Mitigating Inputs of Tire Wear Toxins to Protect Salmonid Habitat on Vancouver Island

Pilot Year & Year 1 Summary Report

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| Acronym | Definition |
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| 6-PPD | N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine |
| 6-PPDQ | N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone |
| BCCF ARRC | British Columbia Conservation Foundation's Applied Research & Restoration Centre |
| BCSRIF | British Columbia Salmon Restoration & Innovation Fund |
| BCWS | Brooklyn Creek Watershed Stewards |
| CGC | Campbell Geospatial Consultants |
| CP MIMS | Condensed phase membrane introduction mass spectrometry |
| DFO | Department of Fisheries & Oceans |
| DI | Deionized (water) |
| DWWP | Drinking Water and Watershed Protection |
| ECVI | East coast of Vancouver Island |
| FBS | Friends of Bowker Creek |
| ID | Identification |
| LC ₅₀ | Lethal concentration that causes 50% mortality |
| MPWS | Millard-Piercy Watershed Stewards |
| MS | Mass spectrometer |
| NALT | Nanaimo Area Land Trust |
| NCC | Nature Conservancy Canada |
| PSF | Pacific Salmon Foundation |
| RDN | Regional District of Nanaimo |
| SFU | Simon Fraser University |
| TWP | Tire wear particles |
| TWT | Tire wear toxins |
| URMS | Urban runoff mortality syndrome |
| UVic CWIL | University of Victoria's Community Water Innovation Lab |
| VIU AERL | Vancouver Island University's Applied Environmental Research Labs |
| WCS | Walley Creek Streamkeepers |

ACRONYM LIST

EXECUTIVE SUMMARY

Entering waterways through stormwater runoff at toxic concentrations (parts-per-trillion or ng/L), a compound discovered in 2020 and known as '6-PPDQ' can cause urban runoff mortality syndrome (URMS) in numerous salmonid species, including coho, Chinook, and steelhead, in a matter of hours. Once coho, Chinook, and steelhead begin to demonstrate symptoms of URMS, the effects are irreversible, and they succumb to their symptoms within 24 to 48 hours.

The discussed 6-PPDQ monitoring program is co-led by the BC Conservation Foundation's Applied Research & Restoration Centre (BCCF ARRC) and Vancouver Island University's Applied Environmental Research Labs (VIU AERL). The three primary objectives of year 1 (2023 – 2024) were to undertake a wide scale 6-PPDQ surveillance of waterways along the east coast of Vancouver Island (ECVI); conduct increased spatial and temporal sampling for 6-PPDQ in ECVI waterways; and assess existing green infrastructure and construct lab-scale wetland microcosms to assess the most effective strategies to mitigate the impacts of 6-PPDQ and stormwater runoff.

Our wide scale grab sample monitoring resulted in the collection of 1,922 samples from 56 waterways and 123 sites across ECVI. The monitoring efforts were undertaken in partnership with 30 organizations (five First Nations and 25 stewardship groups) across Vancouver Island, from Campbell River, south to Victoria and westward as far as Lake Cowichan. Preliminary trends identified fromsamples:

- peak concentrations typically occurred during the rain event, as opposed to before the rain or ~24 hours after the rain began;
- 2. most waterways saw depressed concentrations observed as the winter season progressed and high flows occurred; and
- 3. concentrations were greater in large urban population centres compared to smaller population centres and rural environments.

Increased spatial sampling (i.e., increased number of sample sites in one watershed) and was strategically implemented in 13 systems along ECVI. Sites were added to a watershed once initial sample results indicated that 6-PPDQ was detected at downstream locations with multiple point source inputs. Overall, it appears that there is great variability between systems; the number and location of the point source outfalls impact where 6-PPDQ concentrations peaked.

Increased temporal sampling occurred on three systems over two rain events, with over 270 samples collected. Response time for 6-PPDQ detections varied depending on the system. However, all systems with detections showed variability at hourly intervals, indicating that a finer scale of sampling is required to better understand the dynamics of 6-PPDQ. BCCF ARRC/VIU AERL are planning on installing autosamplers and utilizing VIU AERL's mobile mass spectrometer lab in year 2 (2024 – 2025).

Existing green infrastructure was identified, with one rain garden monitored and sampled. There was little existing green infrastructure in Nanaimo available for sampling on a regular basis (i.e., rainwater/stormwater runoff did not build up enough to sample during regular rain events). The project team will work to expand these efforts in year 2 with the help of partnered First Nations and stewardship groups outside of Nanaimo.

Further, the University of Victoria's Community Water Innovation Lab is in the initial stages of building and testing lab-scale constructed wetland microcosms to assess different materials and strategies to determine the most efficient removal of 6-PPDQ. Results will inform the development and construction of a small pilot wetland that will undergo further 6-PPDQ mitigation testing.

Overall, significantly more sampling will need to be completed to get a full picture of which ECVI systems are most impacted by 6-PPDQ and which sites are most in need for remediation efforts.

1.0 INTRODUCTION

1.1 Tire Wear Toxins & Salmonids

It has been recognized for decades that stormwater runoff has been the cause of acute mortality, now referred to as 'urban runoff mortality syndrome' (URMS), in coho salmon (*Oncorhynchus kisutch*) in the Pacific Northwest (Tian *et al.*, 2020). However, it wasn't until 2020 that the compound responsible for the death of 40 to 90% of Puget Sound's returning coho in its most urbanized watersheds (impacting ~40% of the Puget Sound area) was identified: N-(1,3-dimethylbutyl)-N-phenyl-p-phenylendiamine quinone, commonly referred to 6-PPDQ (Tian *et al.*, 2020). Derived from N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylendiamine (6-PPD), 6-PPDQ is a transformative product (**Figure 1**). It is an antiozonant that has been added to tire rubber since the 1970s to preserve the lifespan of tires (Tian *et al.*, 2020). Overtime, tire treads break down and tire wear particles are left to reside on the roadway until washed away by rainfall.



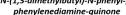


Figure 1. Compound added to tires as an antiozonant, known as 6-PPD (left), and its transformative compound, 6-PPDQ (right) that is toxic to salmonids.

Studies have identified that stormwater runoff is the greatest contributor of 6-PPDQ to urban waterways (Tian *et al.*, 2022; Webowski *et al.*, 2021). Stormwater runoff entering waterways with toxic concentrations (parts-per-trillion or ng/L) of 6-PPDQ can cause URMS to occur in numerous salmonid species in a matter of hours.

Originally, identified to impact coho salmon (Tian *et al.*, 2020), further studies have identified a similar fate to occur for rainbow trout (*O. mykiss*), brook trout (*Salvelinus fontinalis*; Brinkmann *et al.*, 2022), steelhead (anadromous *O. mykiss*), Chinook salmon (*O. tshawytscha*; French *et al.*, 2022), and coastal cutthroat trout (*O. clarkii clarkii*; Shankar *et al.*, 2024). In contrast, laboratory investigations suggest that other species, such as arctic char (*Salvelinus alpinus*), white sturgeon (*Acipenser transmontanus*) (Brinkmann *et al.*, 2022), sockeye salmon (*O. nerka*) (French *et al.*, 2022), and chum salmon (McIntyre *et al.*, 2021) appear to be unaffected when exposed to the same concentrations. Impacts and mortality rates vary between species (Table 1).

Table 1. Overview of toxicology studies, including fish species, life stage, lethal concentration, exposure time required to cause URMS, and mortality rates (Brinkmann *et al.*, 2022, French *et al.*, 2022; Hiki *et al.*, 2021; Hiki & Yamamoto 2022; Lo *et al.*, 2023; McIntyre *et al.*, 2021; Shankar *et al.*, 2024; Varshney *et al.*, 2022). Rows with greyed backgrounds indicate species local to east coast Vancouver Island (ECVI).

| Fish Species | Life Stage | 6PPDq LC ₅₀ * | Exposure Time | Mortality Rates |
|-----------------------------|-----------------------|--------------------------|----------------|------------------------|
| coho salmon | juvenile (~3 weeks) | 41 ng/L | 24 hours | 92% |
| coho salmon | juvenile (1+ years) | 80 – 95 ng/L | 1 – 8 hours | 92% - 100% |
| coastal cutthroat trout*** | juvenile (~weeks) | 320 | 24 hours | 50% |
| rainbow trout | juvenile (2+ years) | 1.000 ng/L | 7 – 60 hours | 50% |
| brook trout | juvenile ~1 year) | 590 ng/L | 1.2 – 20 hours | 100% |
| Arctic char | juvenile (~3 years) | 14,200 ng/L | 96 hours | 0% |
| white sturgeon | juvenile (~4.5 years) | 12,700 ng/L | 96 hours | 0% |
| steelhead** | juvenile (1+ year) | Not stated | 24 – 72 hours | 4% – 42% |
| Chinook salmon | juvenile (~3 weeks) | > 67,306 ng/L | 24 hours | 50% |
| Chinook salmon** | juvenile (< 1 year) | Not stated | 2 – 72 hours | 0% - 13% |
| sockeye salmon** | juvenile (<1 year) | Not stated | 24 hours | 0% |
| chum salmon | juvenile (1+ years) | >320,000,000 | 24 hours | 0% |
| brown Trout | alevins | Not stated | 48 hours | 0% |
| white-spotted char | juvenile (<1 year) | 510 ng/L | 24 hours | 50% |
| Atlantic salmon | alevins | Not stated | 48 hours | 0% |
| zebrafish | embryo | 308,670 | 96 hours | 50% |
| southern Asian Dolly Varden | juvenile (<1 year) | >3,800 ng/L | 24 hours | 0% |
| masu salmon | juvenile (<1 year) | >3,800 ng/L | 24 hours | 0% |
| Japanese medaka | juvenile (41 days) | >34,000 ng/L | 96 hours | 0% |

*LC₅₀ (Lethal Concentration 50) refers to the concentration required to cause mortalities in 50% of the test specimens.

**Fish were exposed directly to untreated stormwater runoff from three separate storm events, no concentrations were stated. Source: French *et al.*, 2022

***Preliminary, unpublished data presented at the World Fisheries Congress in Seattle, WA on March 5, 2024 by Prarthana Shankar

French *et al.* (2022) found that once coho, Chinook, and steelhead began to demonstrate symptoms of URMS (i.e., gaping at surface, loss of equilibrium, swimming along the surface), the effects were irreversible and they would succumb to their symptoms within 24 to 48 hours. Studies are currently underway to understand the mechanism involved in URMS. However, researchers are currently suggesting that potential mechanisms may include, but are not limited to: mitochondrial dysfunction, oxidation, and/or blood-brain barrier disruption (Blair *et al.*, 2021; Mahoney *et al.*, 2022).

It is predicted that climate change will result in increased average temperatures, summer drought periods, rainfall during fall spawn migrations, and reduced snowpack along the ECVI (Capital Regional District, 2017). These changes are imminent and evidence of their impacts to salmon populations have already been documented; for instance, the mass fish mortalities that occurred in late June 2021 in the Nanaimo, Quinsam, Tsolum, and Cowichan Rivers due to freshwater temperatures rising above 24°C (J. Atkinson, Senior Biologist, BCCF ARRC, Pers. Comm. 2024). It is thought that the time of greatest concern for URMS is during increased rainfall throughout fall spawn migrations, when base flows are at their lowest.

1.2 Tire Wear Toxins Project Overview

The ECVI is a prime location to begin sampling for presence of 6-PPDQ on Vancouver Island, as it is home to ~89% of Vancouver Island's residents (Province of BC, 2021). The Island Highway and Highway 1 cross most, if not all, river systems along ECVI. As a result, the Island's waterways are vulnerable to 6-PPDQ exposure, potentially at risk to URMS.

The longer-term goals of the project include: 1) identifying major 6-PPDQ hotspots and prioritize these sites for future mitigation efforts; 2) determine the most efficient means of mitigating 6-PPDQ and identifying potential locations for mitigation efforts; and 3) be part raising awareness of the issue within the community, all levels of government, and tire manufacturers to ensure conservation actions are implemented and tire wear toxins (TWT) are reduced/removed from waterways where they directly threaten vulnerable salmonid species.

In 2023 – 2024, the British Columbia Conservation Foundation's (BCCF) Aquatic Research and Restoration Centre (ARRC) acquired funding from the British Columbia Salmon Restoration and Innovation Fund (BCSRIF), Pacific Salmon Foundation (PSF), British Columbia Conservation and Biodiversity Award, Mitacs, Community Gaming Grant, and the Regional District of Nanaimo (RDN) to undertake Year 1 activities. A pilot year was run in 2022 – 2023 with funding from PSF, RDN, and the Habitat Conservation Trust Foundation.

The program is broken into four key activities. All project activities are occurring in collaboration with Vancouver Island University's Applied Environmental Research Labs (VIU AERL).

1.2.1 WIDE SCALE SURVEILLANCE GRAB SAMPLING

In the pilot study year (2022 – 2023), BCCF ARRC was working with 10 stewardship groups to collect samples between Courtenay and Victoria. In 2023 – 2024, the existing community-based monitoring program was expanded to involve more First Nations and stewardship groups. This allowed collection of samples from a greater area, expanding from Campbell River, south to Victoria. In total, 30 groups (five First Nations, 25 stewardship groups) were trained to collect grab samples during rain events, directed by BCCF ARRC. Participants collected samples from some of the over 10,000 locations where roadways intersect salmon-bearing systems along ECVI (Figure 1).

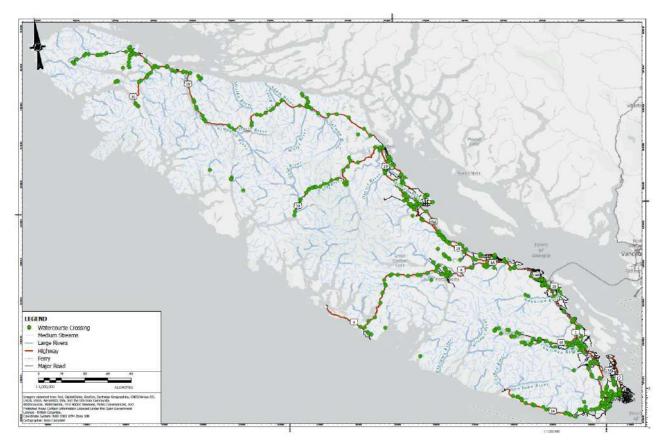


Figure 1. Green dots indicates individual locations where a roadway intersects a salmonidbearing system along ECVI. Map prepared by Campbell Geospatial Consultants (2022).

1.2.2 Investigate Tire Wear Toxin Variation Over Time & Space

There are many unanswered questions related to the dynamics of how 6-PPDQ flushes into and through a waterway. For instance, it is not clear how long it takes for 6-PPDQ in stormwater runoff to reach acutely toxic levels in waterways and how long it lasts.

This activity will characterize the temporal variation of 6-PPDQ concentrations using increased grab sampling efforts, intermittent autosamplers, and ultimately continuous real-time monitoring using a mobilized mass spectrometer.

1.2.3 EVALUATE GREEN INFRASTRUCTURE & NATURE-BASED SOLUTIONS

This activity will be assessing the efficacy of previously installed green infrastructure at removing 6-PPDQ by collecting grab samples, where possible. Additionally, partners at the University of Victoria's (UVic) Community Water Innovation Lab (CWIL) are constructing laboratory scale microcosm systems, to assess options for removing 6-PPDQ from stormwater runoff. These studies will assess various media, designs, and plant species that could ultimately be used in different types of green infrastructure to remove 6-PPDQ more efficiently before the stormwater runoff reaches waterways.

1.2.4 ANNUAL WORKSHOP FOR KNOWLEDGE DISSEMINATION

BCCF ARRC and VIU AERL will be co-hosting a two-day workshop each year to mobilize knowledge, generate awareness amongst the community, and inform policy/decision makers at all levels of government. As 6-PPDQ was only discovered in 2020, many important questions remain. Therefore, this workshop is intended to bring together those working on understanding the fate and distribution of 6-PPDQ, their impacts on ecosystem health, and mitigation strategies. These workshops will be important knowledge-sharing gatherings to ensure that the science is robust, relevant, and accessible so that we can tackle the many dimensions of the issue collectively.

The first workshop was postponed until April 2024 (now part of the 2024 – 2025 fiscal year) due to availability of researchers. A short summary will be shared in the next technical report and a full summary of the event will be prepared and shared with participants and interested parties following the event.

2.0 METHODS

2.1 Study Area

In the pilot study year (2022 - 2023), a total of 14 waterways along ECVI from Courtenay to Victoria were sampled (Figure 2). In year 1 (2023 - 2024) of the study, efforts expanded to

include 56 systems across 123 locations, spanning from Campbell River south to Victoria, as far west as Lake Cowichan, at the headwaters of the Cowichan River (Figure 3).

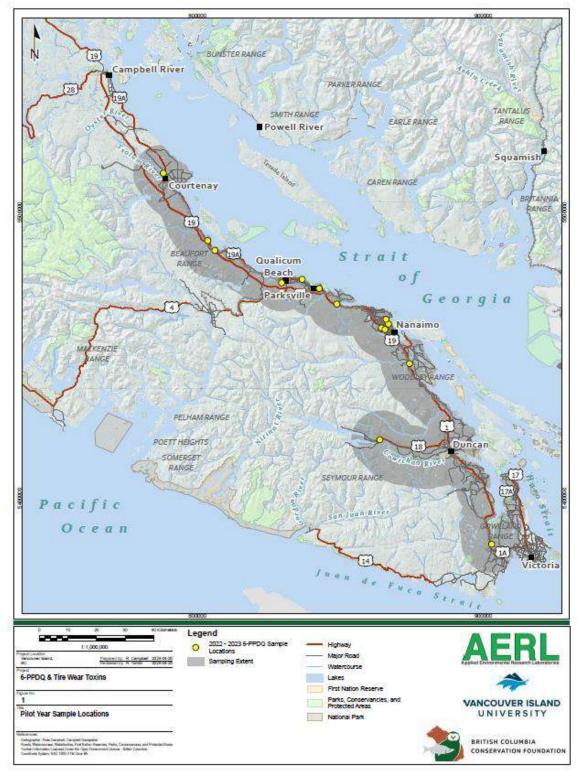


Figure 2. Pilot study year (2022 – 2023) 6-PPDQ sample area extends from Courtenay to Victoria along the ECVI. Map was created by Campbell Geospatial Consultants in April, 2024.

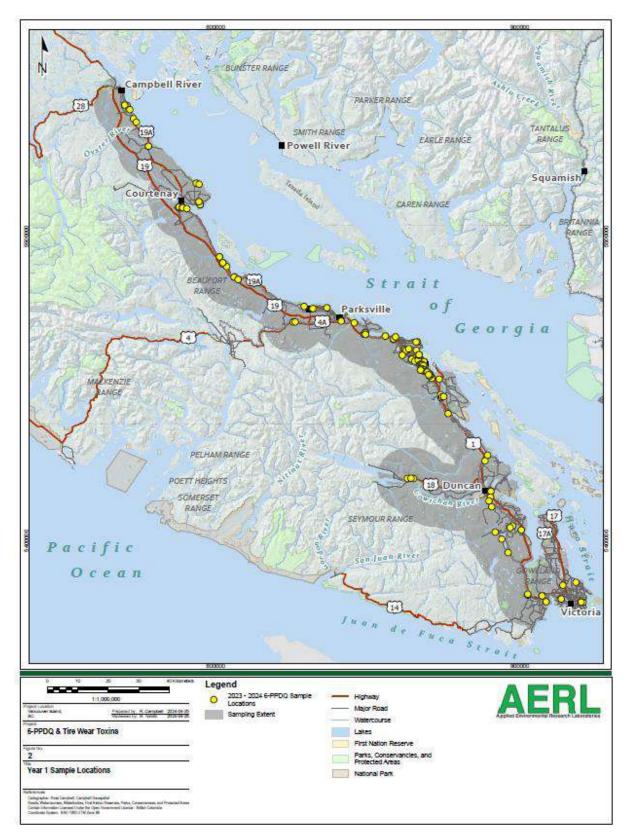


Figure 3. Year 1 (2023 – 2024) 6-PPDQ sample area extends from Campbell River to Victoria. Map was created by Campbell Geospatial Consultants in April, 2024.

All sample locations in ECVI waterways currently or historically supported coho, Chinook, and/or rainbow trout. Towards the end of year 1 the project team began to add systems and sample locations from waterways home to coastal cutthroat trout, as a result of recent research indicating they are also vulnerable to 6-PPDQ (unpublished data; Shankar *et al.*, 2024). Sites in both years were selected based on their proximity to high-velocity and high-volume traffic, safe accessibility, and the availability and knowledge of stewardship groups and First Nations throughout the study area.

Although there is significant variation in microclimates between each of the selected locations, all sites exist within the Eastern Vancouver Island ecoregion, which extends along the coast from the Vancouver Island ranges to the Salish Sea (Nature Conservancy Canada (NCC), 2019). The Vancouver Island ranges protect the east coast from extreme weather systems, providing a temperate climate. In general, ECVI has warm, dry summers and wet, mild winters (NCC, 2019).

The major urban centres along ECVI are Campbell River, Courtenay, Comox, Nanaimo, Duncan, and Victoria; however, townships exist along the entire ECVI (NCC, 2019). As of 2021, Vancouver Island has 868,517 inhabitants, with the majority (772,423) of those living along the ECVI (Province of British Columbia, 2021). As a result, the greatest volume of traffic is likely to occur along ECVI; subsequently, these waterways are likely the most vulnerable to the influence of 6-PPDQ on Vancouver Island.

Each sample location is situated within about 60 to 100 metres (up- or downstream) of a potential 6-PPDQ point source (i.e., bridge, roadway, stormwater drains); the exact sample location at each waterway was selected opportunistically based on safe and permissible access.

2.2 Citizen Science Involvement

As this project involves an extensive geographic boundary and capturing the influx of 6-PPDQ into waterways requires sampling during rain events, it is logistically challenging to capture these events with a small team. Therefore, BCCF ARRC and VIU AERL enlisted the assistance of local streamkeeper groups and First Nations to collect water samples within their region. By mobilizing volunteers, water samples can be collected during the peak rainfall within each region of interest, maximizing the opportunity to capture the 6-PPDQ entering the stream.

In order to keep the citizen science participants informed and engaged in the monitoring program, numerous materials were created, including training videos, frequently asked questions information pages, a bi-annual newsletter, and a project website.

2.3 Wide Scale Surveillance Grab Sampling

A Standard Operating Procedure (SOP) for 6-PPDQ grab sampling was produced and distributed to all sampling participants at training sessions. A copy of the SOP is stored in each of the sampling kits and can be found on the <u>project website</u> and in Appendix A.

For both sampling years, the time at which sample collection occurs is determined by weather conditions. In the pilot year, this study utilized the sample protocols that the Department of Fisheries and Oceans (DFO) followed. In order to sample, a dry period of at least 48 hours had to occur before a rain event greater than 5mm. These constraints ensure that there is time allotted for tire wear particles (TWP) to build up on the roadway before another rain event.

In year 1 of this program, it was still preferable to have at least 48 hours of dry weather prior to sampling to allow TWP to build up on the roadway. However, the BCCF ARRC/VIU AERL team aimed to sample a variety of conditions, considering varying lengths of dry weather and quantities of rain.

BCCF ARRC monitors the weather forecast and emails the sample windows to volunteers for their specific region. Correspondence with available volunteers carries on through the entirety of the sampling efforts till sample pick up. BCCF ARRC regularly communicates with those sampling to respond to any questions that may arise, especially when weather patterns are shifting.

After each group collects grab samples during a rain event, BCCF ARRC arranged for samples to be picked up from each volunteer's homes and transported to VIU AERL for delivery at the lab. Sample pick up typically occurred the day following collection of the last creek sample ('after' sample) to ensure samples were delivered to the lab in a timely manner for analysis.

2.3.1 Types of Grab Samples Collected

Wide scale surveillance monitoring includes a minimum of three creek samples: a 'before' sample, collected prior to the rain starting, as a negative control; a 'during' sample, collected during the rain event, preferably during or shortly following the peak rainfall; and an 'after' sample is collected the following day (~24 hours after the 'during sample') in order to determine if 6-PPDQ is still present in the system or if it has degraded or washed away, if it was present.

A fourth sample is collected as a control at all sites following the BCCF ARRC/VIU AERL protocols; it is referred to as a 'field blank'. A 250 mL bottle of deionized (DI) water is brought

into the field, to collect this sample, and transferred into a new empty bottle while creekside. This sample is collected as a control to test for any potential contamination in the field.

Finally, a fifth sample is collected in each sample event at all sites that have a direct point source available to collect from (i.e., downspout on a bridge, culvert from the road). This sample is collected at the 'during' sample event, when stormwater runoff is flowing, and taken immediately following the 'during' and 'field blank' samples.

2.3.2 DATA COLLECTION APP

An electronic data collection app and form (Figure 4) were developed specifically for the program. The app and form have streamlined processes to reduce the need for data transcription, which is time intensive. Samples are delivered to VIU's AERL shortly after collection. The implementation of this app and form allow the data collected to be immediately available when samples are dropped off, reducing bottlenecks and allowing for quick reporting timeframes.

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| | le_BCCF_AERL_02 49 on_Wexford Creek_DS-Hwy1 | P9232: 12. | | Field Blank Fish Visible? | Point Source | Other |
| | In_BCCF_AERL_02., 48 an_Lower Shawnigen_DS-Ce | | | Yes | 1 | No |
| | le_BCCF_AERL_02 49: on_Beck Creek_DG-Hwy1 | 20923, 12. | | Rain Gauge (mm | 0 | - + |
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Figure 4. (A) Data collection app database screen showing all grab samples previously collected; and (B) a blank data collection form where each individual sample that is collected is input and submitted.

QR codes were used to ensure consistency in data collection with the app. In particular, it is important that sample location names are entered the same each time and that sample identification (ID) names are standardized. For this reason, each of the sample locations had a location ID card created (Figure 5), which includes an overview of the site and how to access it. Additionally, every sample bottle was equipped with a unique QR code (Figure 6).



Figure 5. Example of a sample location ID card that was created for each of the locations sampled as part of the wide scale surveillance activity.



Figure 6. Example of a sample bottle with a unique QR code.

For each sample bottle that is filled, the sample crew fills out the required fields in the data collection app. Based on the sample type selected, the fields in the form will change to reflect the required inputs.

2.3.3 SAMPLE KITS

Prior to sample collection, participants must ensure they have their sample kit (Figure 7) and the data collection app downloaded onto a smart phone or tablet. Each kit provided to participants included a cooler, ice pack, rain gauge (multiple if numerous sites sampled), location ID card(s), empty sample bottles (number varied depending on number of sample sites and if point source samples are possible), one bottle of DI water per site sampled, nitrile gloves, a thermometer, and sampling instructions. Tablets with the data collection app were provided to those groups that requested it. Further, some of the groups within the RDN had additional water quality equipment that was provided by the RDN's Drinking Water and Watershed Protection (DWWP) Program's Community Watershed Monitoring Network. This equipment included a YSI Pro2030 multiparameter water quality sonde, which measures temperature, dissolved oxygen, and conductivity, as well as a HACH 2100Q portable turbidimeter to measure turbidity.



Figure 7. An example of the wide scale surveillance grab sampling kit that was provided to each of the participating groups. Kits were scaled up or down depending on the number of sample locations the group was monitoring.

The sample kits used by the BCCF ARRC/VIU AERL crews were comprised of the same materials as displayed in Figure 7. BCCF ARRC and VIU AERL used their own water quality equipment, which consisted of a YSI ProPlus Quattro, which measured temperature, dissolved oxygen, pH, and conductivity, and a HACH 2100Q portable turbidimeters to measure turbidity. BCCF borrowed a turbidimeter from the RDN DWWP Program for the bulk of year 1; however, one was acquired later in the year.

2.3.4 RAIN GAUGES

Rain gauges were set up at most sample locations for the duration of each set of sampling activities. The purpose of the rain gauge is to assess the accuracy of the nearby weather stations. Therefore, when going to collect the 'before' samples, the rain gauge would be near the sample location in an area with an open canopy. The samplers would check the rain gauge when collecting the 'during' sample, record the value, and dump out the water. The samplers would do the same thing when they would come back to collect the 'after' sample; however, they would remove the rain gauge from the site and keep it with the kit once all samples were collected to avoid theft.

2.3.5 GRAB SAMPLE COLLECTION PROTOCOLS

For each sample bottle filled, a form in the data collection app is recorded. Every sample that is collected requires the samplers to enter their names/initials, scan the sample ID and location ID, and select the sample type (i.e., before, during, after, field blank, point source, other). Depending on the sample type, the data collection app fields will adjust to prompt the samplers to collect the required information. Types of information include: identifying if fish were visible (yes/no), quantity of water in the rain gauge, water temperature, date, time, location, site photo (facing upstream from the sample location), additional water quality data (i.e., pH, dissolved oxygen, specific conductivity, and/or turbidity, if equipment is available), and any comments, if applicable.

Samplers wore nitrile gloves, donning them just before sample collection to limit cross contamination. Standing in the river, facing upstream, the sampler would remove the cap from the sanitized 250mL amber glass bottle with polyethylene foam-backed PTFE-lined polypropylene caps (Cole-Parmar Canada, Montreal, QC) and submerge the bottle upside down. Once submerged, the bottle was turned into the flow, allowing it to fill until full. The water was emptied downstream of the sampler, and this process was repeated two more times for each sample to ensure that the bottle was thoroughly rinsed. Once rinsed, the same steps were repeated to collect the sample. Following the fourth fill, the cap was secured on the bottle and placed back into a cooler bag ready with an ice pack. This process was used for the 'before', 'during', and 'after' samples. With regards to the point source samples, the same type of 250mL amber glass bottle with polyethylene foam-backed PTFE-lined polypropylene caps were used. The 250mL bottles were held directly under the point source rinsed three times and then filled. The sample was then capped and placed in the cooler with an ice pack. All samples were refrigerated (~4°C) until handed off to BCCF ARRC for transfer to VIU AERL for analysis.

'Field blanks', or controls, were collected directly after the 'during' sample is collected. A 250 mL amber glass bottle of DI water, prepared at VIU AERL, is brought into the field to collect this sample. The DI water is handled with the same gloves used to collect the 'during' sample. The DI water is poured directly into another 250 mL amber glass bottle with polyethylene foambacked PTFE-lined polypropylene caps while onsite . This sample is collected as a control to test for any potential contamination in the field during the rain event sampling.

To aid in quality control of the VIU AERL rapid analysis technique, BCCF ARRC and VIU AERL are partnered with DFO. When the weather conditions fit the required DFO protocols, BCCF ARRC/VIU AERL collects duplicates from seven different waterways (10 locations), providing one sample to DFO and one to VIU AERL. Samples collected for DFO are collected in the same manner except they are collected in 250 mL wide-mouthed amber glass bottles with polyethylene foam-backed PTFE-lined polypropylene caps, filled only half full and frozen (~-20 °C), rather than refrigerated.

2.4 Laboratory Analysis

Once delivered to VIU AERL, samples were inventoried. This was done by scanning bottle QR codes using a laptop webcam, the sample information was retrieved and shown on screen to indicate the sample was successfully recorded with the in-field app. Samples were then triaged, with any sample identified as 'other' or 'point source' being separated for batch analysis (due to potentially elevated 6-PPDQ levels). A button in the sample inventory app then allowed the new sample to be added to a continuous inventory list, capturing the time/date when the sample was inventoried and where it is stored in the lab.

When preparing to be measured, another custom application was used to prepare samples for analysis. Samples and QA/QC solutions are made in 40 mL glass vials with PTFE-lined polypropylene caps and DI water when necessary. The desktop app records concentrations of the 6-PPDQ and analogous compounds standard solution, and the internal standard 6-PDDQ- d_5 used in building a 5-point calibration curve for environmentally relevant concentrations (0 to 280 ng/L 6-PPDQ). Samples are poured directly into 40 mL glass vials to almost fill (approximately 38 g), the mass of sample is recorded from a lab balance, then a known volume of internal standard is added before capping the vial. Samples identified as potential high concentration (point source or other) are diluted five to 10 times with DI water in the 40 mL vial before addition of internal standard. Once QA/QC and samples were prepped and recorded via the desktop app, they were brought to the instrument or stored in a fridge before measuring.

6-PPDQ was measured at VIU AERL using condensed phase membrane introduction mass spectrometry (CP-MIMS). In CP-MIMS, a semi-permeable membrane is used to allow for selective introduction and enrichment of hydrophobic contaminants from complex water samples (Figure 8). Typically, polydimethylsiloxane (PDMS, silicone rubber) membranes are employed, which allow for effective measurement of 6-PPDQ, while rejecting bulk sample components in stormwater runoff-affected waters (i.e., water, salts, suspended particulates). When mounted on a stainless-steel 'immersion probe', this allows for a simple 'dip and sip' measurement strategy to quickly measure 6-PPDQ and other contaminants in a large number of samples. Analytes (*e.g.*, 6-PPDQ) that cross the membrane are transported to a mass spectrometer for detection via liquid acceptor solvent flowed through the inside (lumen) of the membrane. For 6-PPDQ, previous work has shown that a miniaturized version of the membrane probe (Monaghan, 2021) provides detection limits as low as 6 ng/L and allows for simultaneous detection of additional PPDQ analogs (Monaghan, 2023).

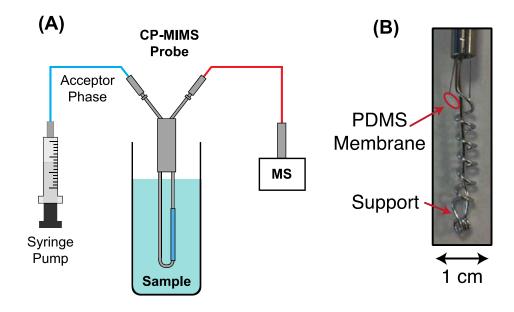


Figure 8. Overview of membrane sampling process with probe directly immersed in a sample is shown in Figure 8A. Permeating analytes are carried to a mass spectrometer (MS) for detection via a continuously flowing solvent acceptor phase. B: Photograph of miniaturized CP-MIMS probe used for 6-PPDQ measurements. Graphics prepared by Joseph Monghan.

To accommodate the large sample numbers involved in this monitoring campaign, the CP-MIMS analysis step was automated using a low-cost 3D-printer (Figure 9). This system uses a simple XYZ stage to automate the 'dip and sip' process, freeing up the analysts' time to collect/prepare additional samples, interpret results, and report back to stakeholders. In its current configuration, the autosampler measures samples in batches of 25, alongside various calibration solutions and QA/QC checks. Each batch takes about three hours to measure, allowing up to 100 samples to be measured per day (176 total measurements, including QA/QC).

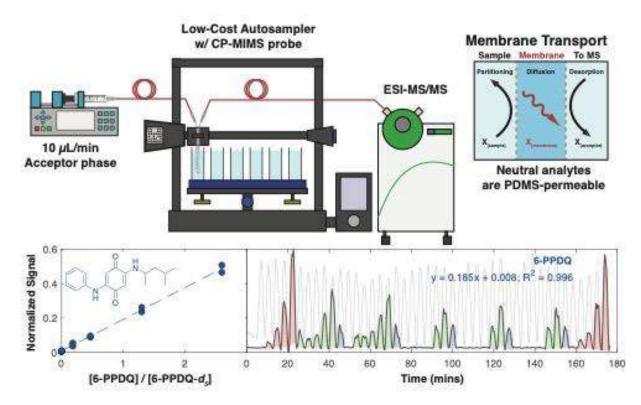


Figure 9. Overview of CP-MIMS analysis using low-cost autosampler derived from a 3D-printer. Graphics prepared by Joseph Monghan.

Various strategies are used to ensure CP-MIMS is providing reliable quantitation for 6-PPDQ. An isotopically labelled standard of 6-PPDQ (6-PDDQ- d_5) is added to every sample and standard to compensate for instrument drift and matrix effects during the membrane partitioning and ionization process. Sample analysis is bracketed by measurement of a calibration check solution and DI water blank every five samples. Whenever significant 6-PPDQ is detected in an environmental sample, replicate analyses are performed (such that $n \ge 3$) to ensure the result is reproducible. 6-PPDQ identification in high concentration samples (\ge 50 ng/L) is further supported by monitoring multiple characteristic fragments during routine analysis, where the

presence of these additional qualifier fragments reinforces the presence of 6-PPDQ in the sample.

2.5 Increased TWT Monitoring Over Time & Space

The second activity associated with the *Mitigating Inputs of Tire Wear Toxins to Protect Salmonid Habitat on Vancouver Island* involves investigating TWT variation over time and space. In order to do this, steps have been taken to increase sampling efforts over time and space at multiple locations within Nanaimo by the BCCF ARRC/VIU AERL team. In year's 2 and 3 of the project, the team will focus efforts on employing autosamplers in multiple waterways across ECVI, as well as utilizing the VIU AERL mobile mass spectrometry lab, which allows for real-time measurements to be collected continuously in the field.

2.5.1 INCREASED SPATIAL SAMPLING

Preliminary testing is required for each system added to the project. Initial sampling begins with a single sample site, located furthest downstream (i.e., exposed to multiple point sources) or two sample locations, one upstream and one downstream of a point source. Following preliminary testing, if 6-PPDQ is detected, additional sites across the watershed are added to provide spatial information on point source impacts. If 6-PPDQ is not detected, another location further upstream may be identified and tested before expanding to multiple sites.

Within Nanaimo, BCCF ARRC/VIU AERL has a dedicated team to conduct increased sampling across most systems that are currently monitored. For some of the new systems that are added, the same approach that is taken with the participating groups (i.e., collecting samples from one to two locations below the furthest downstream potential point source). To begin the year 1 sampling season, the project team built off of sample results from the pilot year of the program and added additional sites across watersheds where 6-PPDQ was detected.

Increased spatial sampling follows the same protocols as the wide scale surveillance monitoring: a before, during, after, field blank, and point source (if applicable) are collected.

2.5.2 INCREASED TEMPORAL SAMPLING

In order to gain a better understanding of TWT dynamics, including how long it takes for them to appear in a system, how long it takes for peak concentrations to occur, and how long it persists are all critical aspects that need to be understood to better inform mitigation strategies. As such, the BCCF ARRC/VIU AERL team has begun assessing multiple locations

identified to have 6-PPDQ in the 'during' samples on several occasions in the wide scale surveillance sampling efforts.

In particular, three waterways (Northfield Creek, Millstone River, Cat Stream) have had increased temporal sampling completed in 2023 – 2024. Grab samples are collected prior to the rain occurring every one to six hours and then once every hour during the rain event. Sampling continued after the rain every one to six hours until the creek's TWT concentrations were near baseline.

Northfield Creek has had two increased temporal sampling events occur; the first was November 21 – 22, 2023 and the second was November 30 – December 5, 2023. Millstone River and Cat Stream had increased temporal sampling occur once from November 30 – December 5, 2023. For the event with three sites, sampling crews sampled at Northfield Creek on the hour, then the Millstone River, followed by Cat Stream, returning to Northfield Creek to again sample on the hour. Increased temporal sampling follows the same protocols as the wide scale surveillance monitoring; however, there are multiple of each the before, during, and after samples. Field blank and point source samples are not collected during high temporal sampling.

2.5.3 AUTOSAMPLERS

In year 1 it was proposed that autosamplers would be deployed in four waterways across ECVI. This initiative is still in progress, as the crew at VIU AERL is working to adapt existing autosamplers to fit the needs of the sampling program. Autosamplers that are available for purchase are primarily made out of plastic materials, including the sample bottles, which is not suitable to collecting 6-PPDQ, as the compound adheres to plastic (Hu *et al.* 2023).

Once the autosamplers are ready, we will work with partnered First Nations and stewardship groups to install and trigger the autosamplers prior to a rain event.

2.5.4 MOBILE MASS SPECTROMETRY LAB

It is planned that the mobile mass spectrometry lab will be deployed in year 2 at locations where 6-PPDQ has been known to occur; these sites are being identified through the wide scale surveillance sampling program.

In year 1, BCCF ARRC/VIU AERL have been investigating different potential locations that the mobile lab could be utilized. The project team has been working to identify locations along ECVI that have the facilities required to run the mobile lab, including two separate plugs (110V, 15

amp) on separate circuits, space to set up a tent for some dry area outside of the van, and (if possible) access to some running water/outside tap for rinsing gear.

2.6 Evaluate Green Infrastructure & Nature-Based Solutions

Green infrastructure is known to be an efficient means of removing multiple toxins and compounds (up to 90%), as well as sediments (up to 80%) from stormwater runoff (Groundwater Foundation, 2022). A few recent studies have indicated that green infrastructure (i.e., rain gardens, bioretention systems) has been shown to be effective at removing TWT, in particular 6-PPDQ, from stormwater runoff (Rodgers *et al.*, 2023). For this reason, this program is looking to both identify and test existing green infrastructure and build lab-scale microcosms to begin testing different materials that could be used in green infrastructure.

2.6.1 MONITORING EXISTING GREEN INFRASTRUCTURE

In year 1, the BCCF ARRC/VIU AERL team worked to identify existing green infrastructure within the Nanaimo region, as well as potential locations along ECVI that First Nations and stewardship groups may be able to sample at a later date. Sites were identified through conversations with partner organizations, local knowledge, and reviewing the City of Nanaimo's GIS mapping (City of Nanaimo, 2011). Sites within Nanaimo that were accessible were sampled, when possible.

2.6.2 NATURE-BASED SOLUTIONS

Project partners at the UVic CWIL are currently assessing nature-based solutions to mitigate the impacts of TWT, specifically 6-PPDQ. UVic CWIL is constructing laboratory scale constructed wetland microcosms (Figure 10) that replicate the functions of wetlands and/or raingardens. The microcosms are constructed of a glass column and filled with glass beads as a baseline, as they are a low porosity substance for adsorption of 6-PPDQ. This process is then going to be repeated with different media, including sand, nonsterile soil, woodchips, and charcoal as components of the microcosm.

To run the system, one side of the microcosm (top) is considered an entrance for solution, which will be comprised of different solutions, including 6-PPD, 6-PPDQ, and a mixture of 6-PPD and 6-PPDQ. The microcosm is gradually filled until the media or substrates are submerged by solution. Following submergence, the effluent will then be discharged from the bottom of the column. Adjustments to the feeding rate of the pump will assess varying retention times.

Results from the microcosm studies will be applied to the design of a small scale constructed wetland for further testing.



Figure 10. Lab-scale microcosm built by Uvic CWIL. Photo: Saman Samadi

2.7 Annual Workshop for Knowledge Dissemination

The annual workshop for 2023 – 2024 was pushed into the 2024 – 2024 fiscal year. It will be hosted at the Vancouver Island University, Nanaimo Campus, on April 29 – 30, 2024. Invitations and workshop organization began in February and March 2023.

3.0 RESULTS

3.1 Citizen Science Involvement

In the pilot year (fall 2022), 10 streamkeeper groups and local First Nations (Table 2) were trained to collect water samples from 10 different systems between Courtenay and Victoria. Each group was trained between mid-August and early September to ensure they were ready for sampling in mid- to late-September when the first substantial rain of the fall season was to be expected.

In year 1, the network expanded to 30 groups (five First Nations, 25 stewardship groups; Table 2) trained throughout the year to collect water samples with the most up-to-date sampling

procedures from 46 waterways along ECVI. Overall, eight of the 30 groups participated in sampling in both the pilot sampling year and year 1 of the program. Similarly to the pilot year, most training occurred in late August to mid-September with anticipation that the fall flush would occur in late September. As the program expanded through the year, additional training sessions were held as new groups joined the sampling network. Therefore, not all groups captured the fall flush in this first year.

Approximately 160 volunteer hours were contributed in the pilot year, valued at \$3,200 (\$20/hour). Year 1 of the sampling program had approximately 672 volunteer hours, valued at \$13,440, contributed by 159 people through training and sampling activities. Therefore, volunteer contributions were scaled up ~4 times from the pilot year to year 1 of the monitoring program.

Table 2. Groups trained to participate in water sampling for 6-PPDQ in the pilot year and year 1 of the project, including the region they are located and waterways they are sampling. A hyperlink to each of the group's webpage is included in the table, if available.

| ECV/I Pagion | Crown Name | | Trained & Sampled | |
|--|--|--|-------------------|--------------|
| ECVI Region | Group Name | Waterways | Pilot Year | Year 1 |
| | Save Simms Creek | Simms Creek | | \checkmark |
| Campbell River | Greenways Land Trust | Willow Creek | | \checkmark |
| | Campbell River Environmental Committee | Woods Creek, Caddisfly Creek | Pilot Year | \checkmark |
| Black Creek | Friends of Black Creek | Black Creek Tributary | | \checkmark |
| Greenways Land Trust Greenways Land Trust Campbell River Environmental Committee Black Creek Friends of Black Creek Comox Little River Enhancement Society Brooklyn Creek Watershed Society Courtenay Millard Piercy Watershed Stewards Tsolum River Restoration Society Beaufort Watershed Stewards | Little River Enhancement Society | Little River | | \checkmark |
| | Brooklyn Creek | | \checkmark | |
| Co | Millard Piercy Watershed Stewards | Piercy Creek | | \checkmark |
| Courtenay | Tsolum River Restoration Society | Towhee Creek | \checkmark | |
| Farmer Davi | | \checkmark | \checkmark | |
| Fanny Bay | Fanny Bay Salmonid Enhancement Society | Rosewall Creek, Cook Creek | ✓ | \checkmark |
| Qualicum Beach | Qualicum Beach Streamkeepers Society | Grandon Creek, Beach Creek, Whiskey Creek, Harris Creek | ✓ | \checkmark |
| French Creek | | French Creek | ✓ | \checkmark |
| Parksville | | Craig Creek, Shelly Creek | ✓ | \checkmark |
| Nanoose Bay | Snaw-Naw-As First Nation | Nanoose Creek | ✓ | |
| | Departure Creek Streamkeepers | Departure Creek | \checkmark | \checkmark |
| | Nanaimo Area Land Trust | Cat Stream | | \checkmark |
| Nanaimo | Snuneymuxw First Nation | Nanaimo River, Haslam Creek, Beck Creek | | √ |
| | Walley Creek Streamkeepers | Walley Creek | | \checkmark |
| Chemainus | Halalt First Nation | Chemainus River, Bonsall Creek | | √ |

| ECVI Region | Group Name | Waterways | Trained & Sampled | |
|----------------|--|---|-------------------|--------------|
| Levi Kegion | Group Name | water ways | Pilot Year | Year 1 |
| Duncan | <u>Cowichan Tribes</u> | Fish gut alley | Pilot Year | \checkmark |
| Duncan | Koksilah Watershed Working Group | Kelvin Creek, Norrie Creek, Busy Creek | | \checkmark |
| Lake Cowichan | Cowichan Lake & River Stewardship Society | Stanley Creek, Hatchery Creek, Tiny Creek, Exeter Creek | \checkmark | \checkmark |
| Mill Bay | Mill Bay & District Conservation Society | Lower Shawnigan Creek | | \checkmark |
| Shawnigan Lake | Shawnigan Basin Society | Upper Shawnigan Creek, West Arm Creek, McGee Creek | | \checkmark |
| | Goldstream Volunteer Salmonid Enhancement Society | Goldstream River | \checkmark | \checkmark |
| | Friends of Bowker Creek | Bowker Creek | | \checkmark |
| Victoria | Esquimalt Lagoon Stewardship Initiative | Colwood Creek | | \checkmark |
| | Gorge Waterway Action Society | Gorge Creek | | \checkmark |
| | PKOLS - Mount Douglas Conservancy | Douglas Creek | | \checkmark |
| | Peninsula Streams Society & Volunteers | Millstream Creek, Gabo Creek | | \checkmark |
| Port Alberni | Tseshaht First Nation | BCCF ARRC/VIU AERL is supporting Tseshaht's pre-existing sampling program | | \checkmark |

3.1.1 INFORMATION & ENGAGEMENT TOOLS

As a large portion of this program involved engagement with citizen scientists, BCCF ARRC/VIU AERL has prepared multiple documents to keep the participants informed and engaged:

A <u>project website</u> was generated to provide a up-to-date background and applicable information on the project. In general, the website gives an overview of the project's goals (short- and long-term), project team and contact information, descriptions of 6-PPDQ and TWT, sample analysis methods, nature-based solutions testing, information about each of the participating First Nations and stewardship groups, volunteer resources, a link to the interactive database, information about the annual workshop, and links to the most up-to-date news, project updates, and the bi-annual newsletter.

The <u>Volunteer Resources</u> page of the project website was created in order to compile all materials created for volunteers to be easily accessible. Training videos for how to download the data collection app and form, along with a document outlining frequently asked questions about the app and sample collection can be found there. Additionally, training videos walking participants through each type of sample that may be collected and the SOP are stored on the *Volunteer Resources* page.

The *News* tab on the project website has a few different types of news. First, the page titled <u>*TWT in the Media*</u> has a long list of news articles that make mention of 6-PPDQ and TWT that have been published since July 2023. The page titled <u>*Project Updates*</u> is still currently under construction but will be a place where interested parties will be able to receive updates on the BCCF ARRC/VIU AERL specific project. The last news related page is titled <u>*Bi-Annual Newsletter*</u> and hosts links to the bi-annual newsletter that is released in August and February each year.

3.2 Wide Scale Surveillance Grab Sampling

The goal of the wide-scale surveillance grab sampling is to identify major TWT sources along ECVI and suitable sites for increased spatial and temporal sampling efforts. All data is for research purposes only and should be verified where consequential for any third-party use. The project team does not warrant in any way that the data will meet end user's requirements, or that the data will be complete, uninterrupted, or error free. For use outside of this project scope (for research or publishing purposes), please contact the project team. All graphs displayed in this report were prepared by VIU AERL.

3.2.1 GRAB SAMPLING

A total of 1,922 grab samples were collected from over six rain events between September 2023 and March 2024. These samples cover 56 waterways and 123 sample sites located between Campbell River and Victoria, expanding as far west as Lake Cowichan. Appendix B shows results from all samples collected from all locations between September 2023 and March 2024.

Several trends were found to be consistent across the majority of sample locations, with a few sites showing different responses to roadway/stormwater runoff.

3.2.1.1 Peak Concentrations

It was most common that the highest concentrations of 6-PPDQ in creek samples were measured in samples collected 'during' a rain event, with very few having 6-PPDQ detected in the 'before' rain samples. The 'after' rain samples were primarily muted compared to the 'during' samples, having reduced in concentration over the approximate 24 hour period since the 'during' sample was collected (see Figure 11 and Figure 12 as an example of this pattern).

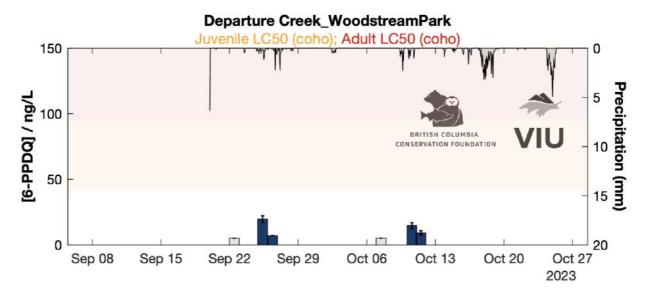


Figure 11. Sample results from samples collected at the Departure Creek at Woodstream Park site in September and October 2023. The 6-PPDQ concentration is expressed along the left axis, while the precipitation from the nearest weather station is found on the right access and runs opposite (top down). Empty bars (black outline, no fill) represent a grab sample that was analyzed, but no 6-PPDQ was detected. The yellow shaded portion of the graph, beginning at 41 ng/L, represents the currently accepted LC₅₀ of juvenile (alevin) coho salmon. The red shaded area of the graph, beginning at 95 ng/L, represents the currently accepted LC₅₀ of smolt and adult coho salmon.

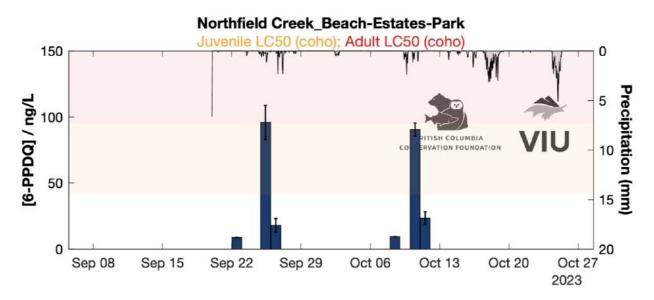


Figure 12. Sample results from samples collected at the Northfield Creek site directly downstream of Departure Bay Road in September and October 2023. The 6-PPDQ concentration is expressed along the left axis, while the precipitation from the nearest weather station is found on the right access and runs opposite (top down). The yellow shaded portion of the graph, beginning at 41 ng/L, represents the currently accepted LC₅₀ of juvenile (alevin) coho salmon. The red shaded area of the graph, beginning at 95 ng/L, represents the currently accepted LC₅₀ of smolt and adult coho salmon.

3.2.1.2 Seasonality

Another pattern consistent across most sites was seasonality, with the highest concentrations measured during the first fall flush, after a significant dry period. Once there was more consistent rain through the winter season, the concentrations observed were often lower and/or below the limit of detection. This effect may be due to a combination of factors, including greater tire wear particle loadings after extended dry periods, as well as less dilution due to lower water levels early in the rainy season. There was a slight increase in 6-PPDQ observed in the samples collected in late-January – early February; this likely due to longer dry periods experienced just prior to this sampling event, as depicted in the precipitation in both Figure 13 and Figure 14.

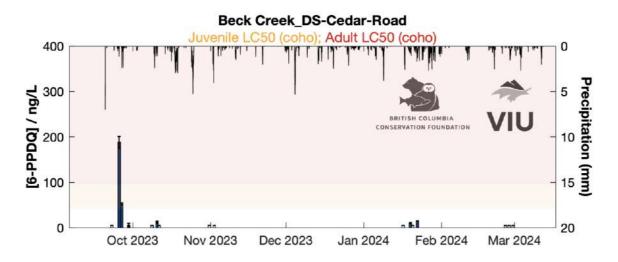


Figure 13. Sample results from samples collected at the Beck Creek site located downstream of Cedar Road between late September 2023 and March 2024. The 6-PPDQ concentration is expressed along the left axis, while the precipitation from the nearest weather station is found on the right access and runs opposite (top down). Empty bars (black outline, no fill) represent a grab sample that was analyzed, but no 6-PPDQ was detected. The yellow shaded portion of the graph, beginning at 41 ng/L, represents the currently accepted LC₅₀ of juvenile (alevin) coho salmon. The red shaded area of the graph, beginning at 95 ng/L, represents the currently accepted LC₅₀ of smolt and adult coho salmon.

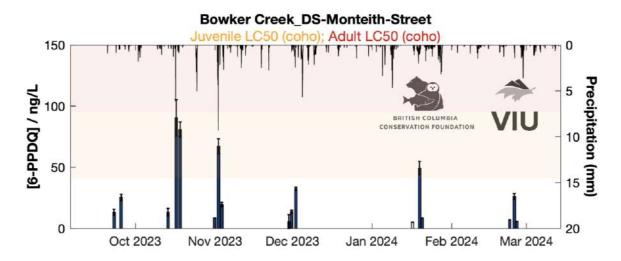


Figure 14. Sample results from samples collected at the Bowker Creek site located downstream of Monteith Street between late September 2023 and March 2024. The 6-PPDQ concentration is expressed along the left axis, while the precipitation from the nearest weather station is found on the right access and runs opposite (top down). Empty bars (black outline, no fill) represent a grab sample that was analyzed, but no 6-PPDQ was detected. The yellow shaded portion of the graph, beginning at 41 ng/L, represents the currently accepted LC₅₀ of juvenile (alevin) coho salmon. The red shaded area of the graph, beginning at 95 ng/L, represents the currently accepted LC₅₀ of smolt and adult coho salmon.

3.2.1.3 Urban Vs. Rural Systems

One final trend that was identified in year 1 was the variation in concentrations observed in urban environments compared to rural environments. Overall, urban environments had greater concentrations of 6-PPDQ measured than rural environments (Table 4); however, 6-PPDQ was still detected in small quantities at a few rural sample locations.

Urban areas (described as 'population centres' by Statistics Canada) are defined as having a population density of no fewer than 400 people per square kilometre and at least 1,000 residents (Statistics Canada, 2011). Population centres vary in size from 1,000 people to over a million; therefore, Statistics Canada (2011) has broken population centres down to provide a better way to describe the variation across urban areas (Table 3).

| Population Centre Grouping | Population Range |
|-------------------------------|------------------------|
| Large urban population centre | ≥ 100,000 people |
| Medium population centre | 30,000 – 99,999 people |
| Small population centre | 1,000 – 29,999 people |
| Rural population centre | < 1,000 people |

Table 3. Statistics Canada (2011) definitions for population centre type and size.

ECVI population centres vary from rural to large urban population centres (> 100,000 people; Statistics Canada, 2022). Larger population centres had a greater number of sample locations tested. In particular, Nanaimo had the greatest number of sample locations and samples collected (Table 4) as a result of having multiple BCCF ARRC/VIU AERL crews able to sample full days (8 hours) during each rain event. All areas sampled outside of Nanaimo were sampled by local First Nations and stewardship groups.

All sites exhibited some samples below the method limit of detection (6 ng/L). The three largest concentrations detected in 2023 – 2024 were 335.1 ng/L, 248.6 ng/L, and 220.9 ng/L, which were collected in Duncan, Colwood, and Nanaimo, respectively. The peak concentration measured in Colwood, a municipality apart of Greater Victoria, was from samples collected October 18, 2023 from Colwood Creek, downstream of Sooke Road; these samples were collected during the second rain event captured in the 2023 – 2024 sampling season. A small lake upstream of the sample location is required to fill prior to flow increasing at the sample location (S. McMillan, personal communication, Aug 31, 2023), which is likely why the peak was not observed in the first fall flush. The peak concentration measured in Nanaimo was from a sample collected in Northfield Creek, downstream of Departure Bay Road, on November 30,

2023 as part of the high interval sampling efforts (see *2.5.2 Increased Temporal Sampling* and *3.3.2 Increased Temporal Sampling*). The peak concentration measured in Fish Gut Alley, a tributary of the Cowichan River, located in Duncan, was collected March 11, 2024; this location was only added in February 2024, so only a few samples were collected to date. The Fish Gut Alley channel is vulnerable to a stormwater retention system that collects stormwater from a large part of Duncan. Two of the three locations that had the peak concentrations were from municipalites that are by definition 'small population centres'. Despite the smaller population sizes, Duncan and Colwood have some of the highest population densities along ECVI.

The population centres with the highest detection frequency were Oak Bay, Colwood, Esquimalt, Saanich, Nanaimo, and Courtenay (Table 4), which includes one large urban, one medium, and four small population centres. Overall, five of the six population centres with the highest detection frequency have population densities greater than 1,000 people per square kilometre. Globally, increased urbanization results in significant land cover changes, largely being covered in impervious surfaces (Hamilton *et al.*, 2021). Shifting land cover from natural, porous earth to impervious surfaces can result in increased stormwater runoff, which can increase concentrations of contaminants in local waterbodies, as well as degrade or destroy habitat as a result of high flow and scouring (Hamilton *et al.*, 2021).

Overall, 475 of the 1,434 creek samples collected before, during, and after rain events between September 2023 and March 2024 had 6-PPDQ detected (33.1% detection frequency). When summarizing all results for each type of population centre (Table 5), detection frequencies decreased by the size of the population centre; the highest detection frequency was in large urban population centres, followed by medium population centres, small population centres, and rural population centres.

| Pop. Centre Grouping | Pop. Centre Name | Pop.* | Pop. Density (pop/km²)* | Systems Sampled (#) | Sample Locations (#) | Samples Collected (#) | 6-PPDQ Detected Samples (#) | Detection Frequency (%) | Max Conc. (ng/L) |
|-------------------------|-----------------------|---------|----------------------------|---------------------------|----------------------------|-----------------------------|--------------------------------------|-------------------------------|------------------------|
| Large urban | Saanich | 117,735 | 1,136.6 | 2 | 2 | 30 | 23 | 76.7 | 95.5 |
| population centre | Nanaimo | 106,079 | 1,222.7 | 14 | 61 | 803 | 329 | 41.0 | 220.9 |
| N 4 a alivura | Comox | 72,445 | 877.7 | 2 | 5 | 41 | 10 | 24.4 | 15.5 |
| Medium | Courtenay | 48,917 | 876.7 | 1 | 5 | 29 | 9 | 31.0 | 24.5 |
| population | Langford | 46,584 | 1,124.4 | 2 | 2 | 25 | 5 | 20.0 | 22.1 |
| centre | Campbell River | 38,108 | 246.0 | 4 | 5 | 42 | 9 | 21.4 | 30.5 |
| | Parksville | 27,330 | 939.5 | 2 | 2 | 36 | 0 | 0.0 | 0 |
| | Duncan | 24,358 | 2,444.5 | 1 | 4 | 12 | 4 | 33.3 | 335.1 |
| | Colwood | 18,961 | 1,073.6 | 1 | 2 | 33 | 29 | 87.9 | 248.6 |
| | Oak Bay | 17,990 | 1,710.1 | 1 | 3 | 25 | 23 | 92.0 | 90.4 |
| | Esquimalt | 17,533 | 2,476.7 | 1 | 1 | 18 | 14 | 77.8 | 52.4 |
| Small | Qualicum Beach | 9,303 | 517.5 | 4 | 4 | 72 | 3 | 4.2 | 17.6 |
| population centre | Shawnigan-Mill Bay | 7,285 | 490.0 | 4 | 6 | 33 | 0 | 0.0 | 0 |
| | Nanoose Bay | 6,540 | 168.8 | 2 | 4 | 54 | 3 | 5.6 | 5.5 |
| | Chemainus | 4,033 | 693.0 | 2 | 2 | 12 | 0 | 0.0 | 0 |
| | Lake Cowichan | 3,325 | 403.5 | 4 | 4 | 54 | 5 | 9.3 | 11.9 |
| | Cowichan Bay | 2,799 | 626.5 | 3 | 3 | 9 | 1 | 11.1 | 10.8 |
| | French Creek | 1,107 | 69.1 | 1 | 1 | 14 | 0 | 0.0 | 0 |
| Rural | Black Creek | 2,080** | 29.0 | 1 | 2 | 24 | 6 | 25.0 | 12.1 |
| population centre | Fanny Bay | 921 | 124.5 | 4 | 4 | 60 | 0 | 0.0 | 0 |

Table 4. Overview of the number of creek samples collected in each population centre on ECVI, including the number of systems sampled, sample locations, samples collected, 6-PPDQ detection frequency, and maximum concentration measured in 2023 – 2024.

*Population values were obtained from Statistics Canada (2022) and represent 2021 populations, the last census.

**Population value is for the Black Creek – Oyster River (Unincorporated) area, not just Black Creek (Statistics Canada, 2022).

| Population Centre Grouping | Total Samples Collected (#) | Total Samples Where 6-PPDQ Detected (#) | Detection Frequency (%) |
|-------------------------------|--------------------------------|--|----------------------------|
| Large urban | 833 | 352 | 42.7 |
| Medium | 137 | 33 | 24.1 |
| Small | 380 | 84 | 22.1 |
| Rural | 84 | 6 | 7.1 |

Table 5. Summary of detection frequencies by population centre type for ECVI 6-PPDQ samplingconducted along ECVI between September 2023 and March 2024.

3.2.2 INTERACTIVE DATABASE

With an immense amount of data anticipated and the large majority of the sample regions being covered by other groups (First Nations, stewardship groups), it was important to find a means of sharing the data. Further, one of the long-term goals of this project is to share the data collected from it with all levels of government to ultimately assist with mitigation planning and justification of green infrastructure installation. As a result, an interactive database was constructed by Campbell Geospatial Consultants (CGC) with guidance from the BCCF ARRC/VIU AERL project team. This database was made public on April 25, 2024 and can be accessed <u>here</u>. The online interactive database has four tabs at the top of the page that can be utilized for different purposes.

Tire Wear Toxins, or the home screen, provides an overview of all data collected (Figure 15). The map, located in the centre of the page, shows all of the maximum 6-PPDQ concentrations measured throughout the study timeframe (September 2023 onward). There are two tabs below the map, so the user can toggle back and forth between the maximum and mean 6-PPDQ concentrations collected to date. A circle on the map represents a single sample location – the larger and darker shade of red the circle is, the greater the concentration detected at that location. Users can manually select a sample location or select a watercourse in the top right corner. Once a site is selected, the database shows all information collected at that site to date in a variety of ways: the 6-PPDQ concentrations measured at that site are broken down by date in the top left corner; the bottom left corner shows the 6-PPDQ concentration gradient and total number of samples collected at the selected site; a graph depicting all sample results (2023 – 2024) is shown on the top right corner of the screen; and the photos taken during sample collection are shown in the bottom right corner of the page.

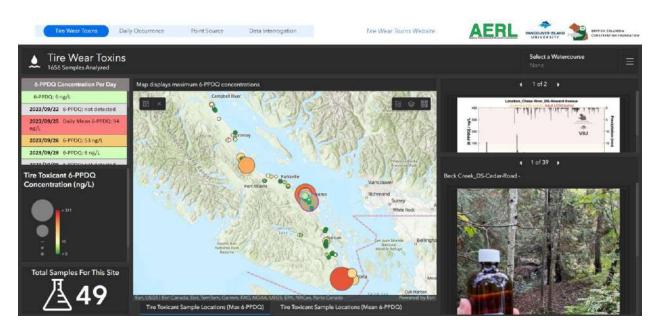


Figure 15. Screenshot of *Tire Wear Toxins* tab in the online interactive database created by CGC (April 2024).

The Daily Occurrence tab shows all samples collected on a given day, across the entire study area (Figure 16). The dates where sampling occurred are listed in the top right corner; the different shades of blue used in this list represent the quantity of precipitation that occurred each day, with darker shades indicating greater precipitation. When the user selects a date, the map populates with all sample data collected on that specific date. The top left corner shows the 6-PPDQ concentration gradient; the larger, darker symbols indicate higher concentrations. The total number of samples tested and samples collected on a specific date (once selected) appear on the left, below the concentration gradient. The user can select a specific location by clicking on the map. Once a site is selected, all of the data collected to date for that site, not just the date selected, is displayed on the graph that appears along the bottom of the page. Sliders at the top of the graph allow the user to zoom in on specific sample events for a closer look. The graph shows the LC₅₀ for juvenile coho (41 ng/L) as a dotted yellow line and the LC₅₀ for adult coho (95 ng/L) as a dotted red line to provide context for the numbers displayed. Precipitation is displayed along the x-axis of the graph; values are shown as negatives only to more easily visualize how much precipitation occurred at the time of sampling. Precipitation data is retrieved on a per-site basis from the nearest available weather station using a third party service (VisualCrossing.com). This data is stored locally and updated after each sampling event to provide precipitation data to the TWT dashboard.

| 1656 Samples Analyzed | | | | | | |
|---|---|--|---------------------------------------|---------------------------------|--|---|
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| uant 6-PPDQ ng/L | H K | S Nanality Town 1 | | - | | Number of Samples: |
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| | | \ | Start - | Hute | | 2023-09-26 |
| | | 74 | 1 A | (stan) | | Number of Samples: 41 |
| Samples on this date | | | • | | | 2023-09-27 Number of Samples: 2 |
| 88 | | | | ar (Se | | 2023-09-28 |
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Figure 16. Screenshot of the *Daily Occurrence* tab in the online interactive database created by CGC (April 2024).

The third tab, *Point Source*, is still under development. It will be laid out the same as the *Daily Occurrence* tab (Figure 17); however, it will only depict sample results collected from potential point sources (i.e., bridge decks, parking lots, stormwater drain, etc.).

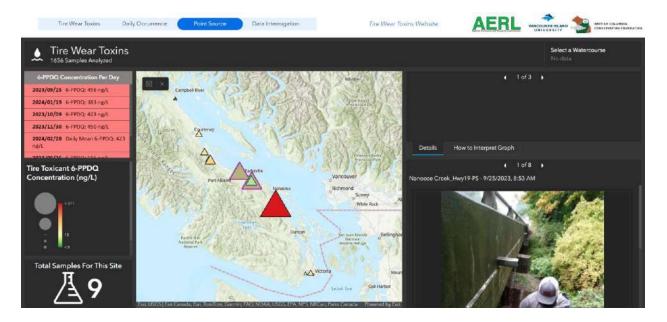


Figure 17. Screenshot of the *Point Source* tab in the online interactive database created by CGC. The final tab, *Data Interpretation*, was generated for users to compare results from different locations throughout the sample area (Figure 18). Along the left side of the screen there are

four windows which can each be populated with timeseries data for a specific stream, facilitating site- or stream-comparison. In the top right corner, the user can select which systems to access. Once selected, all sample locations associated with that watercourse are available in the drop down menu located at the top of each of the four windows. The user can then select a sample location from the drop down menu and all data collected in 2023 – 2024 appears in the window. Sliders are available at the top of each graph window to allow the user to manipulate the timeframe and compare data closer up from different rain events.

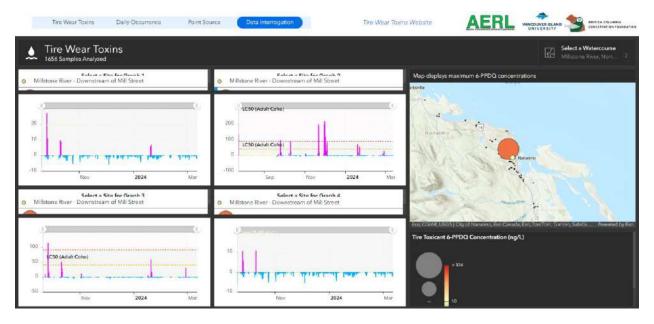


Figure 18. Screenshot of the *Data Interpretation* tab in the online interactive database created by CGC.

The online interactive database constructed by CGC is more desktop- and/or tablet-friendly, rather than mobile-friendly. Therefore, in addition to the desktop-based database, VIU AERL generated a <u>mobile version</u>; however, it does not have the same data interrogation abilities, but allows users to search throughout the study area and visualize sample results from each sample location (Figure 19).

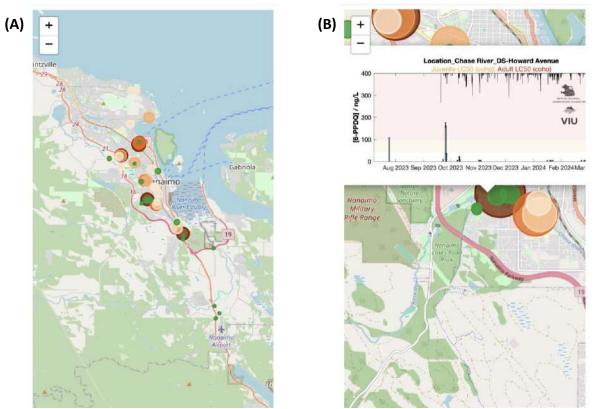


Figure 19. Screenshots of the mobile version of the online interactive database. Figure 17(A) shows an overview of the map that appears with the sample locations; the larger and darker the circle, the greater the 6-PPDQ concentration detected. Figure 17(B) shows a single site selected and the graph showing all data collected in 2023 – 2024 that pops up.

3.3 Increased TWT Monitoring Over Time & Space

As the toxin of concern, 6-PPDQ, was only identified in 2020, there are still many questions regarding the fate and distribution of 6-PPDQ in the natural environment. In order to get a grasp on the largest point sources in each system and how quickly 6-PPDQ flushes into and out of each system, increased spatial and temporal sampling were undertaken.

3.3.1 INCREASED SPATIAL SAMPLING

3.3.1.1 Increased Spatial Sampling Conducted by Partnered Organizations

In general, most locations outside of Nanaimo had one to two sample locations established to start; they were typically located either up- and/or downstream of the lowest potential point source input or up- and/or downstream of the assumed largest potential point source (i.e., highways, large storm drain). Once sampling was undertaken and it was shown that 6-PPDQ was detected, additional sites were added to assess which point sources may have the biggest influence.

In the 2023 – 2024 season, a total of six groups expanded their sampling efforts to include other reaches of their respective watersheds:

Brooklyn Creek Watershed Stewards (BCWS) initiated sampling on Brooklyn Creek late in the 2022 – 2023 pilot season up- and downstream of Balmoral Avenue, in Comox. BCWS continued sampling these two sites through the fall flush of the 2023 – 2024 sampling season. Upon finding 6-PPDQ was detected in small concentrations, two more sites were added upstream. The new sites, located up- and downstream of Pritchard Road, are to determine if there were higher concentrations upstream due to other point sources.

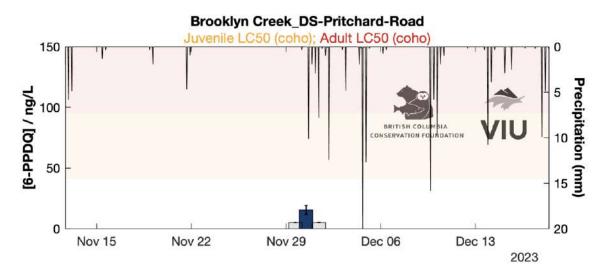


Figure 20. Sample results from samples collected at the Brooklyn Creek site located downstream of Pritchard Road in late November and early December 2023. The 6-PPDQ concentration is expressed along the left axis, while the precipitation from the nearest weather station is found on the right access and runs opposite (top down). Empty bars (black outline, no fill) represent a grab sample that was analyzed, but no 6-PPDQ was detected. The yellow shaded portion of the graph, beginning at 41 ng/L, represents the currently accepted LC₅₀ of juvenile (alevin) coho salmon. The red shaded area of the graph, beginning at 95 ng/L, represents the currently accepted LC₅₀ of smolt and adult coho salmon.

Millard-Piercy Watershed Stewards (MPWS) had begun sampling with the DFO 6-PPDQ monitoring program in 2022, following a coho smolt die-off in May 2021 at their smolt trap located downstream of the Comox Valley Parkway (H. Novak, Personal Communication, December 9, 2022). MPWS began collecting duplicate samples at their Piercy Creek sample locations in 2023; in total, five sites were sampled in 2023 – 2024. Sampling efforts at these sites follow DFO protocols and required weather conditions.

Walley Creek Streamkeepers (WCS) began sampling Walley Creek, downstream of Shores Drive, in the fall of 2023. Overall, 6-PPDQ was detected in three of the first four rain events at this site (Figure 21); all concentrations were relatively consistent, measuring between 0 ng/L (not detected) to 10.6 ng/L, below the LC₅₀ for juvenile coho salmon in the during and after samples collected. Similarly to BCWS, WCS were curious if there were any larger upstream inputs; therefore, a second site was added downstream of McGuffie Drive in January 2024. Results in the first samples collected at the McGuffie Drive site in January 2024 indicated that there are larger point sources further upstream, as suspected. Concentrations at the new site measured between 0 ng/L (not detected) to 22.5 ng/L, still below the LC₅₀ for juvenile coho (Figure 22); however, it will be important to sample this location during the fall flush in 2024 to get a better understanding of potential peak 6-PPDQ exposure.

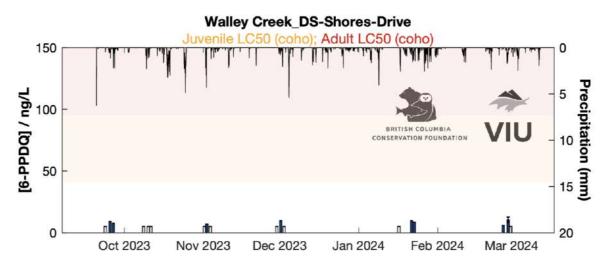


Figure 21. Sample results from samples collected at the Walley Creek site located downstream of Shores Drive between late September 2023 and March 2024. The 6-PPDQ concentration is expressed along the left axis, while the precipitation from the nearest weather station is found on the right access and runs opposite (top down). Empty bars (black outline, no fill) represent a grab sample that was analyzed, but no 6-PPDQ was detected. The yellow shaded portion of the graph, beginning at 41 ng/L, represents the currently accepted LC_{50} of juvenile (alevin) coho salmon. The red shaded area of the graph, beginning at 95 ng/L, represents the currently accepted LC_{50} of smolt and adult coho salmon.

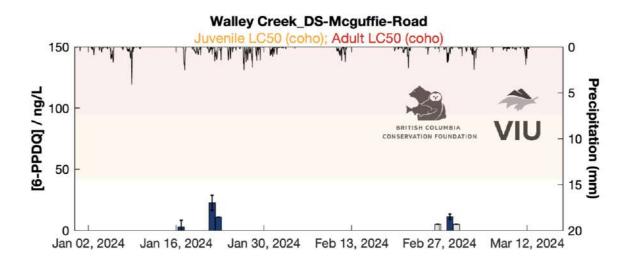


Figure 22. Sample results from samples collected at the Walley Creek site located downstream of McGuffie Road between late September 2023 and March 2024. The 6-PPDQ concentration is expressed along the left axis, while the precipitation from the nearest weather station is found on the right access and runs opposite (top down). Empty bars (black outline, no fill) represent a grab sample that was analyzed, but no 6-PPDQ was detected. The yellow shaded portion of the graph, beginning at 41 ng/L, represents the currently accepted LC₅₀ of juvenile (alevin) coho salmon. The red shaded area of the graph, beginning at 95 ng/L, represents the currently accepted LC₅₀ of smolt and adult coho salmon.

Cowichan Tribes has been sampling Fish Gut Alley, a tributary to the Cowichan River. This tributary is susceptible to high inflows of stormwater runoff, as the stormwater collection tank for a large portion of Duncan overflows directly into Fish Gut Alley. There have been reported fish kills in the tributary, but none have been directly correlated to 6-PPDQ, as most occurred prior to knowledge of the toxin's impacts (T. Kulchyski, Personal Communication, September 2023). Monitoring of this tributary has also occurred through the DFO sampling program. Based on those sample results, additional sites were added upstream and downstream of the stormwater collection tank outfall to better gauge the reach at which this stormwater runoff is impacting the tributary. Regular monitoring of Fish Gut Alley within this sampling program began in March 2024. A sample collected directly in front of the stormwater collection tank overflow on March 11, 2024 was 335.1 ng/L, the greatest concentrations of any measured creek samples in the 2023 – 2024 sampling season.

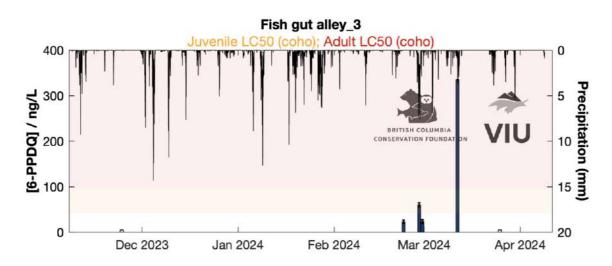


Figure 23. Sample results from samples collected at the Fish Gut Alley site located directly downstream of the stormwater collection tank outflow between December 2023 and March 2024. The 6-PPDQ concentration is expressed along the left axis, while the precipitation from the nearest weather station is found on the right access and runs opposite (top down). Empty bars (black outline, no fill) represent a grab sample that was analyzed, but no 6-PPDQ was detected. The yellow shaded portion of the graph, beginning at 41 ng/L, represents the currently accepted LC₅₀ of juvenile (alevin) coho salmon. The red shaded area of the graph, beginning at 95 ng/L, represents the currently accepted LC₅₀ of smolt and adult coho salmon.

Volunteers associated with the Nanaimo Area Land Trust (NALT) have been monitoring water quality of the Cat Stream as part of the RDN DWWP Program's Community Watershed Monitoring Program (RDN, 2024). As a result of previous water quality monitoring efforts conducted by NALT and preliminary 6-PPDQ monitoring efforts on the Cat Stream taken by BCCF ARRC/VIU AERL, NALT started monitoring two locations in fall 2023.

Finally, the Friends of Bowker Creek (FBC) began monitoring for 6-PPDQ at one location, downstream of Monteith Street, in late September 2023. The peak concentration measured at this site was 90.4 ng/L on October 16, 2023 (Figure 24), which is greater than the LC_{50} for juvenile coho (41 ng/L) and within range for LC_{50} of coho smolts to adults (80 to 95 ng/L). A combination of the sample results from the Monteith Street site and the growing concerns over the artificial turf at Oak Bay High School (Marlow, 2023), located upstream of the Monteith Street site, discussions began with FBC about adding additional monitoring sites to better gauge the artificial turf as a potential point source. Two new sites were added in late February, one just downstream of Bee Street (upstream of the artificial turf field) and one at Oak Bay High School (downstream of the artificial turf field). Since establishing the new sites, only one rain event has been captured. The site downstream of Bee Street had concentrations between 6.2



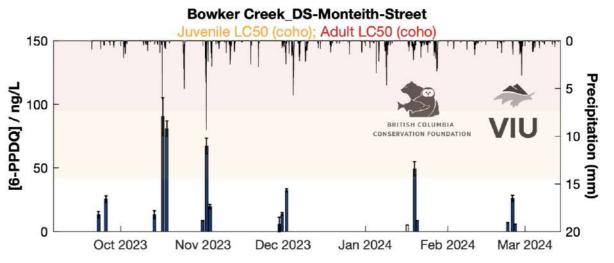


Figure 24. Sample results from samples collected at the Bowker Creek site located downstream of Monteith Street between late September 2023 and March 2024. The 6-PPDQ concentration is expressed along the left axis, while the precipitation from the nearest weather station is found on the right access and runs opposite (top down). Empty bars (black outline, no fill) represent a grab sample that was analyzed, but no 6-PPDQ was detected. The yellow shaded portion of the graph, beginning at 41 ng/L, represents the currently accepted LC₅₀ of juvenile (alevin) coho salmon. The red shaded area of the graph, beginning at 95 ng/L, represents the currently accepted LC₅₀ of smolt and adult coho salmon.

3.3.1.2 Increased Spatial Sampling Conducted by BCCF ARRC/VIU AERL

Preliminary 6-PPDQ sampling that was conducted in the 2022 – 2023 sample season and summer 2023 directed and advised the spatial sampling that BCCF ARRC/VIU AERL conducted in the 2023 – 2024 sampling season. Typically, systems had one sample location to start to see if 6-PPDQ was detected; however, in urban systems there were some cases when multiple locations were added initially, as results indicated that urban systems were more likely to have 6-PPDQ concentrations detected.

Systems that currently only have one or two locations (up and/or downstream of a point source) include Bonnell Creek, Knarston Creek, Haslam Creek, Harewood Creek, Nanaimo River, and four tributaries that flow into Long Lake in Nanaimo. In total, there are 12 sites across these six systems.

Systems that have had increased spatial sampling include Bloods Creek (four sites), Nanoose Creek (three sites), Millstone River (10 sites), Northfield Creek (four sites), Cat Stream (11 sites),

Chase River (10 sites), Beck Creek (four sites), and Wexford Creek (six sites). In total, there are 48 sites across these eight systems.

The Cat Stream is one of the first systems in Nanaimo that spatial sampling was conducted on. Results indicated that there was variability in 6-PPDQ concentrations measured between sites, with indications that the greatest point source was located near Wakesiah Avenue, the furthest upstream location site sampled (Figure 25). This trend was seen during three of the four precipitation events that were captured; the event in November had the lowest concentrations measured and were all approximately the same value, except for the site upstream of Howard Avenue, where no 6-PPDQ was detected.

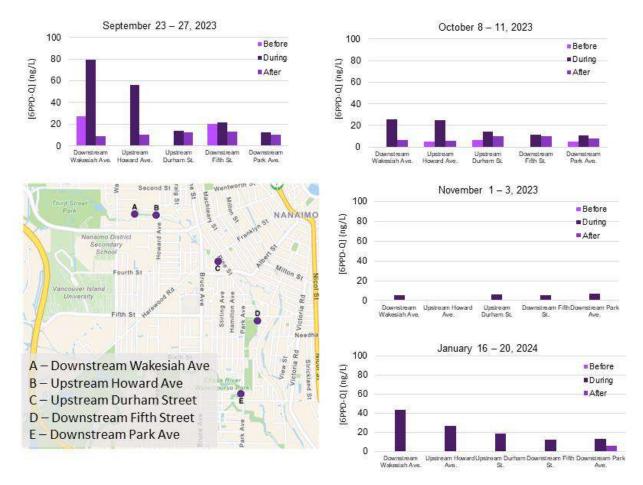


Figure 25. Spatial sampling results at five of 11 established sampling locations on the Cat Stream in Nanaimo, BC for four precipitation events occurring between September 23, 2023 and January 20, 2024; the first three events captured rain, while the fourth was collected during snow melt. Sample locations identified on the map, labelled A – E, are listed moving in a downstream direction. All graphs depict locations with the furthest upstream location on the left, moving progressively further downstream as sites are listed across the x axis (left to right). Graphs prepared by Angelina Jaeger, VIU AERL.

Millstone River was another system in Nanaimo that had significant spatial monitoring conducted (Figure 26). Currently, 10 sample locations are established throughout the watershed, with two of the furthest upstream locations, up- and downstream of Jingle Pot Road, were only added in March 2024.

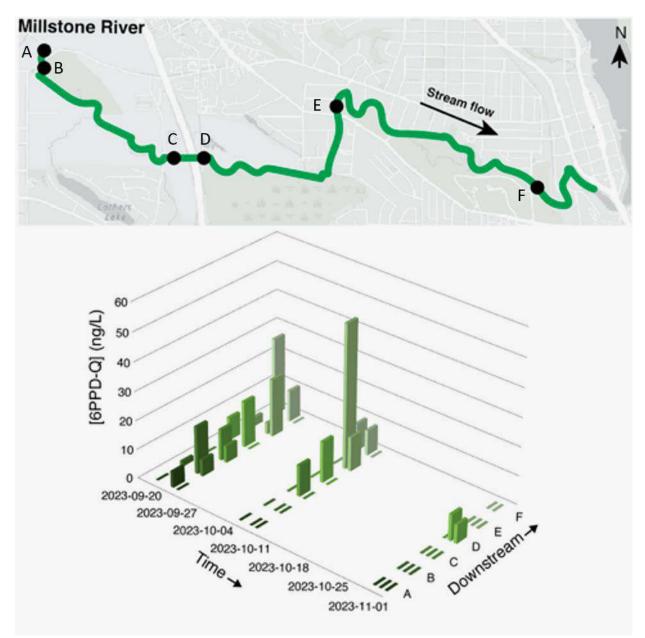


Figure 26. Spatial sampling results at six of 10 established sampling locations on the Millstone River in Nanaimo, BC for four rain events occurring between September 22 and November 3, 2023. Sample locations identified on the map, labelled A – F, are listed moving in a downstream direction and into a progressively more urban setting. All graphs depict locations with the furthest upstream location on the left, moving progressively further downstream as sites are listed across the x axis (left to right). Graphs prepared by Angelina Jaeger, VIU AERL.

In general, the Millstone River sample sites further downstream, located in the more urban setting, had greater 6-PPDQ concentrations measured, with the greatest measurement occurring downstream of Bowen Road (site E; Figure 26); the peak concentration observed at this site was 50.3 ng/L on October 10, 2023. As sampling progressed through the season, sampling results typically had little to no detection of 6-PPDQ at all sample locations along Millstone River, likely due to increased flows in the system.

3.3.2 INCREASED TEMPORAL SAMPLING

One of the largest challenges the project team has faced with grab samples is understanding the most ideal time to collect 'during' rain samples. It was anticipated that 6-PPDQ concentrations are variable and dynamic as stormwater runoff flushes into a stream. It may be that the highest concentration of 6-PPDQ would occur shortly after runoff begins, as it would be the first flush of tire particles off the road surfaces. However, certain streams may show extended 'lag' times from the onset of rain to peak 6-PPDQ levels due to riparian zones, watershed composition, etc. Previous work on the Don River in Toronto exhibited peak 6-PPDQ concentrations 14 to 35 hours after the onset of rain, well-after the peak discharge rate (Johannessen, 2022). To assess these hypotheses, the project team increased sampling efforts and frequency in select streams known to exhibit detectable 6-PPDQ. Two increased temporal sampling efforts were undertaken in the 2023 – 2024 sampling season.

3.3.2.1 Increased Temporal Sampling in a Single System

The first increased temporal sampling effort was conducted at Northfield Creek, downstream of Departure Bay Road. This site was selected as grab sample results indicated 6-PPDQ was detected in every during sample that was collected previously. The first effort included the collection of 29 samples, one collected every one to two hours (Figure 27). Two-hour increments were used at the start and end of the sampling efforts; while raining, sampling occurred every hour. The peak concentration observed was 200 ng/L and occurred approximately three hours after the rain began, but only about one hour after the rain intensity increased and stormwater runoff began. The concentration increased quickly, essentially mirroring the increase and decrease in precipitation. 6-PPDQ concentrations remained above the LC_{50} for adult coho for approximately five hours.

In an effort to assess sample-to-sample variability, back to back samples were collected at 18:00, 19:00 (Nov 21st), and 01:00 (Nov 22nd). While the 01:00 samples exhibit good relative agreement, the two replicate sets exhibited considerably wider variance. These samples were collected minutes apart (e.g. 18:00, 18:04, and 18:05 for the 18:00 samples). This higher

variance suggests rapid changes in 6-PPDQ concentrations over the course of minutes during peak rainfall, with an overall shift of 20.1 ng/L over this five minute period. This variance, along with the hourly sampling indicated that concentrations of 6-PPDQ are dynamic and the timing of sampling makes a significant difference.

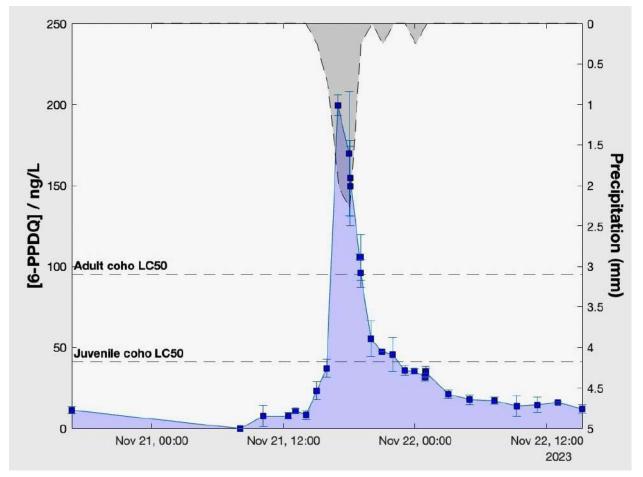


Figure 27. Results of increased temporal sampling at Northfield Creek, downstream of Departure Bay Road, on November 21 and 22, 2023. The 6-PPDQ concentration is expressed along the left axis, while the precipitation from the nearest weather station is found on the right access and runs opposite (top down). The currently accepted LC₅₀ of juvenile (alevin) coho (41 ng/L) and smolt/adult coho (95 ng/L) are expressed as dotted lines, with values indicated on the left axis. Graph prepared by Angelina Jaeger and Joseph Monaghan, VIU AERL.

3.3.2.2 Increased Temporal Sampling in Three Systems

As the first increased temporal sampling indicated that timing is important, the project team then wanted to assess variability between systems and if they all react similarly or not. To assess this, three sample locations were selected based on previous grab sample results, selecting for those sites that have seen 6-PPDQ in previous grab samples. The three sites included Northfield Creek, downstream of Departure Bay Road; Millstone River, downstream of Bowen Road; and Cat Stream, upstream of Howard Avenue.

Over a one-week period, 244 samples were collected from these three streams across several small rain events (two to five millimetres) and a large rain event (25 mm) between November 29 and December 5, 2023. Samples were collected every one to six hours throughout the rain event (i.e., before, during, after). The high sample throughput method that is used, enabled the project team to get results within hours of sample collection, allowing crews to adapt the sampling frequency to ensure that the full in-stream pulse of 6-PPDQ was captured.

During sampling efforts, the largest waterway, Millstone River, showed little to no detectable 6-PPDQ (Figure 28). However, concentrations rose above adult and juvenile coho salmon LC₅₀s during several of the early smaller rain events for the two smaller systems sampled (i.e., Northfield Creek and Cat Stream; Figure 29 and Figure 30). The concentrations were observed to be very dynamic, emphasizing how dependent concentrations are on collection time.

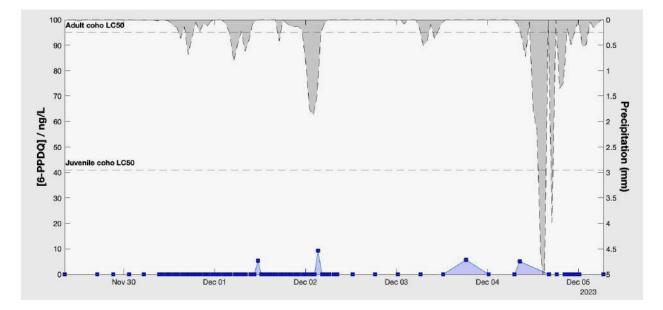


Figure 28. Results of increased temporal sampling at Millstone River, downstream of Bowen Road, between November 29 and December 5, 2023. The 6-PPDQ concentration is expressed along the left axis, while the precipitation from the nearest weather station is found on the right access and runs opposite (top down). The currently accepted LC₅₀ of juvenile (alevin) coho (41 ng/L) and smolt/adult coho (95 ng/L) are expressed as dotted lines, with values indicated on the left axis.

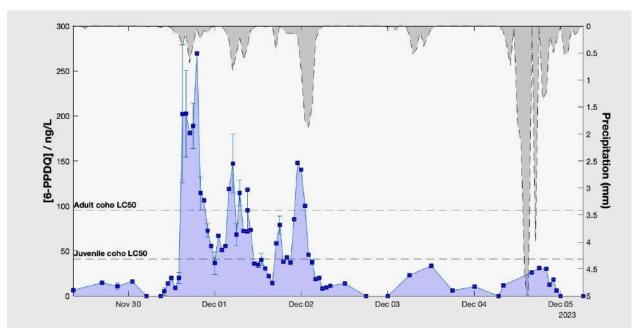


Figure 29. Results of increased temporal sampling at Northfield Creek, downstream of Departure Bay Road, between November 29 and December 5, 2023. The 6-PPDQ concentration is expressed along the left axis, while the precipitation from the nearest weather station is found on the right access and runs opposite (top down). The currently accepted LC_{50} of juvenile (alevin) coho (41 ng/L) and smolt/adult coho (95 ng/L) are expressed as dotted lines, with values indicated on the left axis.

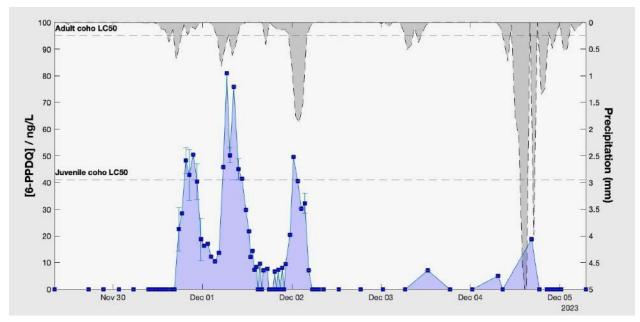


Figure 30. Results of increased temporal sampling at Cat Stream, upstream of Howard Avenue, between November 29 and December 5, 2023. The 6-PPDQ concentration is expressed along the left axis, while the precipitation from the nearest weather station is found on the right access and runs opposite (top down). The currently accepted LC₅₀ of juvenile (alevin) coho (41

ng/L) and smolt/adult coho (95 ng/L) are expressed as dotted lines, with values indicated on the left axis.

Overall, there was variation in how quickly different waterways reacted to the onset of rain. Northfield Creek showed changes immediately after the rain started, while 6-PPDQ concentrations in the Cat Stream peaked several hours after the onset of rain.

These types of experiments are helping build a foundation of understanding where, when, and how long 6-PPDQ may be found in local waterways.

3.3.3 AUTOSAMPLERS

In order to better understand how dynamic 6-PPDQ is within a system, BCCF ARRC/VIU AERL had proposed to install autosamplers in at least four river systems; sites were to be selected based on results from the grab sampling (i.e., selecting sites where 6-PPDQ is consistently detected). The intent is that the samplers would be installed, loaded with bottles, and then triggered just prior to a rain event. Originally, the plan was to have autosamplers collect one sample every two hours over a 48 hour timeframe. However, based on the high interval grab sampling completed in late-November to early December 2023, results indicated that two-hour intervals may not be enough.

The largest challenge faced by the project team was not the timeframe for sampling, but the materials of which commercial autosamplers and the associated bottles used are made of: plastic and silicone rubber. Unfortunately, 6-PPDQ adheres very well to these materials (Hu *et al.*, 2023), therefore, it is a not a viable option for sampling. Further, commercial autosampler systems leave sample bottles uncapped after sample collection. Efforts during our pilot year revealed that 6-PPDQ exhibits significant atmospheric sensitivity, with aqueous concentrations halving every ~50 hours at 20 °C (Monaghan, 2023). As a result, the VIU AERL team has been working to design new autosamplers or adapt commercial systems with materials conducive to sampling for 6-PPDQ, including sealable-glass containers.

In anticipation of the autosamplers being utilized in year 2, the project team looked at identifying potential locations for autosamplers to be installed. In particular, criteria for potential autosampler locations includes: safe access, in a reach of a system that regularly has 6-PPDQ detected during rain events throughout the year and limited risk of vandalism. In total, 19 locations were identified (Figure 31); these sites are located on 16 different systems and are located between Campbell River and Victoria.

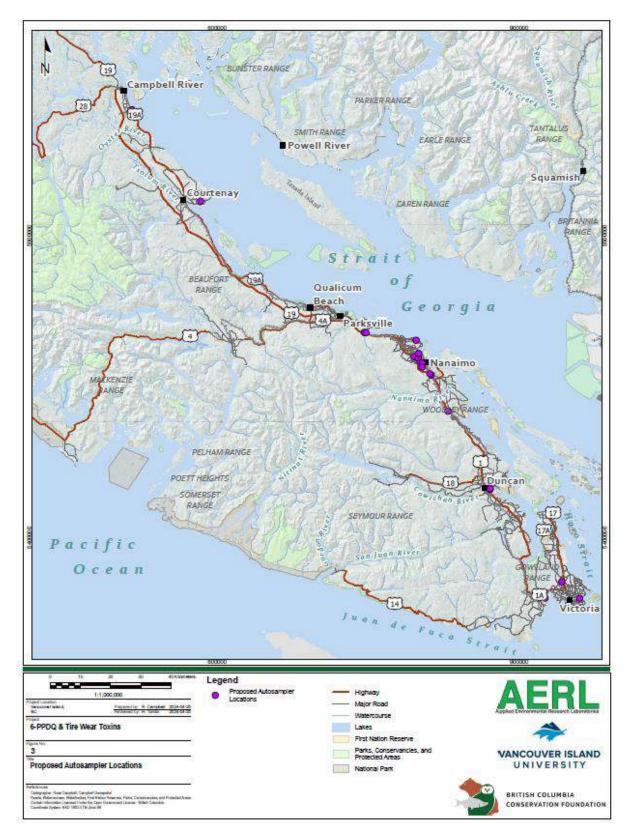


Figure 31. Identified potential locations for autosamplers to be deployed in year 2 (2025 – 2026). Map was created by CGC (April 2024).

3.3.4 MOBILE MASS SPECTROMETRY LAB

To further build on the temporal monitoring of 6-PPDQ's concentration dynamics through a rain event, the project team is working towards deploying VIU AERL's state-of-the-art mobile lab with a MIMS based mass spectrometer system, which would allow for samples to be collected and measured *in situ*, continuously. Employing this method will allow for real-time continuous data collection, rather than intermittent grab sample measurements. This will be an important activity, as the data will be useful for assessing the duration of exposure to acute toxins and for informing appropriate mitigation strategies.

It is currently planned for the mobile lab to be utilized in year 2 (2024 – 2025), therefore the project team created a list of potential locations that would be suitable to park and run the mobile lab. Criteria for a potential mobile lab location(s) includes close proximity to a system that regularly has 6-PPDQ detected during rain events throughout the year, access to two separate plugs (110V, 15 amp) on separate circuits, space to set up a tent for some dry area outside of the van, and (if possible) access to some running water/outside tap for rinsing gear. To assess an entire rain event, the project team would likely be on site 24-hours a day for two to four days (depending on the rain event); therefore, preference for access to shelter and washrooms is a bonus. In total, 12 locations, across 11 systems, were identified as potential sites that may have access to the required site facilities and are adjacent the system of interest (Figure 32).

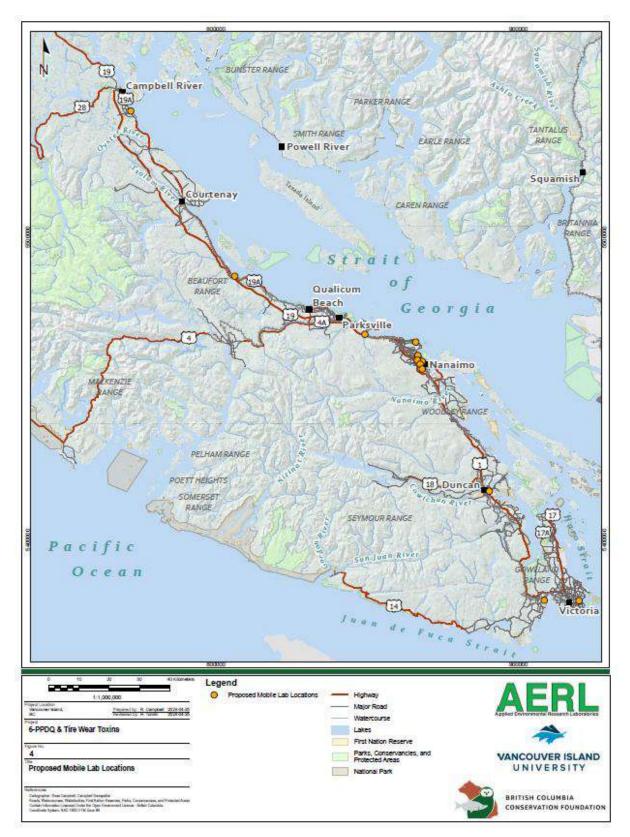


Figure 32. Identified potential locations for the mobile lab to be deployed in year 2 (2025 – 2026). Map was created by Campbell Geospatial Consultants (April 2024).

3.4 Evaluate Green Infrastructure & Nature-Based Solutions

One of the project's long-term goals is to implement project findings and begin installing effective green infrastructure in areas impacted by 6-PPDQ. As part of this program, the project team is undertaking two activities: monitoring existing green infrastructure and assessing potential nature-based solutions by building microcosm wetlands in the lab.

3.4.1 MONITORING EXISTING GREEN INFRASTRUCTURE

To begin, the BCCF ARRC/VIU AERL project team worked to identify locations with existing green infrastructure that could be sampled. In total, 25 locations were identified; these included existing sites and those that are currently being discussed and in the design phase (Figure 34). The green infrastructure monitored in year 1 of the program were located in Nanaimo and sampled by BCCF ARRC/VIU AERL.

Within Nanaimo only four locations were identified; of those four, three rain gardens are currently being planned for, while the other is an existing rain garden that runs adjacent to Boxwood Road and Dufferin Crescent, on both sides of the road. This rain garden has multiple stormwater drains from local parking lots that are directed towards the rain garden; unfortunately, all of the primary stormwater pipes that drain into it have a stormwater drain located directly below it (Figure 33). Therefore, the rain garden appears to only be functional in extremely large rain events, and there was only one rain event where collection from the rain garden itself was possible. Samples were collected directly from the culverts/spouts before pouring into the stormdrain located directly below them in March 2024.



Figure 33. Dufferin Crescent, Nanaimo, parking lot stormwater runoff directed into a rain garden; however, it pours into a stormwater drain rather than the rain garden.

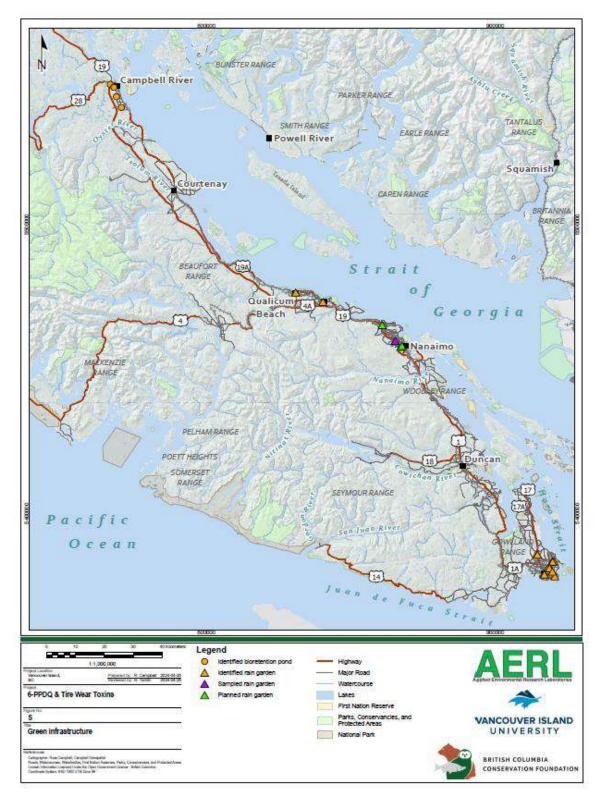


Figure 34. Identified green infrastructure that currently exists or is planned for. The rain garden that was sampled in the 2023 – 2024 sample season is identified on the map. Map was created by Campbell Geospatial Consultants (April 2024).

A challenge observed with regards to sampling rain gardens is finding a means to sample and determine how effective they are preforming; in particular, this is challenging for rain gardens that are built to slowly filter water, as they do not have an outflow to collect from. As a solution, BCCF ARRC/VIU AERL has begun conversations and meetings with the City of Nanaimo, as they are looking at building rain gardens locally. Additionally, conversations have been initiated with Peninsula Stream Society, an organization based in Victoria that is currently installing multiple rain gardens at local schools in their region. BCCF ARRC/VIU AERL is looking at finding opportunities to participate in the design and construction of rain gardens and other green infrastructure so that the team can build in potential means of testing the efficiency of these systems at removing 6-PPDQ.

Additionally, in year 2 (2024 – 2025), BCCF ARRC will begin discussing the opportunity to sample existing green infrastructure that was identified in their specific regions. Specific sampling strategies will be established for each site that is monitored by local First Nations and/or stewardship groups.

3.4.2 NATURE-BASED SOLUTIONS

UVic CWIL began developing the benchtop constructed wetland microcosms throughout year 1. Short-term objectives of the study are to: finalize microcosm design (flow rates, pumping strategies, materials) to ensure results can be predictive of real-world systems, evaluate the capacity of various wetland components (sand, sterile soils, non-sterile soils) against various positive- and negative- controls (e.g. charcoal, glass beads).

Year 1 had some minor setbacks, specifically with design and construction challenges for the microcosm column. Initially, the materials used were primarily comprised of plastic and silicone rubber, which as identified when discussing autosamplers, is not conducive to sampling for 6-PPDQ, as it readily adheres to these materials (Hu *et al.*, 2023).

To date, some preliminary samples have been submitted to VIU AERL for analysis. Further results of the study will be shared in the year 2 technical report.

4.0 DISCUSSION

As the lethal impacts of 6-PPDQ on salmonids ere only discovered in 2020, there are still many questions regarding the dynamics, fate, and distribution of the toxin in the environment. In

response to this, this monitoring program is working to assess some of these spatial and temporal-focused questions.

Through the involvement of local First Nation and stewardship groups across ECVI, a total of 1,922 samples were collected from 56 systems at 123 sites between September 2023 and March 2024. Due to the extensive sampling, the project team was able to identify some preliminary trends in the data, including but not limited to: peak concentrations most often occurred during the first one or two rainfalls of the season; at most locations the concentrations continued to decrease as the season progressed, unless there was a significant period of dry weather; and systems in larger urban population centres see greater concentrations of 6-PPDQ detected when compared to systems in smaller populations centres and rural environments.

Trends identified in year 1 may vary from trends in future sampling years. Precipitation patterns varied a bit from the previous years, with below-average (for the past five years) total precipitation occurring in September 2023 and above-average total precipitation in October 2023, January 2024, February 2024, and March 2024 (Figure 35). Further sampling will be required to determine if results are comparable between each year and total precipitation.

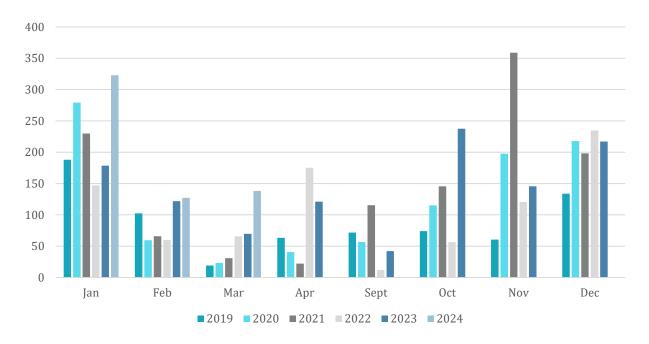


Figure 35. Total monthly precipitation for Nanaimo, BC from January 2019 through March 2024. Year 1 sampling season occurred from September 2023 to March 2024. Data obtained from Environment and Climate Change Canada (WeatherStats, 2024). Preliminary spatial sampling results indicate that the significant variability between systems and where increased concentrations occurs depends on the location of point sources (i.e., they may be further upstream in some locations and dissipate further downstream if significant point sources are not present). Additional sites up- and downstream of known point sources will need to be added and monitored during the fall flush to better understand which inputs have the greatest impact.

One of the largest findings from the first year is how dynamic 6-PPDQ is in a system. Even when multiple samples are collected within minutes of one another, there can be significant variability. To gain an understanding of the variability and capture the 'true' peak 6-PPDQ concentration, finer scale sampling will be required. BCCF ARRC/VIU AERL aims to implement other sampling techniques, including autosamplers and the mobile lab, in year 2 to collect a more detailed visual of how quickly 6-PPDQ is moving into and through a system.

Due to limited opportunities to collect from existing green infrastructure, the project team will work to expand sampling efforts outside Nanaimo and include some of the partnered First Nations and stewardship groups in this sampling. Additionally, BCCF ARRC/VIU AERL will continue to work with existing and new partners on taking part in the design and construction of green infrastructure locally, working to build in different ways for water samples to be collected throughout the infrastructure.

Significantly more sampling will need to be completed to get a full picture of which ECVI systems are most impacted by 6-PPDQ and which sites most need remediation efforts.

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The *Mitigating Inputs of Tire Wear Toxins to Protect Salmonid Habitat on Vancouver Island* program would not have been made possible without the partnership with VIU AERL and the local First Nation and stewardship groups that have been collecting water samples through the worst weather that Vancouver Island has to offer.

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APPENDICES

APPENDIX A - Standard Operating Procedures

Standard Operating Procedures:

6PPD-Quinone Sampling

Version 4

February 2024



BRITISH COLUMBIA CONSERVATION FOUNDATION





Overview

This standard operating procedure (SOP) is to guide the collection of water samples that are to be analyzed for 6PPD-quinone (6PPDQ) contaminants associated with tire particle leachates and roadway runoff. Samples will be processed by the Vancouver Island University's (VIU) Applied Environmental Research Laboratory (AERL) to determine the presence and concentration of 6PPDQ in aquatic habitat of vulnerable salmonid and trout species, including coho salmon (*Oncorhynchus kisutch*), rainbow trout (*O. mykiss*), and Chinook salmon (*O. tshawytscha*), along the Vancouver Island coastline.

This includes pre-trip preparations and field operations. This SOP is intended for use by staff, contractors, and volunteers with the British Columbia Conservation Foundation (BCCF).

Equipment List

Sample kit should include:

- Cooler
- Ice packs
- Nitrile gloves
- Pre-labelled (QR code) 250-mL amber glass bottles
- Thermometer
- Rain gauge
- Water quality equipment (sonde and turbidity metre) (optional)
- Phone or tablet equipped with the data collection app
- Datasheet (in the event that a phone/tablet fails)
- Pencil
- Sample pole (optional)

Preparation

Sample Kit

Amber glass bottles (250 mL) will be used to collect the water samples. **Ensure that the inside of the bottle and lid are** <u>not</u> **touched during handling.** Sample bottles will be prepped by BCCF/AERL prior to sampling activities; they will each include a label that has a distinct QR code.

Ensure that provided ice packs are frozen before heading to the sample location(s).



Figure 1. 6PPDQ sample collection kit

Cautionary note: Please ensure that sample kits and containers are **not** stored near car and/or bicycle tires before, during, or after sampling efforts. The close proximity could result in contamination of the samples. If there is potential of contamination, please include notes in the 'comments' section of the data collection app and/or notify BCCF via email (<u>htomlin@bccf.com</u>).

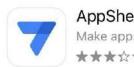
Pre-Trip

Determine the best and safest access location(s) for the sample location(s). Sites can be accessed by parking on the side of highways and roadways and hiking down to the selected waterway(s). Ensure that safety measures are followed and safety equipment is used when accessing the site(s).

Prior to leaving your home and a Wi-Fi connection, be sure to re-open the data collection app, allowing it to update/sync any changes, ensuring that your version is the most up-to-date.

Sample collection app

Prior to heading into the field, ensure that you have downloaded the data collection app onto your phone. The app can be downloaded from the App Store or the Google Play Store by searching for AppSheet.



AppSheet Make apps yourself. Code-free.

OPEN

Figure 2. Image of AppSheet icon and name, which can be downloaded from the App Store or the Google Play Store.

Once downloaded, you will need to make an account as this will allow for better offline performance. Once an account has been created, you can click this <u>link</u> or use the camera on your phone/tablet to scan the QR code below (Figure 3) and open the data collection form. When it opens in the browser, it should then redirect you to AppSheet. Once in AppSheet, select "Install", underneath the AERL logo. These steps should only be required once and the form required for sample collection should stay within AppSheet for all future use.



Figure 3. QR code to access the data collection form on AppSheet.

Prior to every sample date, it is best to re-sync the app to ensure you have the most up-to-date version.

All data that is submitted via the data collection app that was built specifically for this program, is linked to the sample ID and is recorded in a publicly available database (including any photos taken with the app). Once the sample is analyzed, the concentration of 6PPDQ is stored in a private Dropbox folder viewable only by project partners at VIU AERL and BCCF ARRC. The data is then vetted by the researchers and released to the publicly available interactive database.

Sampling Conditions & Effort

BCCF will monitor weather conditions and notify sampling teams when a sampling window is approaching. Samples will be collected at pre-selected locations, typically downstream of a potential 6PPDQ point source (i.e., roadway, bridge) before, during, and after the rain event.

Field Activities

Rain Gauge

Rain gauges will be utilized to capture site specific rainfall and compare those results to nearby weather stations to assess the variability in the region. Not all sample locations will be equip with a rain gauge and it'll be explained to each group which location should have a rain gauge deployed.

For those that are provided a rain gauge for their sample location(s), they will be deployed during the 'before' sample. To deploy the rain gauge, it can be pushed into the ground using the spike on the bottom. It should be placed in an open location with minimal tree cover above it (where possible). If possible, it should be tucked away from footpaths and busy areas to reduce the risk of disturbance/vandalism.

The rain gauge should be checked and the volume should be recorded (see Data Collection below) when on site for the 'during' and 'after' sampling efforts. Following the 'after' sample collection, the rain gauge should be removed and kept with the sampling kit, deployed during the next 'before' sample.

Data Collection

When you open the app, it will be displaying the AERL Map, which shows where all samples have been located to date.

In order to collect data for your sample, select the 'Submit sample' page, located in the bottom right of the screen (Figure 4).



Figure 4. Image depicts location of 'Submit sample' option in the data collection app.

Once on the appropriate screen, fill in the information requested by the data collection app. Each heading below will discuss the data field in more detail and what is required of the data collector. **One submission will be required for each sample collected.**

Samplers: Please record at least the first name of all those that contributed to collecting the sample; last names or initials can be provided, as well.

Sample ID: The sample ID refers to the sample label (QR code) on the bottle. Select the icon on the right side of the sample ID field (Figure 5) and 'allow' the app access to your camera (you should only need to do this the first time). Point your camera at the QR code on the bottle. It should recognize the QR code and auto-populate the field. In the event you accidently scan the wrong QR code (i.e., the wrong bottle), you can highlight what populated the field, delete it, and re-scan the correct bottle. In the event you scan the wrong type of QR code (i.e., the location code), the app will notify you that it is incorrect and you will need to then scan the correct QR code on the bottle.



Figure 5. Image depicting the icon to select in the sample ID field to pull up the phone/tablet camera and scan the bottle's QR code.

Sample Location: Sample locations were pre-determined by BCCF/VIU/project partners. For all sites that a specific group will be sampling, a set of laminated cards will be included in the sample kit. Each card includes the site name, Google Earth birds-eye view of the location, and a site-specific QR code (Figure 6). To fill in the sample location, select the site's corresponding card, click the icon on the right side of the sample location field and point your camera at the QR code on the card. It should recognize the QR code and auto-populate the field. In the event you scan the wrong type of QR code (i.e., a sample code), the app will notify you that it is incorrect and you will need to then scan the correct QR code on the location's card.

Millstone River – Downstream of Bowen Road

QR Code: Location_Millstone River_DS-Bowen-Road



Google Earth Image/Location:



General Directions/Site Description:

Turn off of Bowen Road into the Bowen Park West Field parking lot. Walk along the trail approximately 20-30 m and access the river on the left hand side of the trail.

Figure 6. Sample location card for one of the pre-selected 6PPDQ sample locations. The site card includes the site name, A Google Earth image with approximate location for reference, and a QR code.

Sample Type: Select the type of sample that you are collecting:

- **Before:** Stream sample is to be collected prior to the rain event; see 'Stream Sample Collection' below.
- **During:** Stream sample is to be collected during the rain event; see 'Stream Sample Collection' below.

- *After:* Stream sample is to be collected following the rain event; see 'Stream Sample Collection' below.
- **Field Blank:** Sample is to be collected during the rain event; see 'Field Blank Sample Collection' below.
- **Point Source:** Sample is to be collected during the rain event; see 'Point Source Sample Collection' below.
- **Other:** The sample details will be defined by BCCF/VIU prior to sampling, where necessary; see 'Other Sample Collection' below.

Fish Visible?: Look to see if any fish are present. Select yes, no, or unsure/NA based on your observations.

Rain Gauge: If a rain gauge was deployed at the site, record the volume present in the rain gauge when on site for the 'during' and 'after' samples. If no rain gauge is present, leave this field blank.

Date Time: This field should auto populate anytime the 'Sample ID' field is updated/changed. **Note:** Be sure to double check it to ensure it is correct.

Location: This field <u>should</u> auto populate anytime the 'Sample ID' field is updated/changed. **Note:** Be sure to double check where it shows you on the map to ensure it is accurate. If your location appears as [0.00000 0.0000], please re-open the app and select 'yes' when it asks for your location.

Site Photo: Select the camera icon and choose 'take a photo'. Take a photo directed upstream of the sample location. A site photo will not be required for field blank samples.

Additional WQ [optional]?: If you have the ability to collect water quality data, select yes and fill out the information that the equipment you have will provide. There is space for water temperature (°C), pH, dissolved oxygen (DO; in percentage), specific conductivity (μS/cm), and turbidity (NTU). This information is only applicable to stream samples, it is not required for the field blank and point source samples.

Comments [optional]: This is the space to add any specific comments or concerns with regards to the specific sample. There is no character limit, so if something went wrong when collecting the sample, please provide as much detail as you can.

Once all data is collected, select the 'Save' option in the bottom right of the screen. Once selected, a 'Submission Receipt' should open up (Figure 7). <u>Please take a screen shot</u> of the receipt and submit to the Google Drive that was shared with your group.

| ← AERL Sub | mission Receipt | C |
|---------------|-------------------|---------------|
| Fish Visible? | | |
| No | | |
| Sample ID | | |
| JMHT_BetaT | estSample_023 | |
| Sample Kit | | |
| HT - test | | |
| Sampler | | |
| HT - test | | |
| Sample Type | | |
| During | | |
| Data Time | | |
| 2023-7-17 2:4 | 40 PM | |
| Location | | |
| | Arrante Constants | |
| Site Photo | | |
| 1 | | |
| | ß | 8 |
| MAP | Sample Database | Submit sample |

Figure 7. Screenshot of the 'Submission Receipt' page on the data collection app.

Note: If you are collecting information with your phone's data off, you will need to submit the data when you acquire Wi-Fi. To do so, open the app and select the curled arrow in the top right corner. When there is data to upload, it will show the number of files that need to be uploaded in a small orange circle (Figure 8). Once the data is uploaded, the circle will disappear. **To ensure no data is lost, please be sure to take a screenshot of the 'Submission Receipt' page for reference.**

| AERL Submission Receipt | 0 |
|-------------------------|---|
|-------------------------|---|

Figure 8. Image depicting the curled arrow and number of files (1) that still require uploading. The icon will appear in the top right corner of the screen following data submission.

To collect another sample, just select the 'Submit sample' tab to begin filling out the next site's specific data.

Stream Sample Collection

Data collection required for stream samples includes: sample ID, sample location, sampler(s) names(s), sample type (before, during, after), fish visible, date time, location (GPS), comments [optional], a site photo, rain gauge [site specific], and additional water quality [optional].

Stream sample collection methods:

- Wearing nitrile gloves, open the amber glass bottle and ensure that the inside of the bottle and cap are <u>not</u> touched.
- 2. Use the water on site to rinse the bottle and lid three times.
- 3. With the cap removed, submerge the bottle upside down.
- 4. When the bottle is submerged, turn it into the flow and allow it to fill until it is full.
- 5. When full, secure the lid on the bottle and place in the cooler with the frozen ice packs.

Note: If in a location where safe access is an issue, use a sample pole/scoop to collect the sample. Ensure the sample pole/scoop is rinsed three times prior to using it to rinse the sample bottle and lid. Use the same technique for collection of submerging the scoop upside down and turning it into flow, as would be done with the sample bottle.

Field Blank Sample Collection

Data collection required for field blank samples includes: sample ID, sample location, sampler(s) name(s), sample type (field blank), date time, location (GPS), and comments [optional].

The purpose of collecting field blanks is to detect any onsite contamination that may be occurring during sample handling. Field blanks are simply samples of deionized water that are handled (i.e., collected, filtered, and preserved) in the same conditions that the stream and point source samples are. You will be provided with one 250 ml amber glass bottle full of deionized water for each site and each rain event. Field blanks are to be collected at the same time as the 'during' sample.

When opening and handling the amber glass bottles, ensure that the inside of the bottles and caps are <u>**not**</u> touched.

Field blank sample collection methods:

1. For each location you will be sampling, you will need to bring an empty, pre-labelled (QR coded) 250 ml amber glass bottle (the deionized water will be poured into on site).

- 2. When at the site, the person collecting the stream 'during' sample will do so first.
- 3. After the stream sample is collected (with the same nitrile gloves still on), open both field blank bottles (one empty and one full).
- 4. Pour the full bottle of deionized water into the empty one and recap both.
- When full, secure the lid on the bottle and place in the cooler with the frozen ice packs. Continue to store field blanks samples with the rest of the samples collected until sample pick-up.

Note: A new 'Submit sample' form needs to be filled out for each field blank sample collected at each site. Ensure you **scan the QR code on the empty** field blank bottle (i.e., the one you will pour your sample into and will be submitted to the lab).

Point Source Sample Collection

Data collection required for point source samples includes: sample ID, sample location, sampler(s) names(s), sample type (point source), date time, location (GPS), a site photo, additional water quality [specifically turbidity; optional], and comments [optional].

Point source samples are to be collected for known point sources at the pre-selected sample locations. Each point source will have its own location card in the sample kit; therefore, you will need to use the separate QR code for the point source location.

Point source sample collection methods:

- Wearing nitrile gloves, open the amber glass bottle and ensure that the inside of the bottle and cap are <u>not</u> touched.
- 2. Use the water flowing from the point source to rinse the bottle and lid three times.
- 3. After rinsing, fill the bottle until it is full.
- 4. When full, secure the lid on the bottle and place in the cooler with the frozen ice packs.

Note: A new 'Submit sample' form needs to be filled out for each point source sample collected at each site. Ensure that you <u>scan the QR code on the point source bottle and the specific</u> <u>point source location card</u> when submitting data.

"Other" Sample Collection

Data collection required for point source samples includes: sample ID, sample location, sampler(s) names(s), sample type (other), date time, location (GPS), comments [optional], a site photo, rain gauge [site specific], and water quality parameters.

"Other" samples will only be collected when requested/directed by the BCCF/VIU team. These samples may include routine samples (i.e., collected on a set schedule, regardless of rain events), when testing green infrastructure, etc.

"Other" sample collection will follow the same methods as all other sample collection, unless otherwise specified by the BCCF/VIU project team:

- Wearing nitrile gloves, open the amber glass bottle and ensure that the inside of the bottle and cap are <u>not</u> touched.
- 2. Use the water flowing from the identified "other" sample location to rinse the bottle and lid three times.
- 3. After rinsing, fill the bottle until it is full.
- 4. When full, secure the lid on the bottle and place in the cooler with the frozen ice packs.

Note: A new 'Submit sample' form needs to be filled out for each "other" sample collected at each site. Ensure that you scan the QR code on the bottle and the specific "other" location card when submitting data.

Sample Handling & Handoff

When returning home from sample collection, store water samples either in a cooler with frozen icepacks or in a fridge until handoff. Ensure that the samples **do not freeze**.

Following a rain event, all samples from each sample site will need to be handed off to BCCF. After completing sample collection, contact Haley Tomlin by email (<u>htomlin@bccf.com</u>) or phone (778-269-0749) to arrange for sample drop off or pick-up.

Once samples are collected, BCCF will hand them off to VIU's AERL for 6PPDQ analysis.

Data Results & Updates

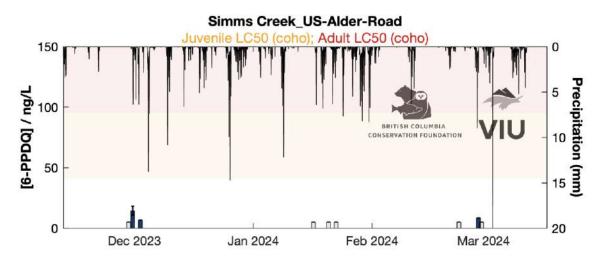
The BCCF and AERL project team anticipates that results will be available to volunteers within one to two weeks of sample pick-up. Results will be directly uploaded onto the sampling program's online GIS dashboard, which will be released winter 2023. A link will be provided to your group to access the dashboard. In addition to the online results database, the highlighted results will be shared bi-annually in a digital newsletter that will be sent to the entire group of project volunteers, partners, and funders. Further, there will be a yearly workshop where results will be shared and up-to-date research will be shared with volunteers, partners, and funders interested in attending.

APPENDIX B – Graphical Results for all Sample Locations 2023 – 2024

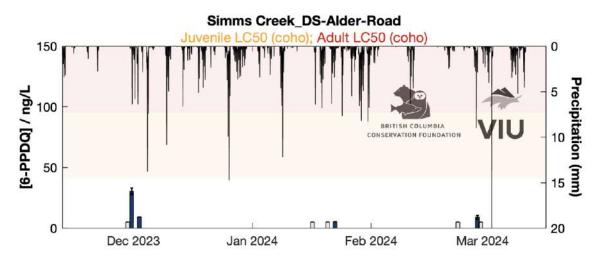
Sample sites will be subdivided by region of Vancouver Island for ease of navigating this appendix. For all graphs the following applies:

The 6-PPDQ concentration is expressed along the left axis, while the precipitation from the nearest weather station is found on the right access and runs opposite (top down). Empty bars (black outline, no fill) represent a grab sample that was analyzed, but no 6-PPDQ was detected. The yellow shaded portion of the graph, beginning at 41 ng/L, represents the currently accepted LC50 of juvenile (alevin) coho salmon. The red shaded area of the graph, beginning at 95 ng/L, represents the currently accepted LC50 of smolt and adult coho salmon. Graph prepared by Joseph Monaghan, VIU AERL.

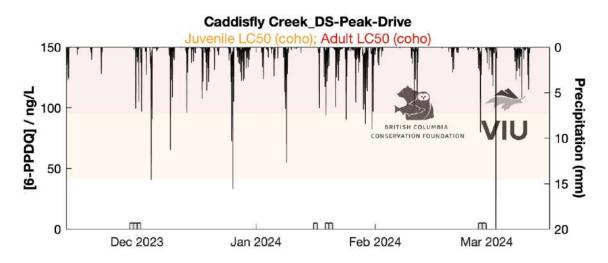
CAMPBELL RIVER



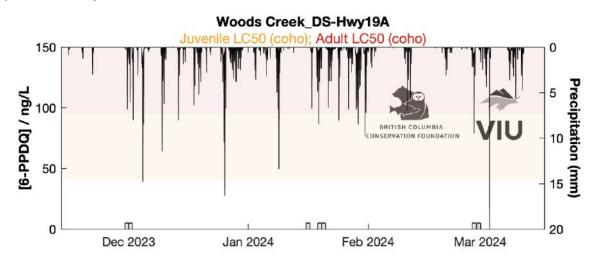
Graph 1. Simms Creek, upstream of Alder Road from December 2023 to March 2024.



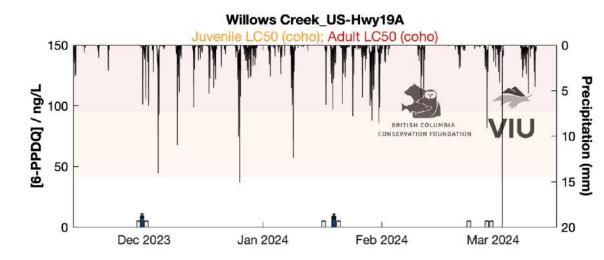
Graph 2. Simms Creek, downstream of Alder Road from December 2023 to March 2024.



Graph 3. Caddisfly Creek, downstream of Peak Drive from December to March 2024.

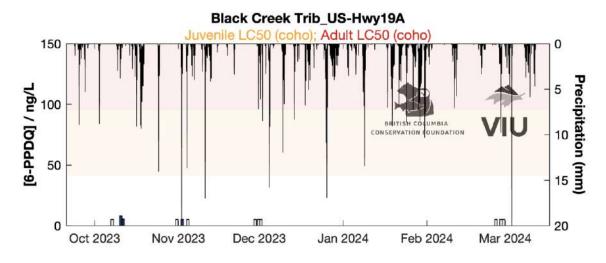


Graph 4. Woods Creek, downstream of Highway 19A from December to March 2024.

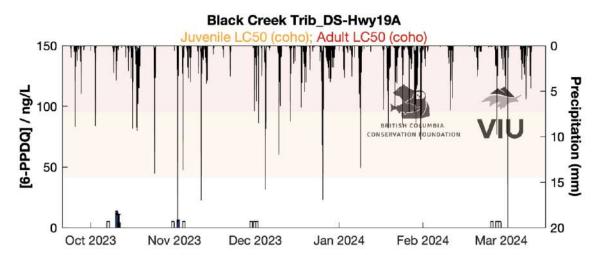


Graph 5. Willow Creek, upstream of Highway 19A from December to March 2024.

BLACK CREEK

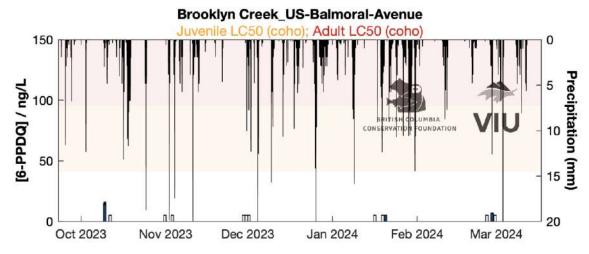


Graph 6. Black Creek tributary, upstream of Highway 19A from October 2023 to March 2024.

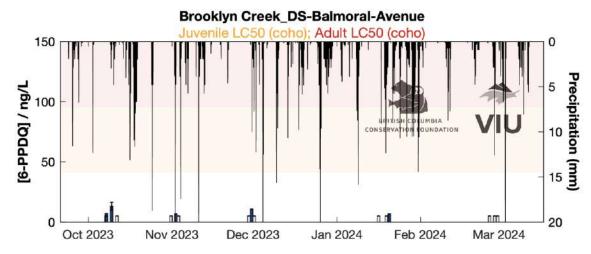


Graph 7. Black Creek tributary, downstream of Highway 19A from October 2023 to March 2024.

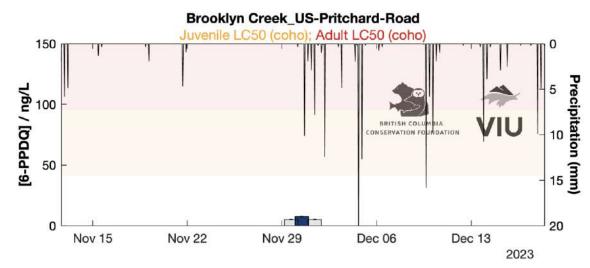
COMOX



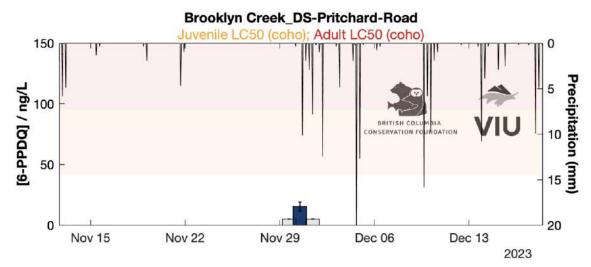
Graph 8. Brooklyn Creek, upstream of Balmoral Avenue from October 2023 to March 2024.



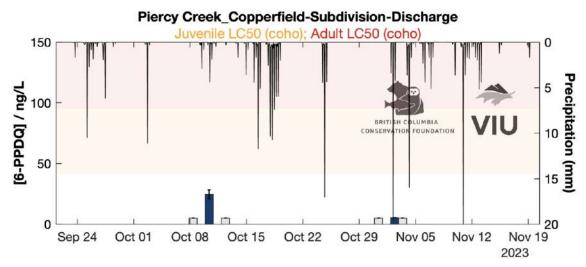
Graph 9. Brooklyn Creek, downstream of Balmoral Avenue from October 2023 to March 2024.



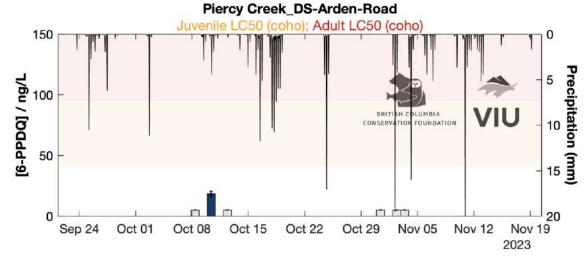
Graph 10. Brooklyn Creek, upstream of Pritchard Road in late-November 2023.



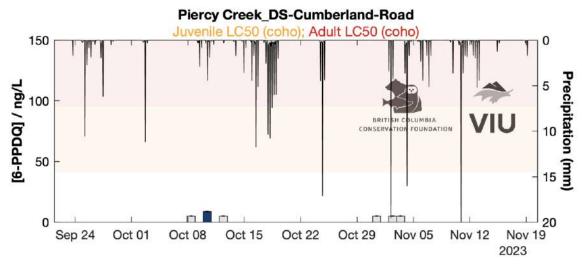
Graph 11. Brooklyn Creek, downstream of Pritchard Road in late-November 2023.



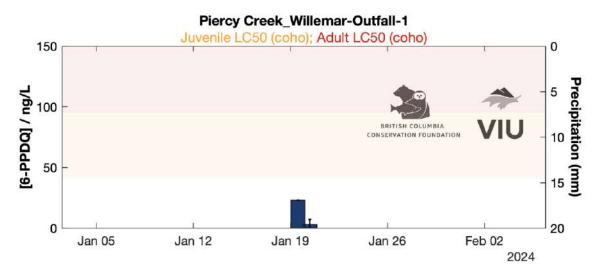
Graph 12. Piercy Creek, downstream of Copperfield Subdivision Discharge from October to November 2023.



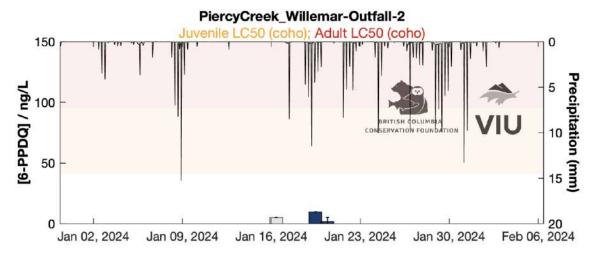
Graph 13. Piercy Creek, downstream of Arden Road from October to November 2023.



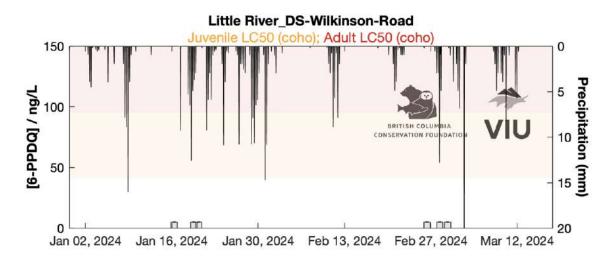
Graph 14. Piercy Creek, downstream of Cumberland Road from October to November 2023.



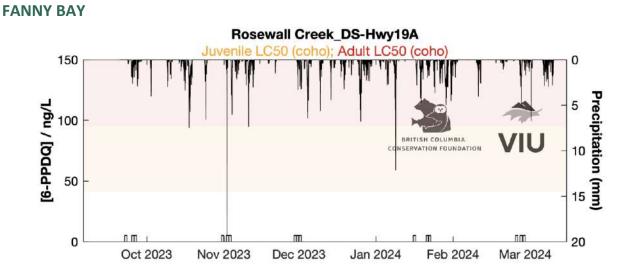
Graph 15. Piercy Creek, Willemar Outfall 1 in January 2024.



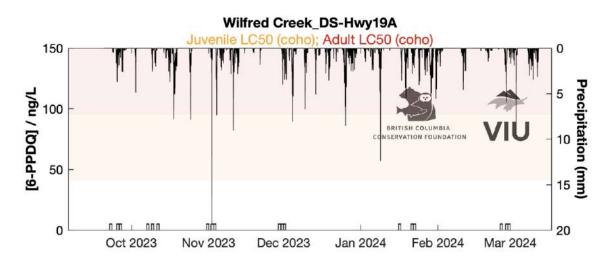
Graph 16. Piercy Creek, Willemar Outfall 2 in January 2024.



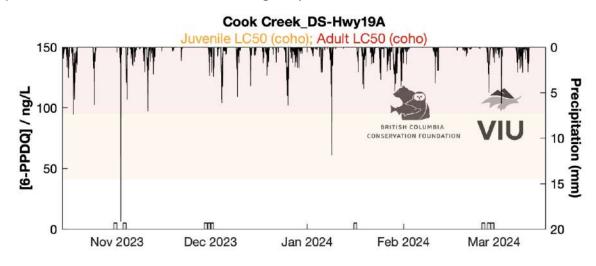
Graph 17. Little River, downstream of Wilkinson Road from January to February 2024.



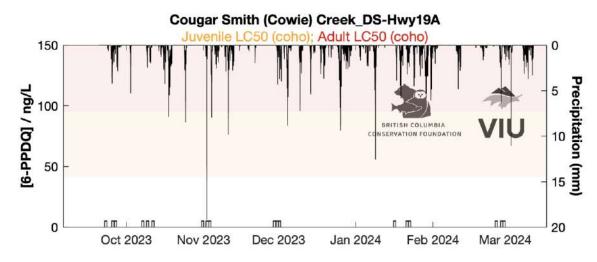
Graph 18. Rosewall Creek, downstream of Highway 19A from October 2023 to March 2024.



Graph 19. Wilfred Creek, downstream of Highway 19A from October 2023 to March 2024.

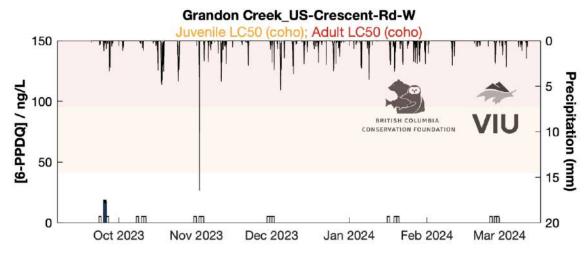


Graph 20. Cook Creek, downstream of Highway 19A from November 2023 to March 2024.

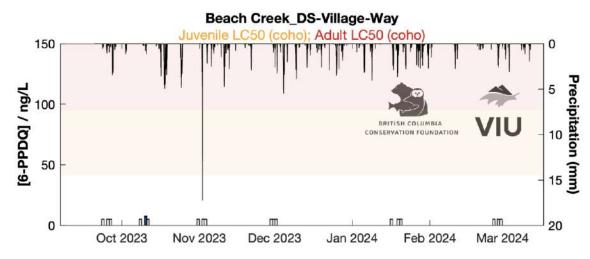


Graph 21. Cowie Creek, downstream of Highway 19A from October 2023 to March 2024.

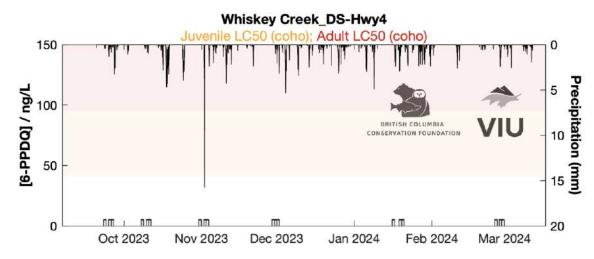
QUALICUM BEACH



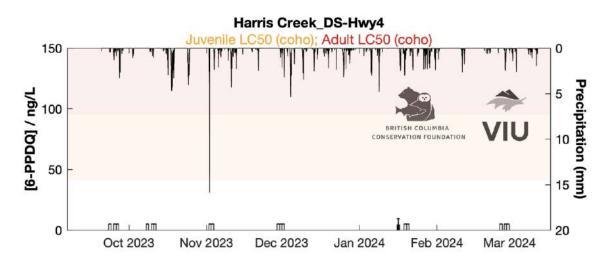
Graph 22. Grandon Creek, upstream of Crescent Road W from October 2023 to March 2024.



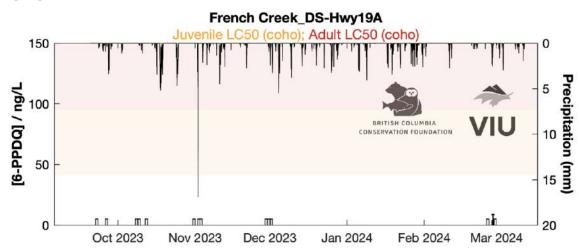
Graph 23. Beach Creek, downstream of Village Way from October 2023 to March 2024.



Graph 24. Whiskey Creek, downstream of Highway 4 from October 2023 to March 2024.



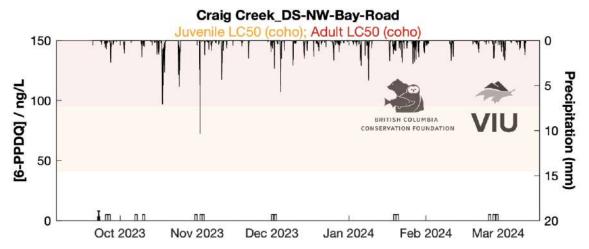
Graph 25. Harris Creek, downstream of Highway 4 from October 2023 to March 2024.



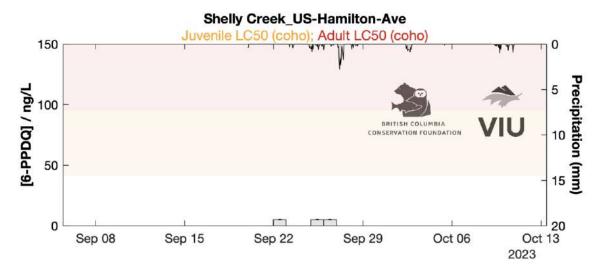
Graph 26. French Creek, downstream of Highway 19A from October 2023 to March 2024.

FRENCH CREEK

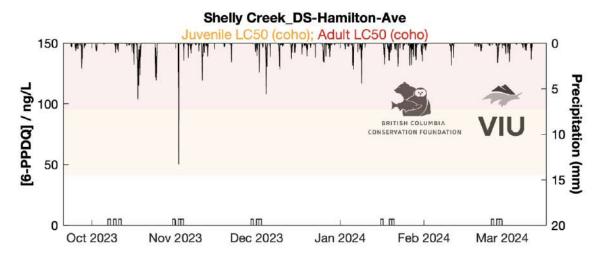
PARKSVILLE



Graph 27. Craig Creek, downstream of Northwest Bay Road from October 2023 to March 2024.

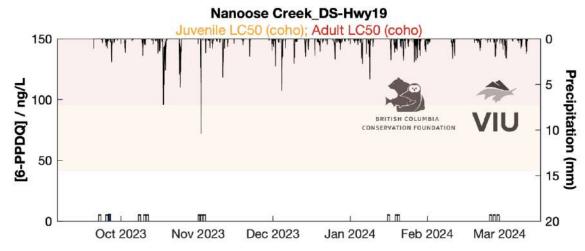


Graph 28. Shelly Creek, upstream of Hamilton Avenue in September 2023.

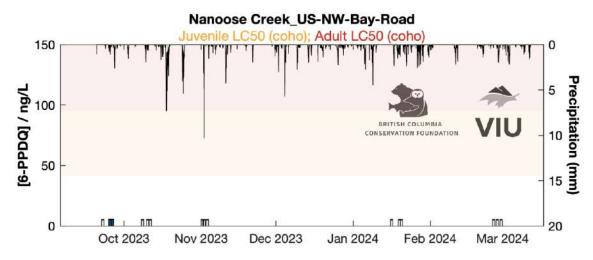


Graph 29. Shelly Creek, downstream of Hamilton Avenue from October 2023 to March 2024.

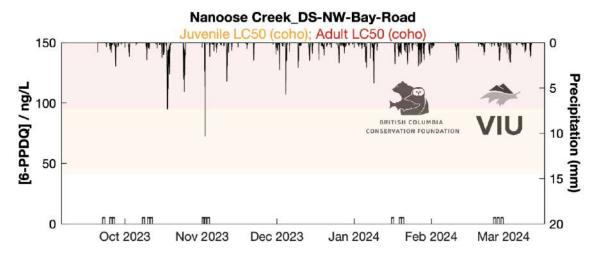
NANOOSE BAY



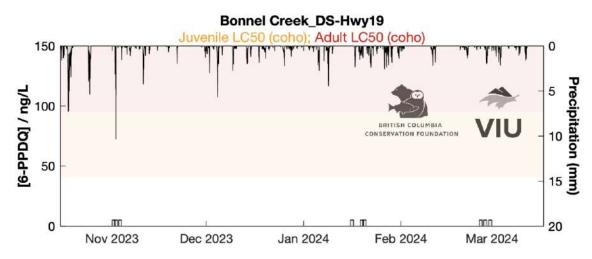
Graph 30. Nanoose Creek, downstream of Highway 19 from October 2023 to March 2024.



Graph 31. Nanoose Creek, upstream of Northwest Bay Road from October 2023 to March 2024.

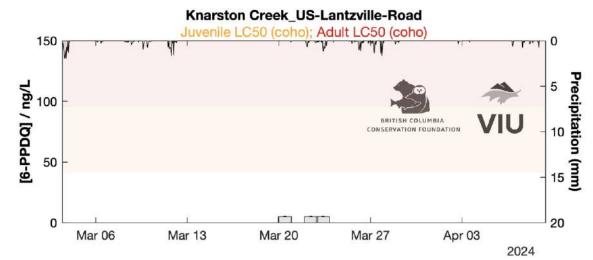


Graph 32. Nanoose Creek, downstream of Northwest Bay Road from October 2023 to March 2024.

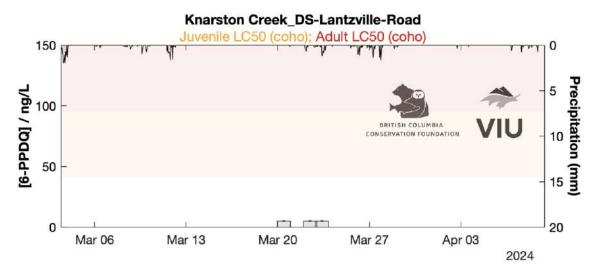


Graph 33. Bonnel Creek, downstream of Highway 19 from November 2023 to March 2024.

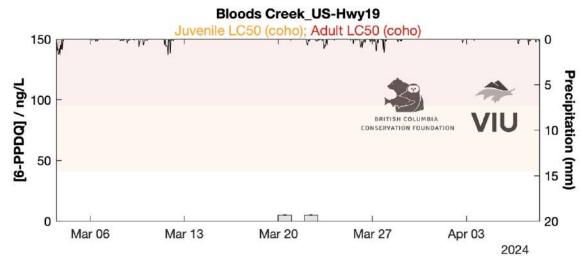
NANAIMO



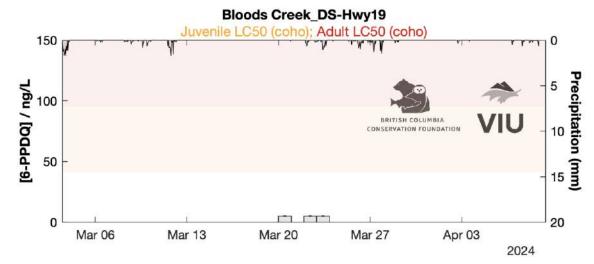
Graph 34. Knarston Creek, upstream of Lantzville Road in March 2024.



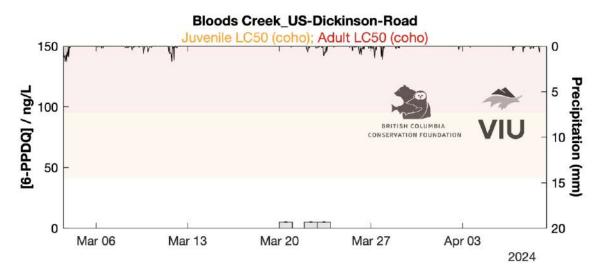
Graph 35. Knarston Creek, downstream of Lantzville Road in March 2024.



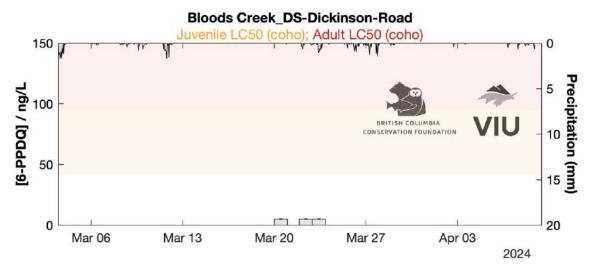
Graph 36. Bloods Creek, upstream of Highway 19 in March 2024.



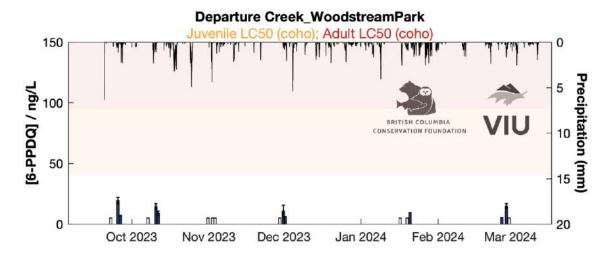
Graph 37. Bloods Creek, downstream of Highway 19 in March 2024.



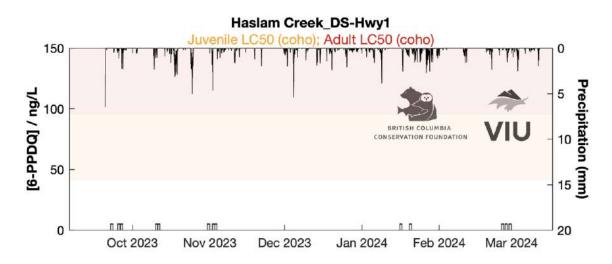
Graph 38. Bloods Creek, upstream of Dickinson Road in March 2024.



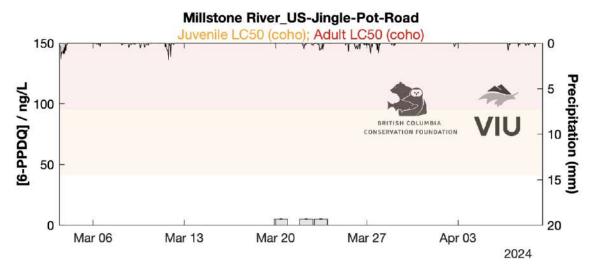
Graph 39. Bloods Creek, downstream of Dickinson Road in March 2024.



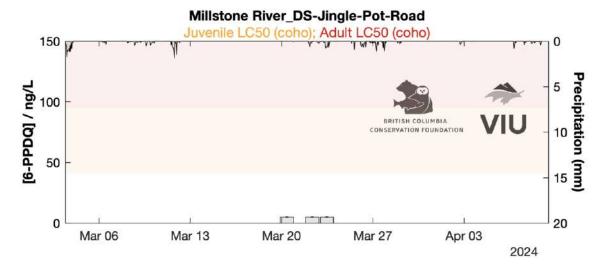
Graph 40. Departure Creek, in Woodstream Park from October 2023 to March 2024.



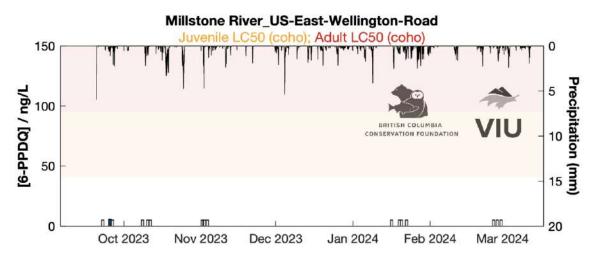
Graph 41. Haslam Creek, downstream of Highway 1 from October 2023 to March 2024.



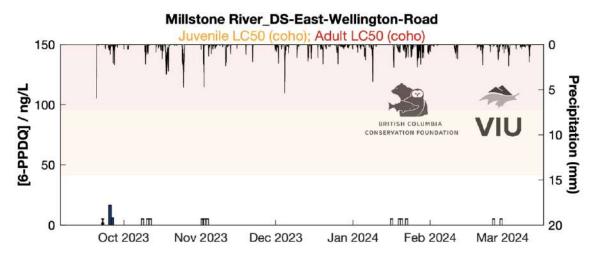
Graph 42. Millstone River, upstream of Jingle Pot Road in March 2024.



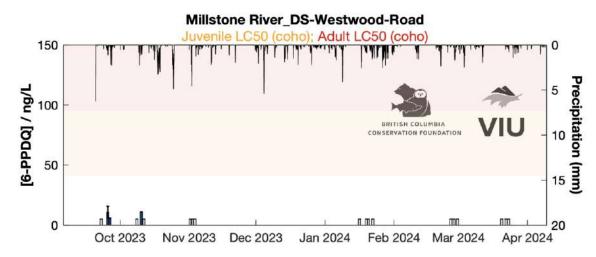
Graph 43. Millstone River, downstream of Jingle Pot Road in March 2024.



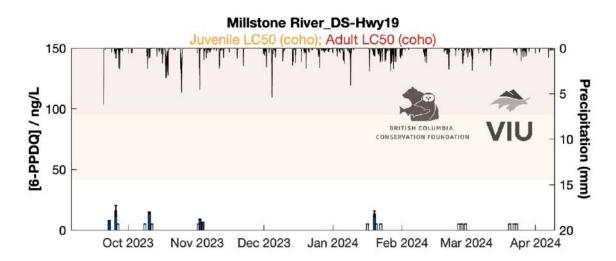
Graph 44. Millstone River, upstream of East Wellington Road from October 2023 to March 2024.



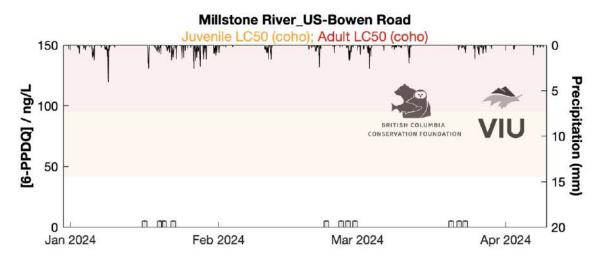
Graph 45. Millstone River, downstream of East Wellington Road from October 2023 to March 2024.



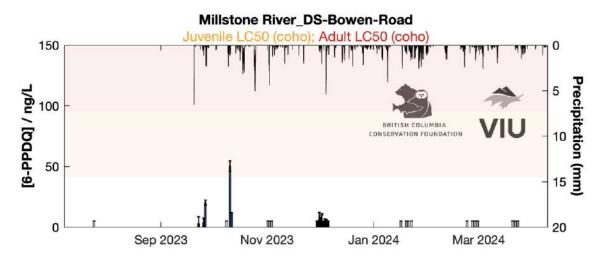
Graph 46. Millstone River, downstream of Westwood Road from October 2023 to March 2024.



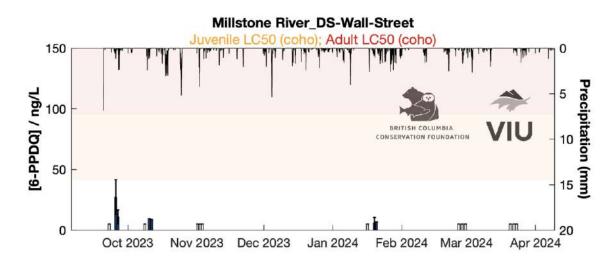
Graph 47. Millstone River, downstream of Highway 19 from October 2023 to March 2024.



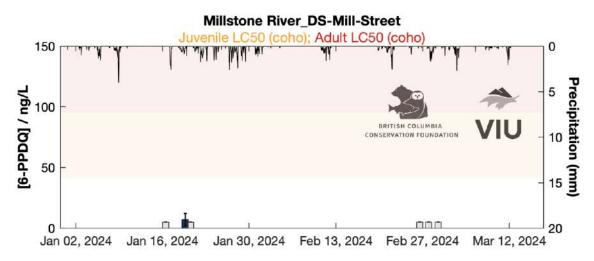
Graph 48. Millstone River, upstream of Bowen Road from January to March 2024.



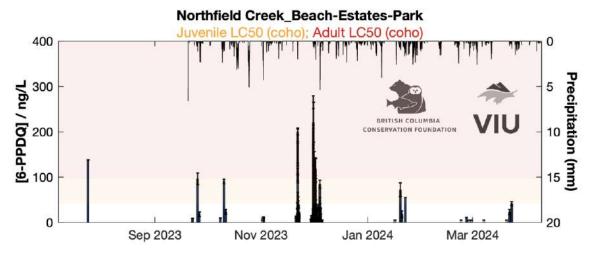
Graph 49. Millstone River, downstream of Bowen Road from September 2023 to March 2024.



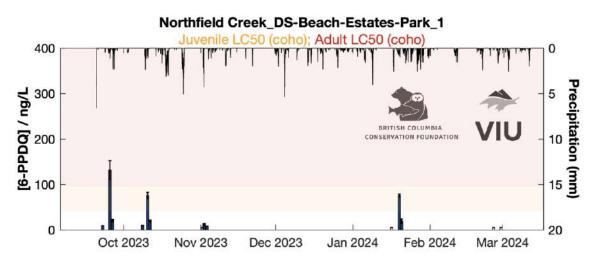
Graph 50. Millstone River, downstream of Wall Street from October 2023 to March 2024.



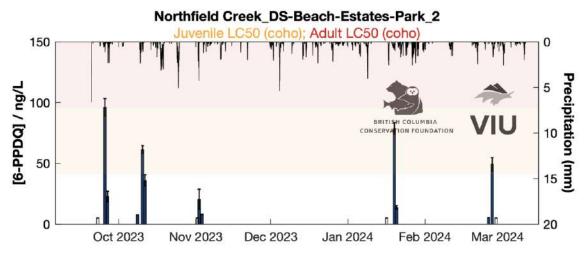
Graph 51. Millstone River, downstream of Mill Street from January to February 2024.



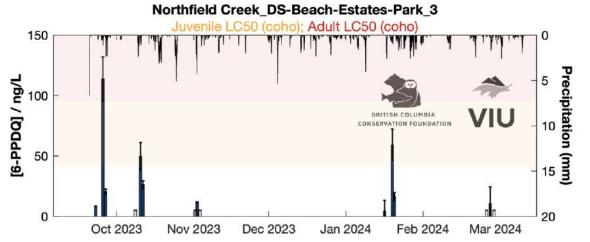
Graph 52. Northfield Creek, downstream of Departure Bay Road from September 2023 to March 2024.



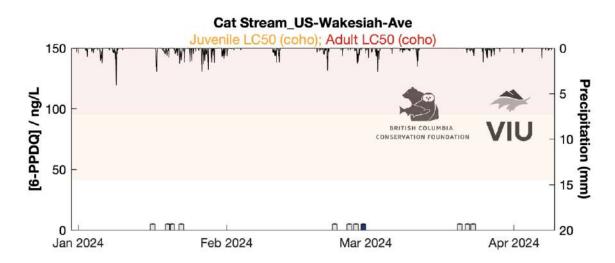
Graph 53. Northfield Creek (site #1), downstream of Departure Bay Road from October 2023 to March 2024.



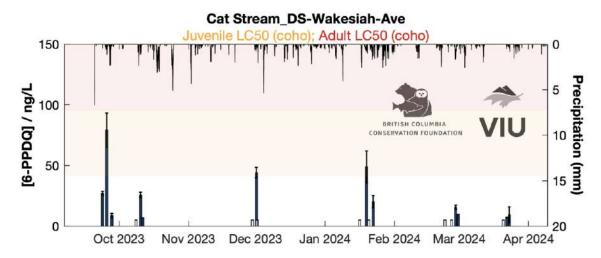
Graph 54. Northfield Creek (site #2), downstream of Departure Bay Road from October 2023 to March 2024.



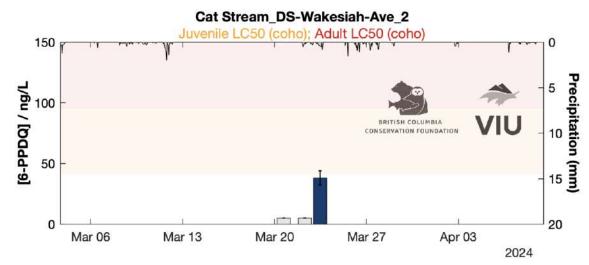
Graph 55. Northfield Creek (site #3), downstream of Departure Bay Road from October 2023 to March 2024.



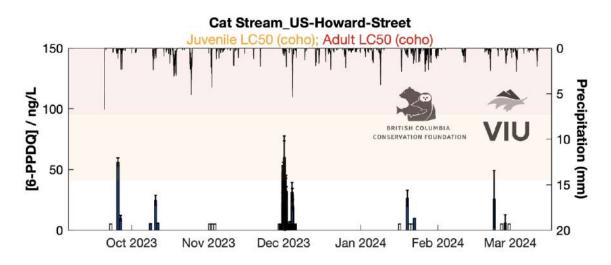
Graph 56. Cat Stream, upstream of Wakesiah Avenue from January to March 2024.



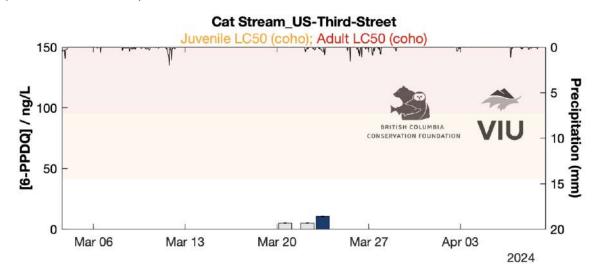
Graph 57. Cat Stream, downstream of Beaconsfield Street from October 2023 to March 2024.



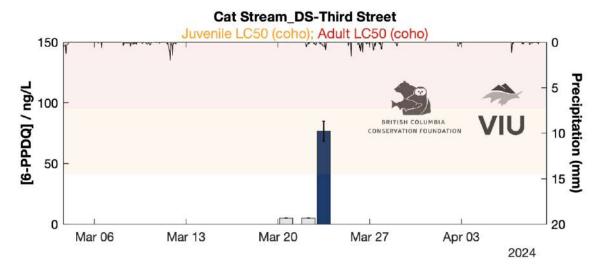
Graph 58. Cat Stream, downstream of Wakesiah Avenue in March 2024.



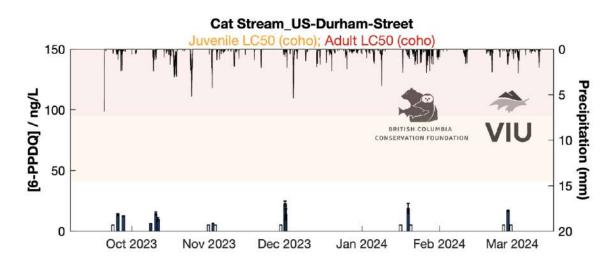
Graph 59. Cat Stream, upstream of Howard Avenue from October 2023 to March 2024.



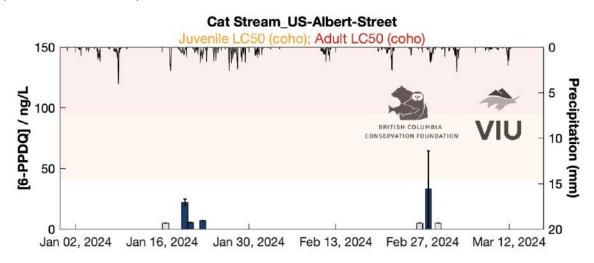
Graph 60. Cat Stream, upstream of Third Street in March 2024.



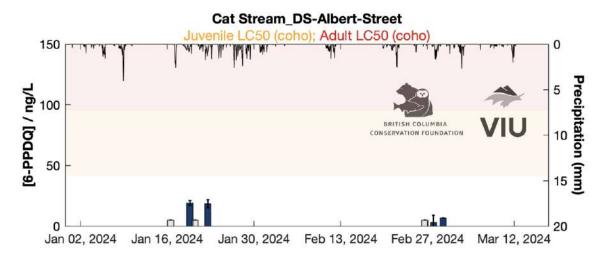
Graph 61. Cat Stream, downstream of Third Street in March 2024.



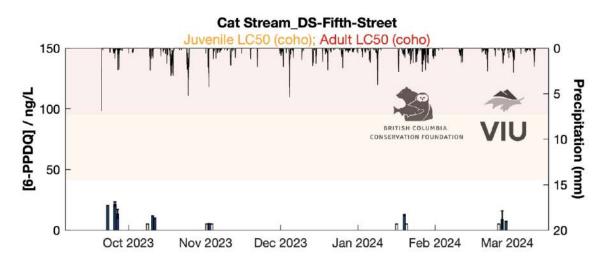
Graph 62. Cat Stream, upstream of Durham Street from October 2023 to March 2024.



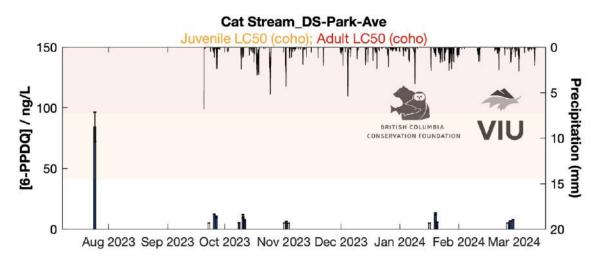
Graph 63. Cat Stream, upstream of Albert Street from January to February 2024.



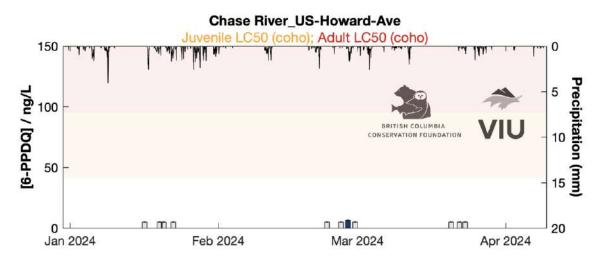
Graph 64. Cat Stream, downstream of Albert Street from January to February 2024.



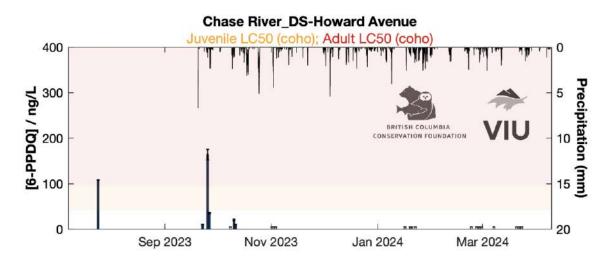
Graph 65. Cat Stream, downstream of Fifth Street from October 2023 to March 2024.



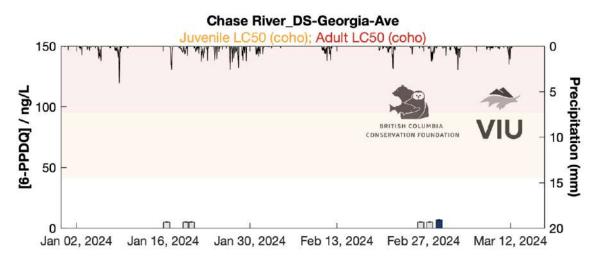
Graph 66. Cat Stream, downstream of Park Avenue from August 2023 to March 2024.



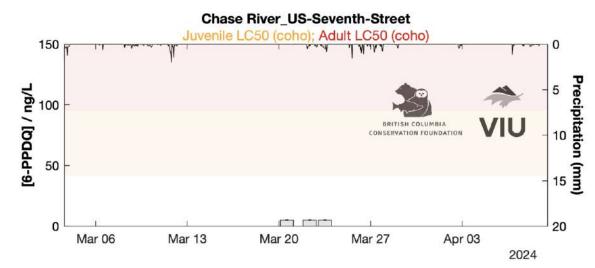
Graph 67. Chase River, upstream of Howard Avenue from January to March 2024.



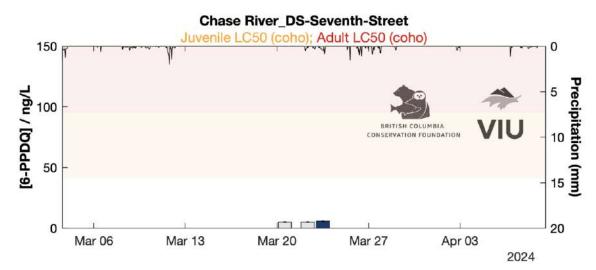
Graph 68. Chase River, downstream of Howard Avenue from September 2023 to March 2024.



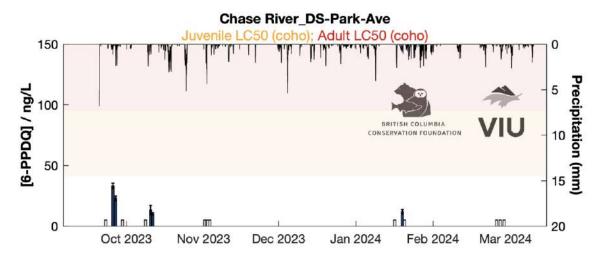
Graph 69. Chase River, downstream of Georgia Avenue from January to March 2024.



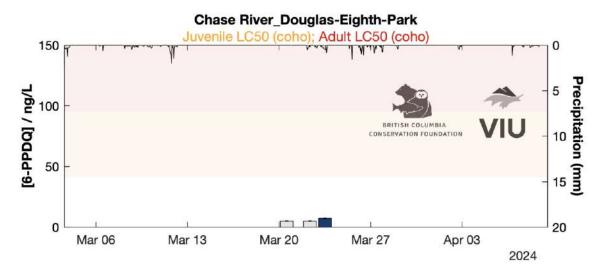
Graph 70. Chase River, upstream of Seventh Street in March 2024.



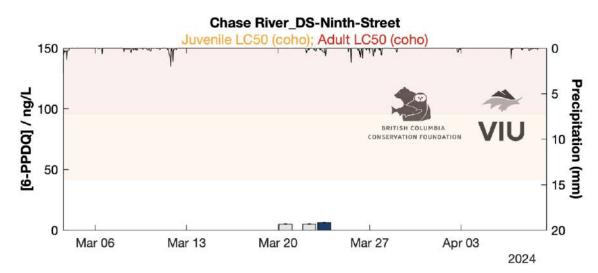
Graph 71. Chase River, downstream of Seventh Street in March 2024.



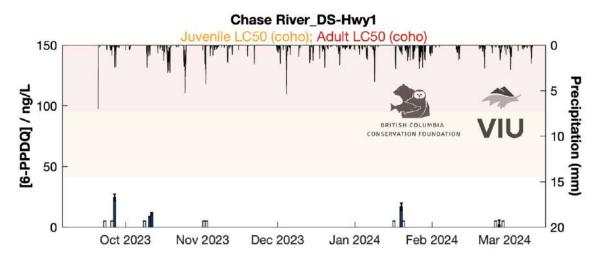
Graph 72. Chase River, downstream of Park Avenue from October 2023 to March 2024.



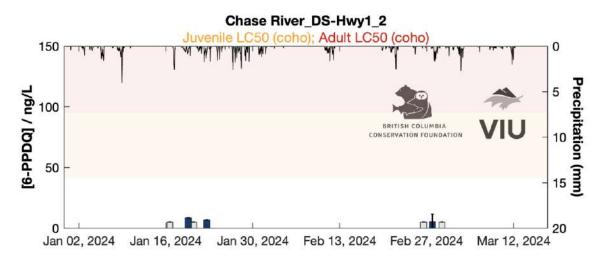
Graph 73. Chase River, Douglas and Eighth Park in March 2024.



Graph 74. Chase River, downstream of Ninth Street in March 2024.



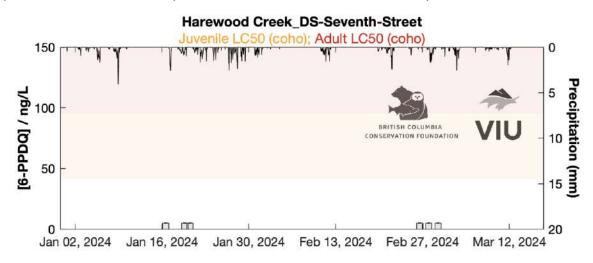
Graph 75. Chase River, downstream of Highway 1 (~300m) from October 2023 to March 2024.



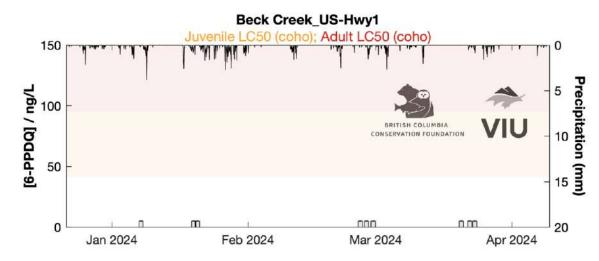
Graph 76. Chase River, downstream of Highway 1 (~50m) from January to March 2024.



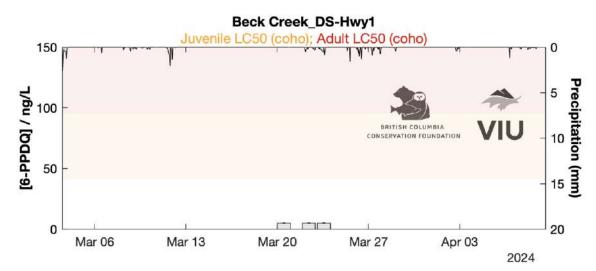
Graph 77. Harewood Creek, upstream of Seventh Street from January to March 2024.



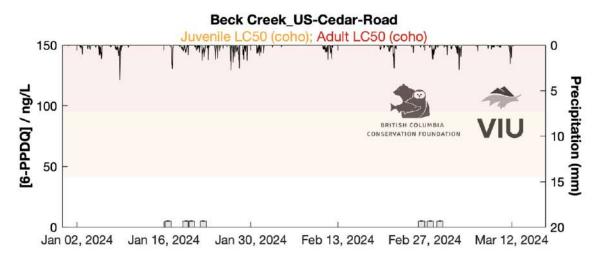
Graph 78. Harewood Creek, upstream of Seventh Street from January to March 2024.



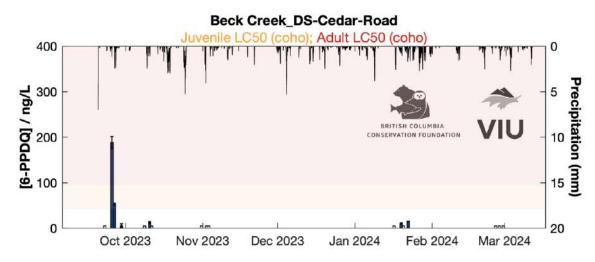
Graph 79. Beck Creek, upstream of Highway 1 from January to March 2024.



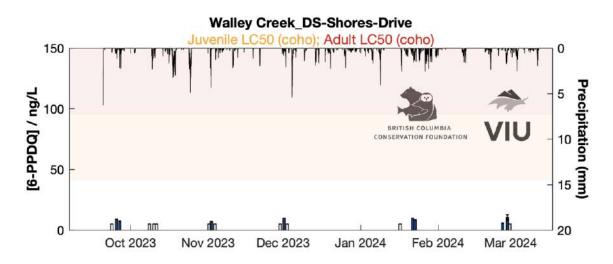
Graph 80. Beck Creek, downstream of Highway 1 in March 2024.



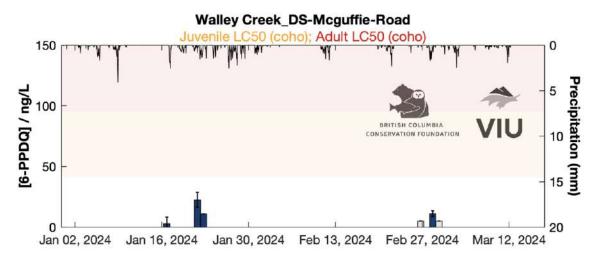
Graph 81. Beck Creek, upstream of Cedar Road from January to March 2024.



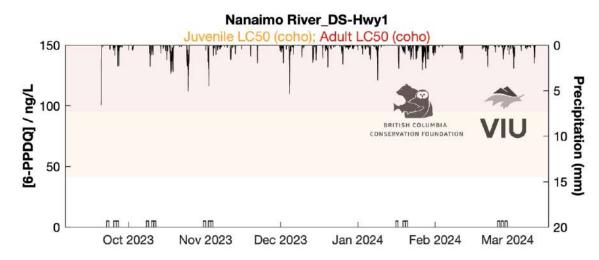
Graph 82. Beck Creek, downstream of Cedar Road from October 2023 to March 2024.



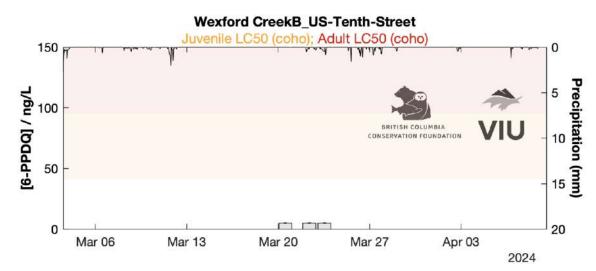
Graph 83. Walley Creek, downstream of Shores Drive from October 2023 to March 2024.



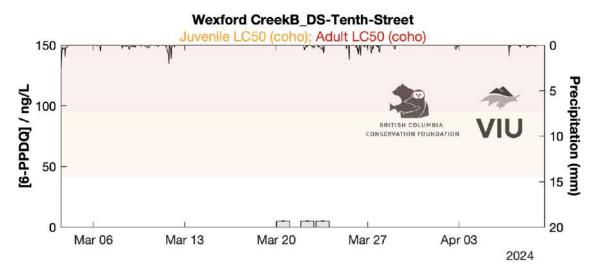
Graph 84. Walley Creek, downstream of McGuffie Drive from January to March 2024.



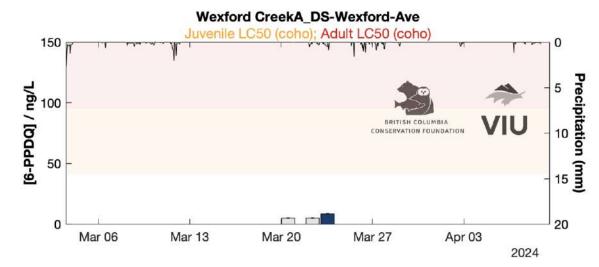
Graph 85. Nanaimo River, downstream of Highway 1 from October 2023 to March 2024.



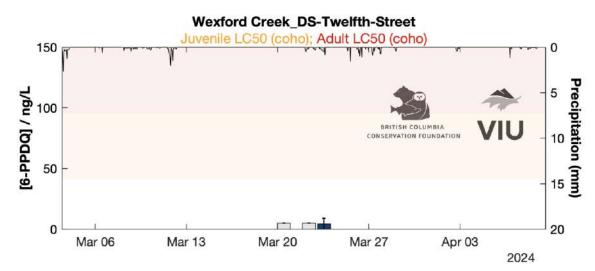
Graph 86. Wexford Creek, upstream of Tenth Street in March 2024.



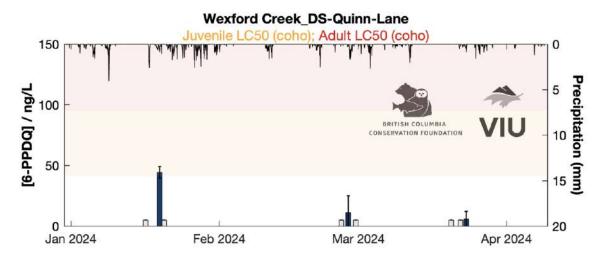
Graph 87. Wexford Creek, downstream of Tenth Street in March 2024.



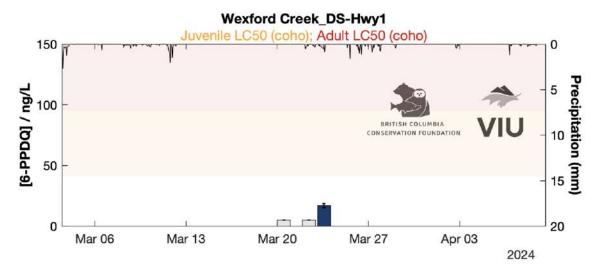
Graph 88. Wexford Creek, downstream of Wexford Avenue in March 2024.



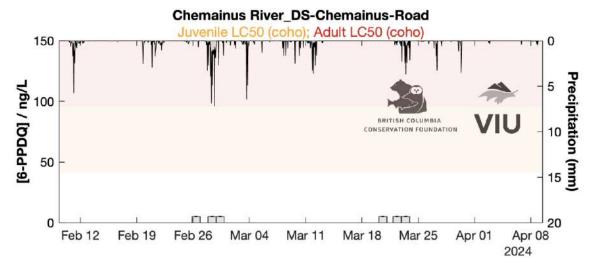
Graph 89. Wexford Creek, downstream of Twelfth Street in March 2024.



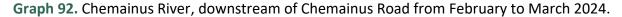
Graph 90. Wexford Creek, downstream of Quinn Lane from January to March 2024.

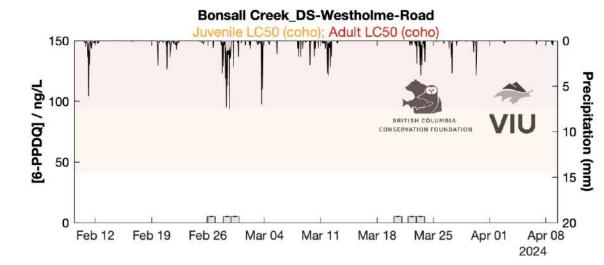


Graph 91. Wexford Creek, downstream of Highway 1 in March 2024.



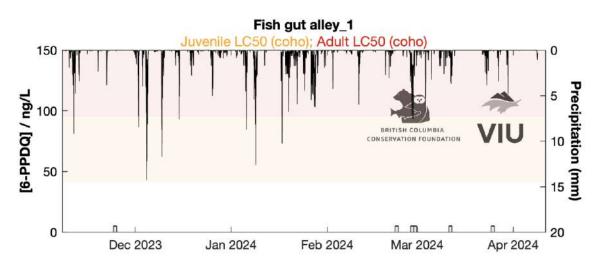
CHEMAINUS



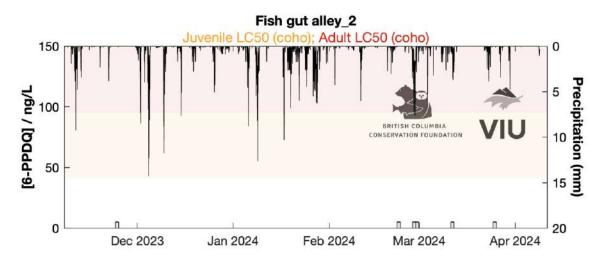


Graph 93. Bonsall Creek, downstream of Westholme Road from February to March 2024.

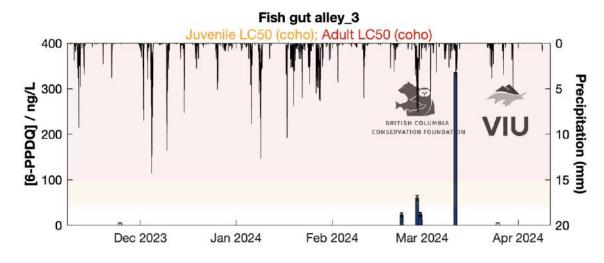
DUNCAN



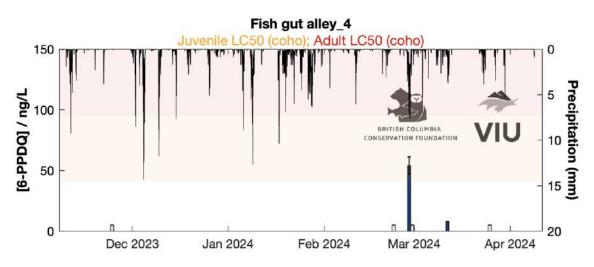
Graph 94. Fish Gut Alley (Site #1) from December 2023 to March 2024.



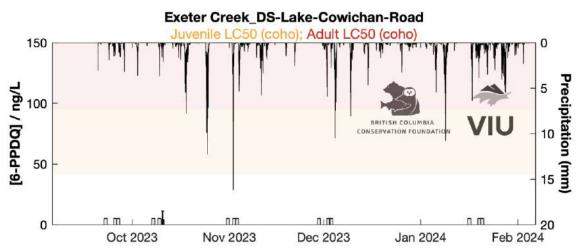
Graph 95. Fish Gut Alley (Site #2) from December 2023 to March 2024.



Graph 96. Fish Gut Alley (Site #2) from December 2023 to March 2024.

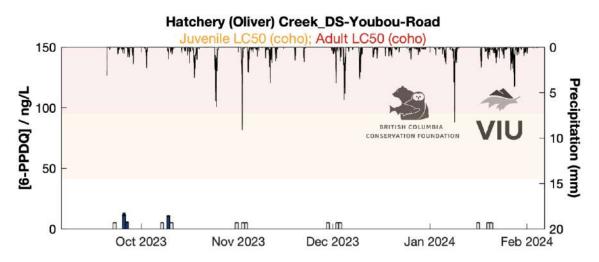


Graph 97. Fish Gut Alley (Site #4) from December 2023 to March 2024.

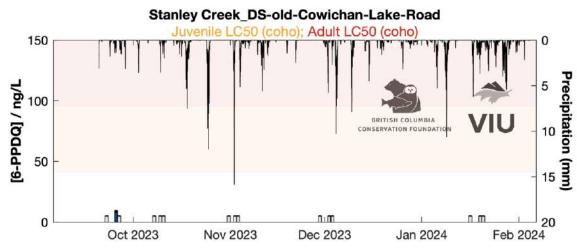


Graph 98. Exeter Creek, downstream of Lake Cowichan Road from October 2023 to February 2024.

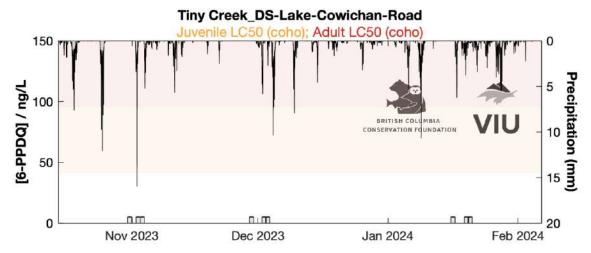
LAKE COWICHAN



Graph 99. Hatchery Creek, downstream of Youbou Road from October 2023 to February 2024.



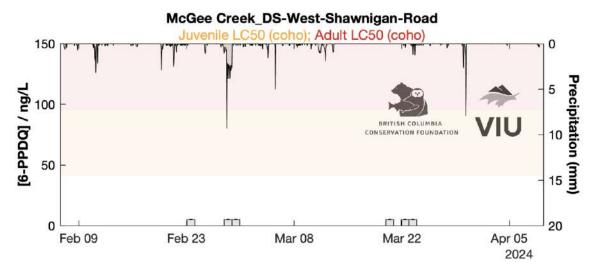
Graph 100. Stanley Creek, downstream of old Cowichan Lake Road from October 2023 to February 2024.



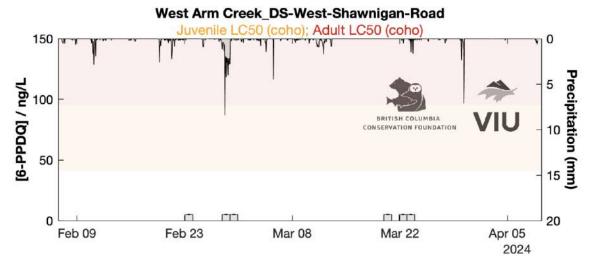
Graph 101. Tiny Creek, downstream of old Cowichan Lake Road from October 2023 to February 2024.

SHAWNIGAN CREEK – MILL BAY

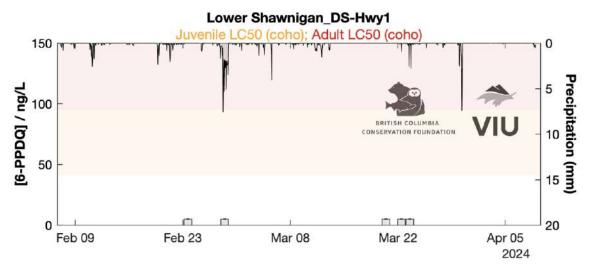
Graph 102. Upper Shawnigan Creek, downstream of Sooke Lake Road from February to March 2024.



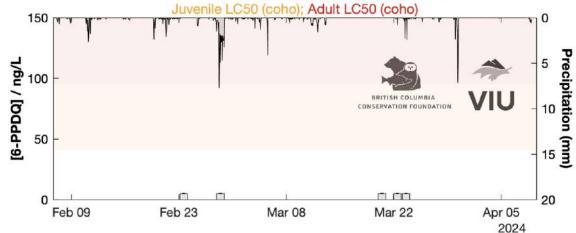
Graph 103. McGee Creek, downstream of West Shawnigan Road from February to March 2024.



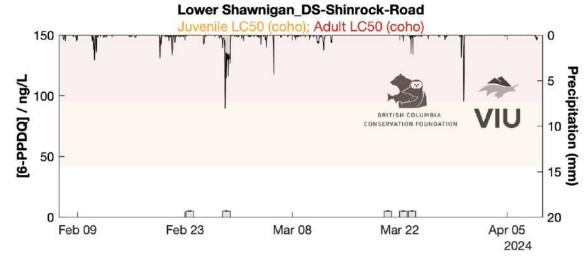
Graph 104. West Shawnigan Creek, downstream of West Shawnigan Road from February to March 2024.



Graph 105. Lower Shawnigan Creek, downstream of Highway 1 from February to March 2024. Lower Shawnigan_DS-Cameron-Taggert-Road

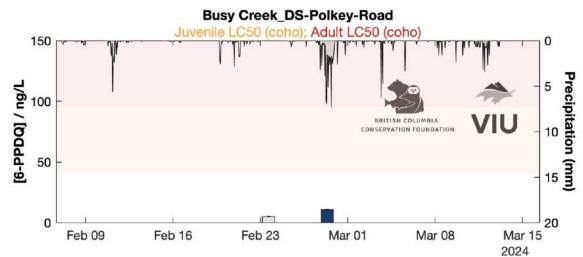


Graph 106. Lower Shawnigan Creek, downstream of Cameron Taggert Road from February to March 2024.



Graph 107. Lower Shawnigan Creek, downstream of Shinrock Road from February to March 2024.

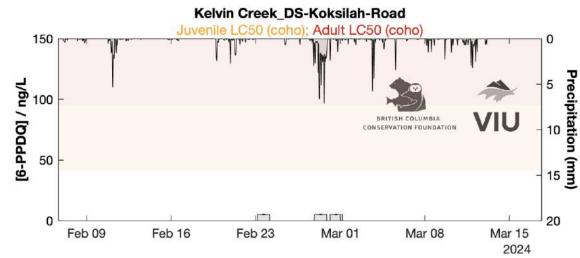
COWICHAN BAY



Graph 108. Busy Creek, downstream of Polkey Road from February to March 2024. Norrie Creek_DS-Koksilah-Road



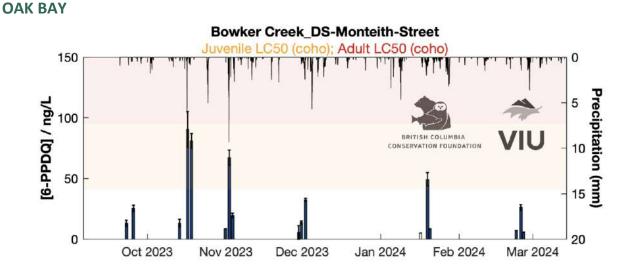
Graph 109. Norrie Creek, downstream of Koksilah Road from February to March 2024.



Graph 110. Kelvin Creek, downstream of Koksilah Road from February to March 2024.

Gorge Creek_DS-Gorge-Stormwater-Outfall Juvenile LC50 (coho); Adult LC50 (coho) 150 0 [6-PPDQ] / ng/L 100 BRITISH COLUMBIA 50 0 20 Oct 2023 Nov 2023 Dec 2023 Jan 2024 Feb 2024 Mar 2024

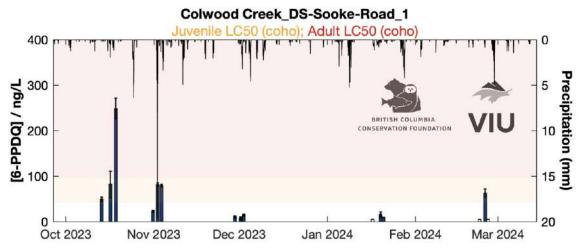
Graph 111. Gorge Creek, downstream of Gorge Stormwater Outfall from October 2023 to March 2024.



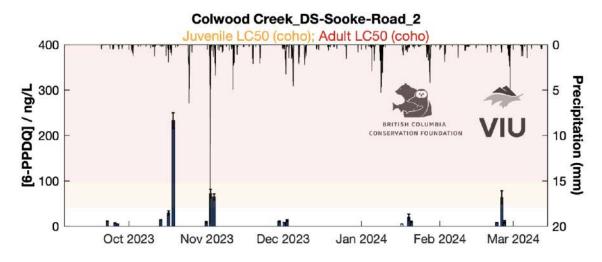
Graph 112. Bowker Creek, downstream of Monteith Street from October 2023 to March 2024.

ESQUIMALT

COLWOOD

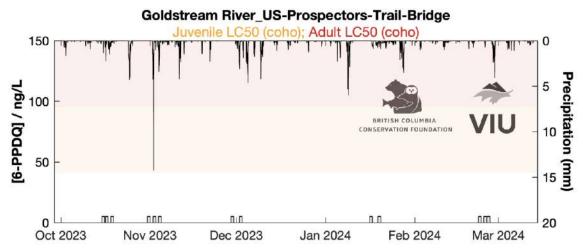


Graph 113. Colwood Creek, downstream of Sooke Road (upstream of point source) from October 2023 to March 2024.

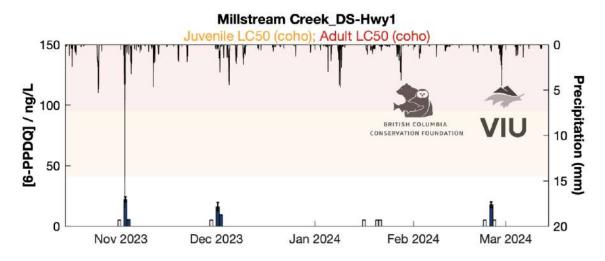


Graph 114. Colwood Creek, downstream of Sooke Road (downstream of point source) from October 2023 to March 2024.

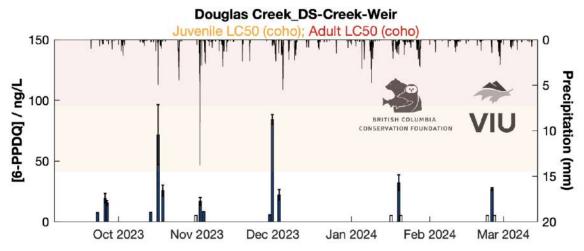
LANGFORD



Graph 115. Goldstream River, upstream of Prospectors Trail Bridge from October 2023 to March 2024.

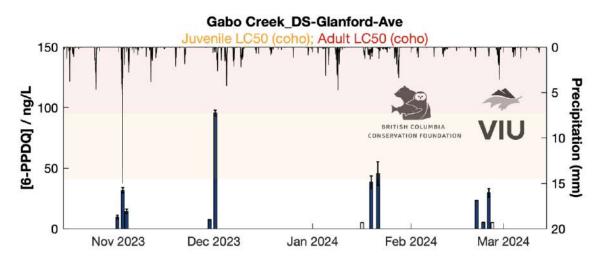


Graph 116. Millstream Creek, downstream of Highway 1 from November 2023 to March 2024.



SAANICH

Graph 117. Douglas Creek, downstream of the creek weir from October 2023 to March 2024.



Graph 118. Gabo Creek, downstream of Glanford Avenue from October 2023 to March 2024.