dietrich. 2018. Dietary exposure to a binary mixture of polybrominated diphenyl ethers alters innate immunity and disease susceptibility in juvenile Chinook salmon (*Oncorhynchus tshawytscha*). Ecotoxicology and Environmental Safety 163: 96-103.

Bethune, C., K. Julshamn, and A. K. Lundebye. 2005. A preliminary comparison of polybrominated diphenyl ether concentrations relative to lipid content and to levels of dioxins and dioxin-like polychlorinated biphenyls in Norwegian farmed Atlantic Salmon (*Salmo salar*).International Journal of Food Science and Technology 40:143–148.

Boecklen W.J., C.T. Yarnes, B.A. Cook., and A.C. James. 2011 On the use of stable isotopes in trophic ecology. Annual Review of Ecology, Evolution, and Systematics 42: 411-440.

Bunting, L., P.R. Leavitt, C.E. Gibson, E.J. McGee, and V.A. Hall. 2007 Degradation of water quality in Lough Neagh, Northern Ireland, by diffuse nitrogen flux for a phosphorous-rich catchment. Limnology and Oceanography 52: 354-369.

Chen, M.F., S.M. O'Neill, A.J. Carey, R.H. Conrad, B.A. Stewart, K.R. Snekvik, G.M. Ylitalo, and P.K. Hershberger.(2018). Infection by *Nanophyetus salmincola* and toxic contaminant exposure in out-

migrating Steelhead from Puget Sound, Washington: Implications for early marine survival. Journal of Aquatic Animal Health 30(2): 103-118.

City of Yelm Wastewater Treatment and Water Reclamation Facility. 2005. Fact Sheet for NPDES Permit WA0040762. Accessed October 17, 2019 from the Washington Department of Ecology PARIS Database. https://apps.ecology.wa.gov/paris/DownloadDocument.aspx?id=15983

Ecology 2006 Washington State Polybrominated Diphenyl Ether (PBDE) Chemical Action Plan: Final Plan. Washington State Department of Ecology, Olympia WA. https://fortress.wa.gov/ecy/publications/summarypages/0507048.html

Ecology and King County, 2011. Control of Toxic Chemicals in Puget Sound: Assessment of Selected Toxic Chemicals in the Puget Sound Basin, 2007-2011. Washington State Department of Ecology, Olympia, WA and King County Department of Natural Resources, Seattle, WA. Ecology Publication No. 11-03-055. www.ecy.wa.gov/biblio/1103055.html

Fire Mountain Farms Inc. General Permit for Biosolids Management Permit No, BT9902, June 2,2017.http://nebula.wsimg.com/1763460680f21f81b642edccd5209fba?AccessKeyId=B8C3326AE00C91BC095C&disposition=0&alloworigin=1

Hobbs, W. 2014. Quality Assurance Project Plan. Wenatchee River PCB and DDT Source Assessment. WA Department of Ecology, Olympia WA. Publication No. 14-03-117.

Hobbs, W. and M. Friese. 2016. Wenatchee River PCB and DDT Source Assessment. Department of Ecology, Olympia, WA. Publication No. 16-03-029.

Hobbs, W.O., S.A. Collyard, C. Larson, A.J. Carey and S.M. O'Neill. 2019. Toxic burdens of freshwater biofilms and use as a source tracking tool in rivers and streams. Environmental Science and Technology 53 (19): 11102-11111.

Hodgson, Sayre. Nisqually Tribe, personal communication, November 2019.

Huckins, J.N., J.D. Petty, and K. Booij. 2006. Monitors of organic chemicals in the environment: Semipermeable membrane devices. Springer, New York, USA. 223p.

Jacobson, K.C., M.R. Arkoosh, A.N. Kagley, E.R. Clemons, T.K. Collier, and E. Casillas. 2003. Cumulative effects of natural and anthropogenic stress on immune function and disease resistance in juvenile Chinook salmon. Journal of Aquatic Health 15: 1-12.

Joy, J. 2006. Standard Operating Procedure for Manually Obtaining Surface Water Samples. Washington State Department of Ecology, Olympia, WA. SOP EAP015. www.ecy.wa.gov/programs/eap/quality.html

Klungle, M.M., J.H. Anderson and M.S. Zimmerman. 2018. Nisqually River Juvenile Salmonid Monitoring Report: 2009 – 2015. Washington Department of Fish and Wildlife, Olympia, WA. Report No. FPA 18-07.

Leavitt, P.R., C.S. Brock, C. Ebel, and A. Patione. 2006. Landscape-scale effects of urban nitrogen on a chain of freshwater lakes in central North America. Limnology and Oceanography 51: 2262-2277.

Loge, F., M.R. Arkoosh, T.R. Ginn, L.L. Johnson, and T.K. Collier. 2005. Impact of environmental stressors on the dynamics of disease transmission. Environmental Science and Technology 39: 7329-7336.

MacKay, D., W.Y. Shui and K.C. Ma. 1997. Illustrated Handbook of Physical-Chemical Porperties and Environmental Fate for Organic Chemicals: Pesticide Chemicals. CRC Press, Boca Raton, FL: Vol. 5.

Meador, J.P., F.C. Sommers, G.M. Ylitalo, and C.A. Sloan. 2006. Altered growth and related physiological responses in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) from dietary exposure to polycyclic aromatic hydrocarbons (PAHs). Canadian Journal of Fisheries and Aquatic Sciences 63 (10): 2364-2376.

Meadows, J.C., K.R Echols, J.N Huckins, F.A Borsuk, R.F. Carline, and D.E. Tillitt. 1998. Estimation of uptake rate constants for PCB congeners accumulated by semipermeable membrane devices and brown trout (*Salmo trutta*). Environmental Science and Technology, *32*(12), pp.1847-1852.

Nisqually Steelhead Recovery Team. 2014. Nisqually River Steelhead Recovery Plan. Draft. July. Seattle, WA. Prepared for the Nisqually Indian Tribe, Olympia, WA.

Osterberg, D.J. and G. Pelletier. 2015. Puget Sound Regional Toxics Model: Evaluation of PCBs, PBDEs, PAHs, Copper, Lead, and Zinc. Washington State Department of Ecology, Environmental Assessment Program. Olympia, WA: pp 125 plus appendices.

Peck, K.A., D.P. Lomax, O.P. Olson, S.Y. Sol, P. Swanson, and L.L. Johnson. 2011. Development of enzyme-linked immunosorbent assay for quantifying vitellogenin in Pacific salmon and assessment of field exposure to environmental estrogens. Environmental Toxicology and Chemistry 30(2): 477-486.

Peterson, B.J. and B. Fry. 1987. Stable isotopes in ecosystems. Annual Review of Ecology and Systematics 18: 293-320.

Pierce County. 2005. Muck Creek Basin Plan. Prepared by Pierce County Public Works and Utilities, Water Programs Division. Tacoma, WA.

Pierce County. 2012. Public Review Draft Nisqually River Basin Plan Volume 1 – Basin Plan & FSEIS. Prepared by Pierce County Public Works and Utilities, Water Programs Division. Tacoma, WA.

Puget Sound Steelhead Marine Survival Workgroup. December 2015. Salish Sea Marine Survival Project – Puget Sound Steelhead Marine Survival: 2013-2015 research findings summary. Long Life the Kings, Seattle, WA. www.marinesurvivalproject.com

R Core Team. 2017. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Salish Sea Marine Survival Project. 2018. Puget Sound Steelhead Marine Survival: 2017 – 2019 Research Work Plan. Long Live the Kings, Seattle, WA. www.marinesurvivalproject.com

Segner, H., M. Wegner, A.M. Moller, B. Kollner, and A. Casanova-Nakayama. 2012. Immunotoxic effects of environmental toxicants in fish – how to assess them? Environmental Science and Pollution Research 19: 2465-2476.

Spromberg, J.A. and J.P. Meador. 2005. Relating results of chronic toxicity responses to population-level effects: Modeling effects on wild Chinook salmon populations. Integrated Environmental Assessment and Management 1(1): 9-21.

Thompson, D.R., S.J. Bury, K.A. Hobson, L.I. Wassenaar, J.P. Shannon. 2005. Stable isotopes in ecological studies. Oecologia 144: 517-519.

U.S. Geological Survey. 2017. National Water Information System data available on the World Wide Web (USGS Water Data for the Nation), accessed October 15, 2019, at https://waterdata.usgs.gov/wa/nwis/uv?site_no=12089500, https://waterdata.usgs.gov/wa/nwis/uv?site_no=12086500, https://waterdata.usgs.gov/wa/nwis/uv?site_no=12088000, https://waterdata.usgs.gov/wa/nwis/uv?site_no=12087000.

Varanasi, U., E. Casillas, M.R. Arkoosh, T. Hom, D.A. Misitano, D.W. Brown, S.-L Chin, T.K. Collier, B.B. McCain, and J.E. Stein. 1993. Contaminant exposure and associated biological effects in juvenile Chinook salmon (*Oncorhynchus tshawytscha*) from urban and non-urban estuaries of Puget Sound. U.S. Department of Commerce, NOAA Tech. Memo., National Marine Fisheries Service – Northwest Fisheries Science Center -8. 112p.

Washington Department of Ecology PARIS database, Discharge Monitoring Reports. Accessed October 15, 2019, at https://apps.ecology.wa.gov/paris/DischargeMonitoringData.aspx

Washington Dept. of Fisheries. 1975. "WRIA 11 : Nisqually River Basin." In *A Catalog of Washington Streams and Salmon Utilization*. Seattle, Wash.: Washington Dept. of Fisheries. http://docs.streamnetlibrary.org/Washington/DFW/StreamCatalog/11-WRIA.pdf.

APPENDIX

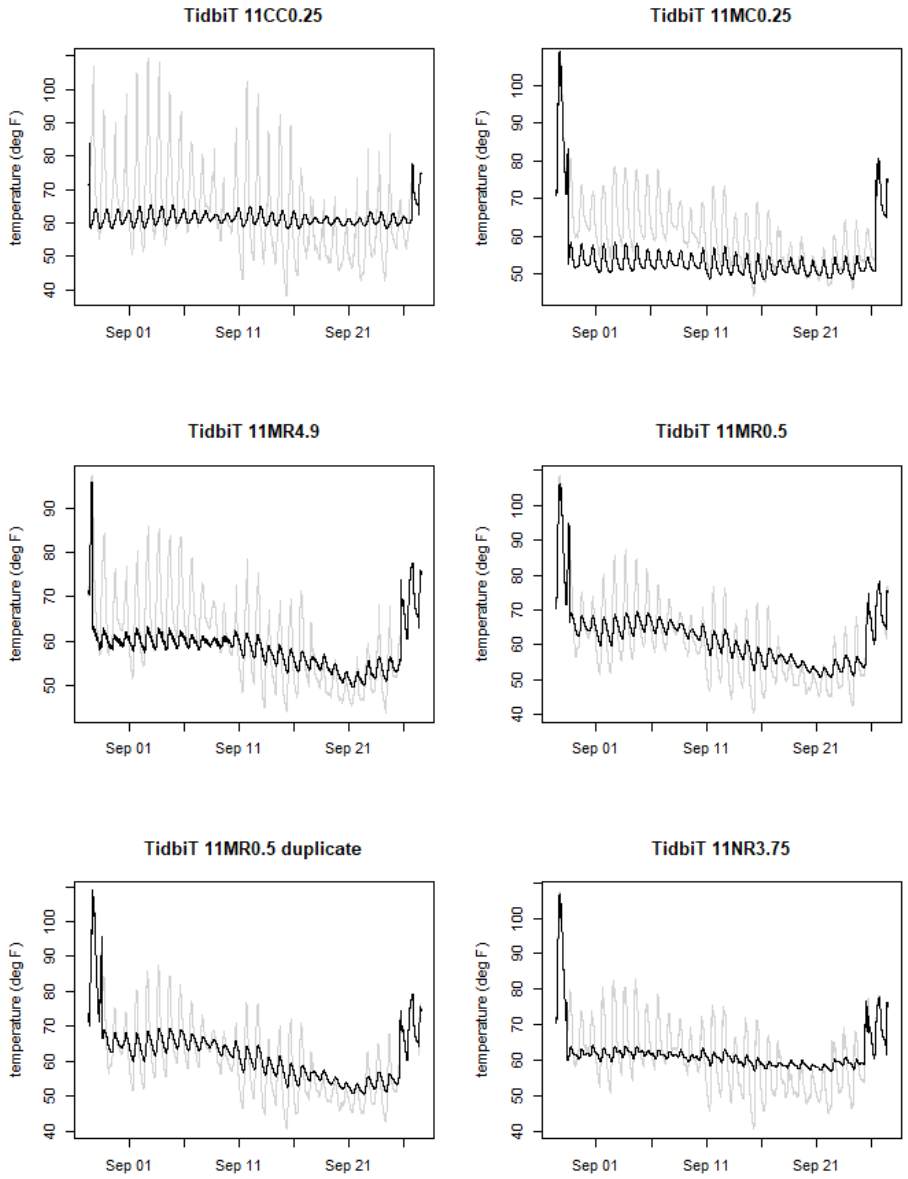

Figure A 1. Temperature data (degrees Fahrenheit) recorded by StowAway® TidbiT™ temperature loggers during the study. A TidbiT was attached to each SMPD cannister to monitor water temperature and another was placed on land near the deployment site to monitor air temperature. This data is used to confirm that the SPMD remained submerged during deployment; gray lines indicate air temperature while black lines indicate water temperature.

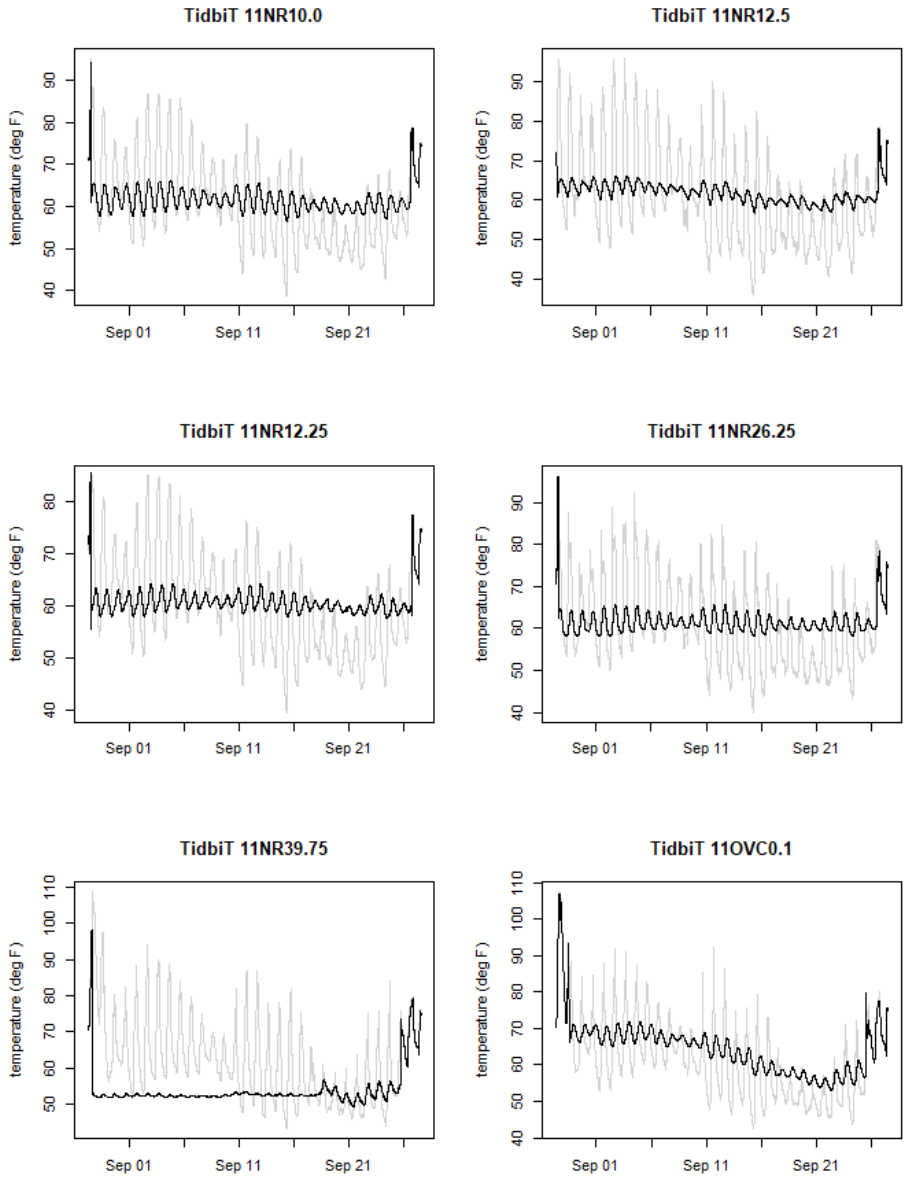

Figure A1 continued. Temperature data (degrees Fahrenheit) recorded by StowAway® TidbiT™ temperature loggers during the study. A TidbiT was attached to each SMPD cannister to monitor water temperature and another was placed on land near the deployment site to monitor air temperature. This data is used to confirm that the SPMD remained submerged during deployment; gray lines indicate air temperature while black lines indicate water temperature.

Table A1. Measurements of dissolved organic carbon (DOC), total organic carbon (TOC), total suspended solids (TSS) and turbidity in surface water grab samples collected on the days of, deployment (d), mid-way through the study (m) and at retrieval (r).

		DOC (mg/L)				TOC (mg/L)				TSS (mg/L)				turbidity (NTU)			
Site	Site name	d	m	r	mean	d	m	r	mean	d	m	r	mean	d	m	r	mean
11NR3.75	Lower NR	0.8	0.7	0.6	0.7	0.9	0.7	0.8	0.8	8	6	8	7	4.8	6.7	16.0	9.2
11MC0.25	Muck Crk	1.1	1.1	1.0	1.1	1.3	1.2	1.1	1.2	1	1[a]	1[a]	1[a]	0.5[a]	0.5[a]	0.5[a]	0.5[a]
11NR12.25	Thompson Crk	0.7	0.6	0.5	0.6	0.7	0.6	0.6	0.6	6	3	7	5	5.2	5.0	15.0	8.4
11CC0.25	Centralia Canal	0.8	0.7	0.6	0.7	0.8	0.7	0.6	0.7	8	6	8	7	7.3	9.7	24.0	13.7
11NR12.5	Smolt Trap	0.9	0.8	0.6	0.8	1.1	0.8	0.8	0.9	9	3	6	6	15.0	6.5	18.0	13.2
11NR21.6	McKenna	0.8	0.7	0.6	0.7	0.9	0.8	0.7	0.8	4	4	5	4	6.2	8.3	20.0	11.5
11NR26.25	Diversion Dam	0.8	0.6	0.5	0.6	0.8	0.7	0.6	0.7	4	4	9	6	6.7	8.7	21.0	12.1
11OVC0.1	Ohop Crk	4.2	4.2	4.3	4.2	4.3	4.4	4.9	4.5	4	4	5	4	4.4	4.2	5.4	4.7
11MR0.5	Lower MR	1.8	1.7	2.0	1.8	1.9	2.0	2.3	2.0	1	2	1	1	1.3	0.9	1.5	1.2
11MR4.9	Upper MR site	1.7	1.8	1.8	1.7	1.8	2.0	1.9	1.9	1	2	1	1	0.6	0.5[a]	0.7	0.6
11NR39.75	Background	0.7	0.6	0.5	0.6	0.7	0.6	0.6	0.6	3	4	6	4	6.8	11.0	25.0	14.3

[a] U-qualified, signifies values were below detectable limits