Summary of Pilot Sampling and Protocol Development for the Columbia River Mainstem Fish Tissue and Water Quality Monitoring Program – Bonneville Dam to Canadian Border

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Background

There is currently no dedicated monitoring program on the Columbia River that specifically monitors the status and trends of contaminants in fish and water quality. The lack of a dedicated contaminant monitoring program impedes evaluation and decision making regarding the health of the river. This is despite the fact that a recent economic evaluation of the Columbia Basin under current conditions identified "Ecosystem Services" as by far the single largest category of economic evaluation in the Basin at $189 Billion dollars (Flores et al 2017). Therefore, this pilot study was conducted as part of a larger effort to develop and implement a Columbia River Mainstem Fish Tissue and Water Quality Monitoring Program (Monitoring Program) aimed at tracking the status and trends of toxics in fish, water, sediments, and invertebrates in the Columbia River mainstem from Bonneville Dam to the Canadian border. This Summary Report summarizes a pilot study within the Bonneville Pool that collects fish and sediment data and tests methods to begin status and trends monitoring and improve upon methods for the larger program.

The Columbia River provides important cultural, economic, and ecological services to a significant portion of the United States. The importance of the Columbia River ecosystem to Tribal sovereignties in the Columbia River Basin (CRB) is well documented, see summary in NRC (2004). Anadromous and resident fish species and other wildlife are integrated into the cultural traditions of all Tribes in the CRB. Salmon are an integral part of Tribal religion, culture, and physical sustenance (Sams, 2007). Fisheries and other water-related resources

(e.g., irrigation water supply) have significant economic and recreational value to Tribal and non-Tribal entities (CRITFC, 1996; IEAB 2005). A 2005 report commissioned by the Northwest Power and Planning Council estimates, "The $109 million generated in the Pacific Northwest states of Washington, Oregon, and Idaho of personal income [from CRB anadromous salmonid production] may support about 3,633 jobs." (IEAB, 2005).

A more recent economic evaluation of the Columbia River Basin identified "Ecosystem Services" as the single largest economic value of the Columbia River Basin and valued it at $189 Billion in 2017 dollars (Flores et al., 2017).

Despite concerns about the effect of contaminants on the aquatic ecosystem (USEPA, 2009), the disproportionate effects of contaminants on members of Tribal sovereignties (Harper and Walker, 2015), and the known effects of contaminants on species protected under the Endangered Species Act (ESA) (Lundin et al., 2019, Lundin et al., 2021; MacNeale et al., 2010), efforts to measure the pollution by toxic chemicals in the Columbia River remain limited. Recent funding sources from the Columbia Basin Restoration Program (CBRP), administered by US EPA Region 10, and the Washington Department of Ecology have brought an increase in attention and sampling to contaminants in the Columbia River mainstem. However, the lack of a dedicated contaminant monitoring program impedes evaluation and decision making regarding the health of the river.

In 2022, we completed a Framework (Counihan et al 2022) for the long-term monitoring of toxics in the mainstem Columbia river from Bonneville Dam to the Canadian border (962 kilometers or 598 miles). The Framework provided expert guidance for the development of a long-term program that provides the basis for assessing the status and trends of contaminants in fish, sediment, water, and other media in the Columbia River. Over the long-term, it is expected that the Monitoring Program will evolve with new and emerging science and community needs. The framework includes the vision, goals, and objectives for the Program; technical planning; community outreach and engagement, and adaptive management. The Framework document outlines the contaminants of concern and a list of priority fish species to be sampled at each site. It also outlines the sampling design (GRTS - a random spatial sampling frame), sample allocation and other important considerations for a long-term program.

Purpose

The primary purpose of this Bonneville Pilot Implementation study was to both test the design and approach of a long-term monitoring program, as outlined in the Framework document described above; and to collect, process, and analyze fish and sediment samples from Bonneville Reservoir, a 50-mile reach of the Columbia River.

This Pilot Study provides information and experience for the necessary planning and documentation needed to conduct aquatic monitoring in a large river like the Columbia, over a long-term basis. Our goal was to further develop a Monitoring Program through this field sampling, analytical and reporting effort. This work directly informs the development of the Monitoring Program by providing on the ground testing and information regarding media specific Quality Assurance Project Plan (QAPPs), Standard Operating Procedures (SOPs), Health and Safety Plans (HASPs), Invasive Species Spread and Prevention Plan (ISSPP), laboratory contracting, performance plan and data review, and other plans and permits acquired

We will continue working collaboratively with the Project Team and key stakeholders (States, Tribes, Federal Agencies) and others to address both the details about how a long-term monitoring program may be

implemented and to generate high quality, up to date data on contaminants, in the Bonneville pool of the Columbia River.

This report summarizes the results of our pilot sampling and testing of sampling protocols in the Bonneville pool reach following the sampling design, as outlined in the Framework document and QAPP. Here we provide a summary overview of the step-by-step sampling methods for fish and sediment, a Quality Assurance review of the laboratory data that was generated, as well as reporting lessons learned. The detailed protocols are provided in the supplementary materials, including in the previously submitted revised QAPP. This report is meant to summarize methods and approach and provide insights into future monitoring efforts but is not intended to fully evaluate and nor interpret the reported contaminant data found herein.

Methods

Site Selection

To assess contamination in sediments and resident fish species across spatial and temporal scales covered in the planned Program, the Project Team concluded that there was a need for a sampling design that probabilistically allocated sampling locations across the study area in a spatially balanced way. Previous studies have probabilistically allocated samples to reaches of the Columbia River that include this Pilot Project's study area. For example, Herger et al. (2016) in a prior assessment of contaminants in fish tissues in the Columbia River used a linear Generalized Random Tessellation Stratified (GRTS) sample frame to allocate sampling locations from Bonneville Dam (rkm 234) to Grand Coulee Dam (rkm 957). A sample design that is based on GRTS is a true probability design where each point has a known, non-zero probability of being included in the draw. Importantly, a GRTS design supports design-based inferences to the entire area or subsets of the study area, thus enabling an estimate of contaminant levels in the media sampled across the entire sampling frame. Additional details can be found in Diaz-Ramos et al. (1996), Stevens (1997), Stevens & Olsen (1999), and Stevens & Olsen (2004).

The Project Team concluded that a sample frame and sample design to allocate resident fish collection locations across the study area should be based on a linear GRTS design. Specifically, the sample frame was based on a river-center line geographic information system (GIS) data layer developed from the high-resolution version of the National Hydrography Dataset (for examples of linear GRTS sample frames see: https://archive.epa.gov/nheerl/arm/web/html/design_intro.html#strms). Some stakeholders have sampling locations that are important to them and/or that provide context to previous studies (e.g., Tribal fishing locations sampled in: USEPA, 2002). Depending on the nature of these non-probabilistically selected locations (e.g., they were not selected because of known issues with contaminants), some proportion may be considered as contributing to the information derived from the probabilistic sites and may have value as sites for targeted, localized trends. Thus, the sample sites selected here were a mix of both- randomly selected from this sample frame in a manner that ensures the distribution of sites throughout the entire study reach (Stevens & Olsen, 2004), as well as two sites of historical importance and with previously existing data. Since fish samples should be collected from shoreline habitats (Herger et al., 2016), the sample locations in the linear GRTS sample frame was further allocated to either the left or right banks of the river.

Sediment contamination concentrations have been shown to be related to the sedimentation characteristics of the river channel (Counihan et al., 2014). The sedimentation in river channels varies laterally and

longitudinally in the Columbia River based on the hydrogeomorphology of river reaches. Dams and other manmade structures also affect sedimentation patterns. Given the variability of sedimentation characteristics in the Columbia River, the Project Team concluded that using a linear GRTS sample frame (i.e., fish sampling sites) may not characterize the variability in sediment contaminants, thus an area-based GRTS sample frame could be used to allocate sediment collection locations across the study area (for examples of areal GRTS sample frames see: https://archive.epa.gov/nheerl/arm/web/html/design_intro.html#strms). Until that time when there is a hydrodynamic model that predicts the location of the various habitats in the mainstem Columbia based on flow and sediment type, the above area based sampling design is the best option.

Site Identification and Verification

Site verification is the process of determining if the randomly selected sites can be sampled in the field. Constraints to sampling a particular site can be accessibility (both physically reaching a site or safety issues), permission (Federal Endangered Species concerns, Tribal areas, or wildlife areas may cause restrictions in sampling specific areas), or mapping errors. This section describes the process for selecting sampling locations.

Sample locations were selected from a river-centerline GIS data layer (or "sample-frame") developed from the National Hydrography Database (NHD). There is a unique set of primary "base" sites and 'oversample' sites. All base sites in the GRTS selection will eventually be sampled unless the validation processes find them to be either non-target, meaning not located on the Columbia River (an unlikely scenario) or unsampleable. If a base site is deemed non-target or unsampleable, an oversample site will be used as a replacement. The randomly selected base and oversample sites have mid-channel coordinates of latitude/longitude in decimal degrees. The actual sample collection site for this project for all samples (water quality and fish tissue) will occur typically not more than 30m from the shore. This approximates the littoral zone and the most biologically active area.

Site validation is required to determine whether a site can and should be sampled (its "sampling status"). Site validation includes an evaluation of the sample locations for position errors, possible safety hazards, accessibility, and restrictions to fishing. Office-based validation and field reconnaissance occur before the sample event. Each site is evaluated for 'target' or 'non-target' status. If the site is positioned on the Columbia River Mainstem the site is considered target otherwise it is 'non-target' (e.g., the shore location falls at/in a tributary confluence). Next, evaluate whether each site is sampleable or non-sampleable based on safety, access, and fishing restrictions.

Sediment Sampling

We attempted to sampled ten sites for sediment in the Bonneville pool of the Columbia River in fall 2023 and spring-summer 2024. Sampling locations for sediment collections were randomly selected locations from an area based GRTS sample frame that has been developed for Bonneville Reservoir (see:https://www.monitoringresources.org/Sites/Master/Detail/2). Sediment samples were collected from a boat using a standard ponar benthic grab sampler, see Photo 1, deployed from a bow-mounted crane and winch. Samples were collected in a downstream to upstream order. If a site was deemed inaccessible by boat a replacement site was chosen from a list of oversample points previously generated from the GRTS sample frame. Individual ponar grab samples collected within a strata were deposited in a stainless steel bowl and then composited. Two sites were rock or bed rock and sampleable material could not be collected there, and one site was too shallow to access/ intermittently dry. The sediment in the dredge was deposited into a clean

stainless steel pan and sediment scooped with a stainless steel spoon into a clean 500 ml sample jar with Teflon lid and placed into a cooler on ice at each site. If needed, sediment was allowed to settle and clear water poured off. The dredge, stainless steel pan and spoon were cleaned using Liquinox soap, a brush and then thoroughly rinsed with native water in between sampling locations. Once back at the lab, samples were placed in freezer until shipment to the contract laboratory for analysis.

Photo 1. Ponor grab sampler used of sediment sampling in Bonneville Reservoir, Columbia River.

Table 1. Sediment sampling locations for pilot in Bonneville Reservoir. Sampling locations are from an areal GRTS sample frame developed for Bonneville reservoir that can be referenced here: https://www.monitoringresources.org/Sites/Master/Detail/2. Latitude and longitude are in decimal degrees using the North American Datum of 1983 (NAD83).

Site Name (this report)	Lon/lat	GRTS ID (from grand population)	Successful Collection	Depth (m)
Bonres_1	-121.887218°W 45.677229°N	BonRes-000003	yes	27.5
Bonres_2	-121.846482°W 45.702842°N	BonRes-000010	yes	50
Bonres_3	-121.799169°W 45.706605°N	BonRes-000002	yes	49.5
Bonres_4	-121.712633°W 45.697288°N	BonRes-000007	yes	35
Bonres_5	-121.589076°W 45.716715°N	BonRes-000004	yes	40.2
Bonres_6	-121.417400°W 45.693306°N	BonRes-000012	yes	24.8
Bonres_7	-121.294550°W 45.695206°N	BonRes-000008	no	na
Bonres_8	-121.261596°W 45.673773°N	BonRes-000013	yes	4
Bonres_9	-121.195281°W 45.613978°N	BonRes-000001	no	104
Bonres_10	-121.176053°W 45.604834°N	BonRes-000005	no	77

Figure 1. Map of Sediment Sampling Sites in Bonneville Pool, Columbia River.

Anadromous Fish Passage and Sampling Considerations

The passage of multiple stocks of Endangered Species Act (ESA) listed salmonids through the Columbia River, both as juvenile out-migrants and adult returns, makes fish sampling in the reservoir particularly challenging. Fish collection permits and methods must address the efficiency and safety of the target fish species while also minimizing contact with non-target species, particularly those that are ESA listed. Water temperature is a particular concern, as well as handling, for all fish, but particularly so for cold-water dependent pacific salmon species. Hence, collection timeframes were carefully planned to minimize both exposure to migratory ESA species, avoid periods of warm water temperatures and maximize collection efficiency. Figure 2 below outlines ten-year averages of Chinook, Coho, and Steelhead return numbers by date, and the window of warm water temperature concern.

Seasonal Considerations for Fish Sampling

While fish may be captured year-round in the mainstem of the Columbia River, the metabolic activity, nutritional demand and physical activity slows with water temperature. Cold temperatures can lead to poor collection success, while warmer water temperatures at a fish's upper tolerance can greatly increase handling stress. Careful attention to water temperature and thus collection time windows were monitored, in accordance with state and Federal permits. Efforts were made to target fish collection outside of high ESA fish densities, high river flows, and high temperatures. Figure 2 below highlights those multiple considerations in the Bonneville Pool area.

Figure 2. Running timing of Anadromous salmon returns to Booneville Dam, ten year average, 2013-2022.

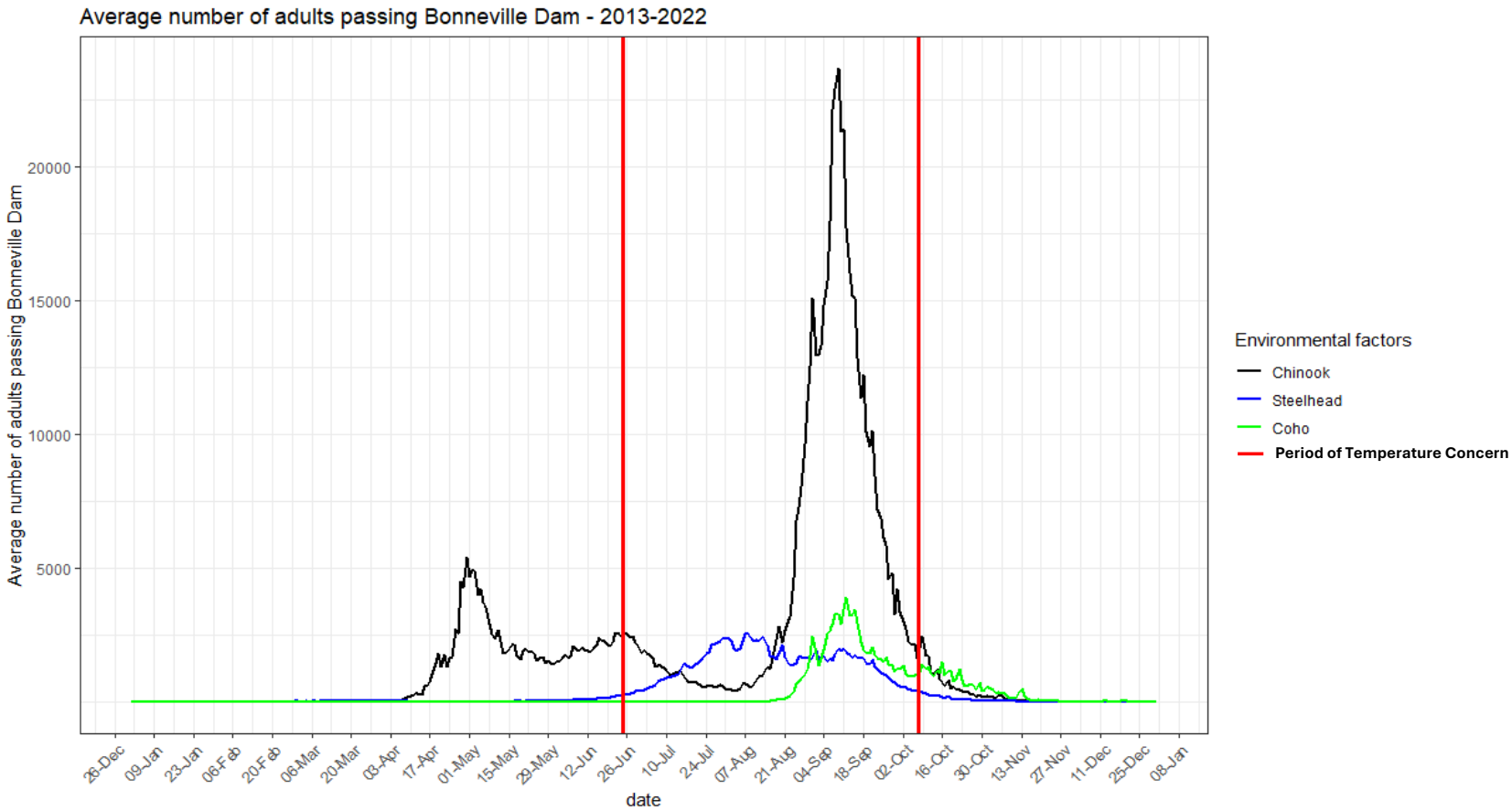
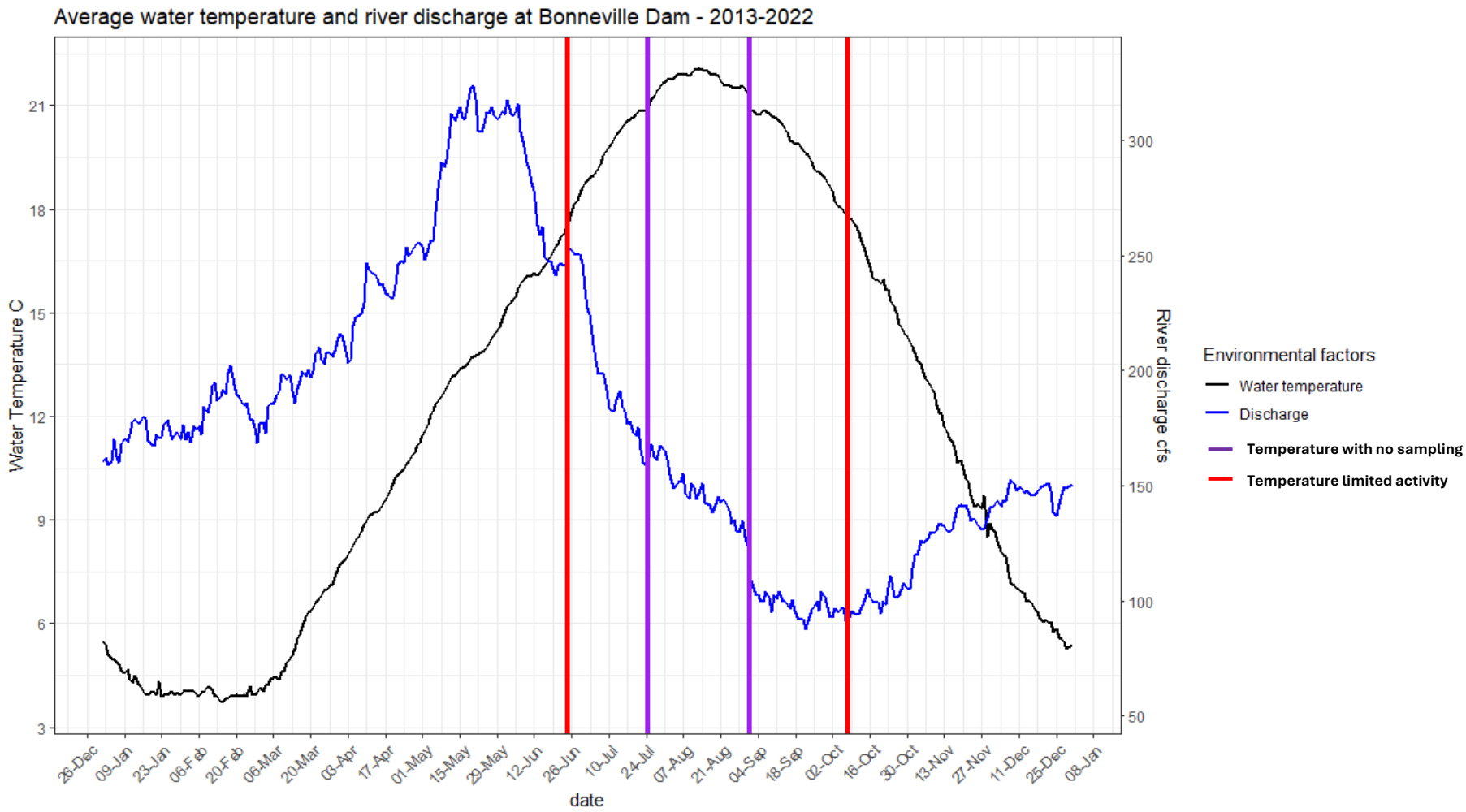


Figure 3. Ten-Year Average plots of Columbia River Discharge and Temperature for Water Year.



Adult Salmon Samples

Adult salmon were purchased from tribal fishers who caught them within the Bonneville pool area. These fish were caught via various gill nets up and downstream from a given boat ramp, precise locations within the reservoir could not be identified. Adult salmon in the Columbia River are highly mobile. While clean handling technique could not be controlled by each Tribal fisherman, the handling of fish prior to receipt and sampling by the USGS is nevertheless reflective of what human consumers are exposed to. We selected four different seasonal time periods to purchase fish starting in early August 2023 through October2023, to document fish from across the salmon stocks runs. Chinook salmon adults were purchased, and tissue plugs were taken for laboratory analysis. We then collected adult fish from three other time periods over the late Summer and Fall _2023. Five chinook salmon fillets were typically composited to create one composite sample. One sample collection also purchased five coho salmon adults for a single Coho salmon composite sample. A total of 6 composite adult salmon samples were collected and submitted. These represented 2 wild-only Chinook composite samples, 2 mixed wild-hatchery Chinook composites, 1 hatchery Chinook only composite, and 1 mixed-hatchery-wild Coho composite sample.

Juvenile Salmon Samples

For the collection of juvenile anadromous fish, “natural mortalities” observed and recovered from the Fish Passage and Counting facilities at Bonneville Dam were retained, labeled and frozen on site. These natural mortalities from the facilities were opportunistic samples, where composites were created when at least 3 whole body juveniles of the sample species and similar collection dates were composited, as available. When staff at the facility observed juvenile salmon mortalities, which were infrequent, they noted the date, time, condition, placed them in an individual zip-lock bag and placed them in a freezer for later transfer to USGS. Juvenile composite samples were collected throughout the juvenile migration season (e.g., April to June 2023). The condition of fish collected from the Fish Passage Center mortalities was documented as either A) freshly dead; silver, bright and firm, B) recently dead; a little darkening, but firm, or C) obvious signs of decay. All juvenile salmon samples collected were either condition A or B, 43 and 57%, respectively. However, no condition C fish were selected for laboratory analysis. From this collection time window, 3 composite whole-body juvenile Chinook samples were generated. Two of these juvenile Chinook composites (from the May to June dates) were all hatchery fish, and the third sample from June was a three wild-only juvenile Chinook composite sample.

Table 2. Bonneville Reservoir Fish Sampling Site Locations and Dates.

Site Name	Latitude	Longitude	Sampling Dates
Alternate 1 Blackberry Bch	45.6861	-121.8584	10/10/2023, 10/17/2023, 4/17/2024
Alternate 4 Eighteenmile Isld	45.68968	-121.43712	10/18/2023, 4/17/2024
Chamberlain	45.69526	-121.31044	10/19/2023
Drano Lake	45.7155622	-121.6169084	10/17/2023
Hatchery	45.72719	-121.54427	10/18/2023
InSitu	45.6965	-121.4585	10/18/2023
Memaloose	45.6994	-121.35786	10/19/2023
Rocky Island	45.64638	-121.20378	10/20/2023, 4/18/2024
Squally Point	45.66411	-121.21596	10/19/2023, 4/18/2024
The Dalles	45.60855	-121.18981	10/20/2023
Wind Mountain	45.70245	-121.76031	10/10/2023, 10/17/2023

Resident Fish

For the collection of resident fish in Bonneville Reservoir, we determined the sampling locations using a probabilistic site selection method where sample sites are selected randomly from a pool of possible sites in Bonneville Reservoir, see Site Selection discussion above. As part of the Columbia River Mainstem Fish Tissue and Water Quality Monitoring Program framework, linear Generalized Random Tessellation Stratified (GRTS) sample frames are being developed that offer scenarios showing the distribution of sampling locations across the study area from Bonneville Dam to the Canadian border. The sampling frames are based on a river-center line GIS data layer developed from the high-resolution version of the National Hydrography Dataset (NHD-Plus HR, see: https://www.usgs.gov/national-hydrography/nhdplus-high-resolution). For the pilot study, the sampling frame included every km-long segment of the Columbia River from Bonneville Dam to The Dalles Dam. From this sample frame, 10 samples were randomly selected. Fish and resident fish tissues were then collected from these 10 fish

collection areas. Note Table 2 lists 11 sites, that includes an “oversample” site after no fish were collected at one of the targeted sites.

Fish collection areas were defined as follows. The mid-channel position of the GRTS sample point was located. Left and right banks were then located alternately from these mid-point locations, perpendicularly from the direction of channel orientation towards each shoreline. The bank site locations were sampled on the right bank for even-numbered sites, or the left bank for odd-numbered sites. Long rectangular sections were sampled from within 30 m of the shoreline and extending upstream 500m of the shoreline starting point. If insufficient fish were collected in the upstream rectangular sampling frame, a second rectangular sampling frame beginning at the shoreline point and extending downstream was additionally sampled. Fishing gear were deployed in these rectangle frames until the target sample size was met, or the level of effort was exceeded. Fishing gear began first with minnow traps and slinky pot (see Photo 2) deployment, followed by baited long lines and ultimately electrofishing in the target frame if needed. Overall, minnow pots and slinky pots were surprisingly ineffective, despite the use of various baits and attractants. Electrofishing was by far the most successful method of fish collection.

Fish collection in the Columbia River poses the risk of encountering fish species listed as threatened or endangered. As such, fish collection permits from both the Federal government, in this case NOAA Fisheries for Federally endangered salmon, and the State of Oregon for state level permits were secured. These permits came with collection restrictions and instructions, including the use of the least invasive collection methods first, and only progressively more aggressive methods as needed. We first attempted sampling for fish using the alternate methods of hoop nets, minnow traps and hook and line in early October 2023, however, these techniques at this time of year (e.g., water temperatures and daylight levels falling) were not successful. We then requested from the permitting agencies to use boat electrofishing. Once sampling by electrofishing the following week in October, we were able to collect foraging fish at almost all of the ten sites, however, we were only able to collect enough predator fish at five sites. We believe this is because with the falling water temperatures, that most predators were spending their time in deeper waters beyond where the electrofishing was effective. Thus we returned to the sites where we did not get our full samples in April and were able to collect fish at all the sites besides the two that were unsamplable, due to thick macrophytes beds and increased depth with very little habitat.

All fish were placed in live wells on the boat, then once collection was completed, fish were sorted into the various species and fish appropriate for tissue collection were placed in large buckets and transferred alive to the mobile laboratory for processing. Individual fish were processed, euthanized with a sharp blow to the head, length and weights were measured, and assigned to the appropriate Forage or Predator composite sample, according to species prioritization outlined below in Appendix A. For Predators, a tissue plug was removed from the left side of the fish just below the dorsal fin, weighted, and composited equally across the composited fish. For forage fish composite samples, whole fish were composited. Tissues were placed the tissue plug in a clean sampling jar; 5 or more

individual fish are composited into the same labeled jar and then placed on dry ice for transport to the freezer at the laboratory. Fish sex and external anomalies were inspected and recorded.

A detailed list of all sediment and fish sites is provided in Appendix B.

Analytical Chemistry Methods

Following collection of the sediment and fish tissue samples, samples were placed on wet ice for the day (<8 hours) and transported to the laboratory holding facility in Lake Oswego, OR or placed immediately on dry ice if the field storage was going to be >8 hours before transfer to a freezer. Samples were then frozen at -18C until shipping to the laboratory. Due to several project delays (permitting, temperature windows, weather, contracting and payment delays between the funding agencies and laboratories), most samples exceeded their holding times of 1 year. Samples were shipped on dry ice to SGS-AXYS Analytical, with the sediment and dissected tissue samples being sent to their Sydney, BC laboratory and the whole fish samples to Wilmington, NC for homogenization prior to forwarding to Sydney, BC.

The samples were extracted and cleaned up for all three organic-method chemistry suites according to SGS AXYS Method MLA-013: Analytical Method for The Determination of: Polybrominated Diphenyl Ethers, PCB Congeners, Chlorinated Pesticides, Technical Toxaphene, Toxaphene Congeners/Parlars and Polychlorinated Dibenzodioxins and Furans using Co-Extraction Techniques. Following this common extraction, analysis of the PCBs followed a method analogous to EPA 1668C method of SGS AXYS Method MLA-210: Analytical Method for the determination of 209 PCB Congeners by GC-MS/MS, which had equal or superior detection limits and performance. A summary of this and other analytical methods are provided in Appendix C- Analytical Method Summary, below. For PBDEs, analysis procedures were in general accordance with “USEPA Method 1614A: Brominated Diphenyl Ether Congeners in Soil, Sediment and Tissue by HRGC/HRMS” as documented in SGS AXYS Method MLA-033. Organochlorine analysis followed AXYS Method MLA-228: Analytical Procedures for Organochlorine Pesticides by GC-MS/MS.

See Appendix C for more analytical method details.

Photo 2. Commercial "slinky" or black cod pot used in Columbia River.

Results

Fish Collection

Two of the original fish sites in the GRTS sample draw could not be sampled, the first from excessive and thick macrophyte beds that prevented boat access and successful pot deployment and the second was too deep with a shoreline cliff face that provided very little and poor fish habitat. As a result, two "oversample" sites were identified from the original GRTS draw, and those were sampled as Alternate sites. Resident Fish collection in the Bonneville demonstrated that resident fish can be caught across a number of micro-habitats, but densities and capture efficiency varied widely by location and date.

Smaller, resident fish in the “forage” fish target size category was dominated by Northern Pikeminnow (*Ptychocheilus oregonensis*) and Prickly Sculpin (*Cottus asper*). Fish in the “predator” resident fish category was overwhelmingly dominated by Smallmouth Bass (*Micropterus dolomieu*) and rarely by Yello Perch (*Perca flavescens*) or larger Northern Pikeminnow. Table 3 provides a summary of the retained fish species and composite counts of those species, in the Forage and Predator categories for Resident fish.

Table 3. Total Resident Fish Collected via Electrofishing.

Site Name	Collection Date	Sample Type	Species	Count
Alternate 1	10/17/2023	Forage	SMB	7
	10/10/2023	Predator	SMB	2
	10/17/2023	Forage	AS	20
	10/17/2023	Forage	NPM	20
	10/17/2023	Predator	SMB	1
	4/18/2024	Predator	SMB	5
Alternate 4	10/18/2023	Predator	SMB	2
	10/18/2023	Forage	PS	10
	4/17/2024	Predator	SMB	2
	4/17/2024	Consumption	LSS	5
	4/17/2024	Forage	SMB	10
Chamberlain	10/19/2023	Predator	YP	3
	10/19/2023	Predator	SMB	1
	10/19/2023	Forage	PS	10
	10/19/2023	Forage	BK	7
Drano Lake	10/17/2023	Predator	SMB	2
	10/17/2023	Predator	YP	5
	10/17/2023	Forage	NPM	10
	10/17/2023	Forage	PS	2
Hatchery	10/18/2023	Predator	SMB	5
	10/18/2023	Forage	NPM	12
InSitu	10/12/2023	Predator	SMB	3
	10/12/2023	Predator	NPM	1
	10/18/2023	Predator	SMB	5
	10/18/2023	Forage	PS	9
Memaloose	10/19/2023	Predator	NPM	2
	10/19/2023	Forage	NPM	10
The Dalles	10/20/2023	Predator	SMB	3
	10/20/2023	Forage	PS	5
Squally Pt.	10/19/2023	Forage	NPM	10
	10/19/2023	Forage	SMB	10
Rocky Island	10/20/2023	Forage	SMB	10
	10/20/2023	Forage	PS	5
	4/18/2024	Predator	SMB	4

	4/18/2024	Consumption	LSS	5
Wind Mountain	10/17/2023	Predator	YP	3
	10/17/2023	Forage	PS	8
	10/17/2023	Forage	SMB	10

[SMB, Smallmouth Bass; AS, American Shad; NPM, Northern Pikeminnow; PS, Prickly Sculpin; LSS, Largescale Sucker; YP, Yellow Perch;]

Analytical Chemistry- Quality Assurance Summary

A detailed description of each of the analytical chemistry methods (Organochlorines, PCBs, PBDEs) is provided in Appendix C below. Overall performance and quality of the laboratory analytical chemistry data was reviewed with close examination of the Quality Control and Environmental sample data from similar dates and times. For each of the four categories of pollutant measured (PCBs, PBDEs, Organochlorines and Mercury), overall Quality Assurance data indicates high quality data, with blank and relative difference data indicating better than usual for these trace level organic compound methods, but spike recovery data more variable and larger than typical targets. Detection frequencies in the blanks above the Quantitation Limit (QL) were 2.7% for the Organochlorines, 3% for PCBs, and 13.7% for the PBDEs, and 0 for the mercury blanks at the detection limit, as outlined below in Table 4.

Table 4. Blank Data Summary Tables.

Organochlorine Laboratory Blanks				
No. of Blank Samples	Number. measured results	Below DL	Below QL	Detections above QL
110	3	0	0	3

PCB Laboratory Blanks				
No. of Blank Samples	Number measured results	Below DL	Below QL	Detections above QL
630	122	179	282	19

Polybrominated Diphenyl Ethers- Laboratory Blanks Summary				
No. of Blank Samples	Number measured results	Below DL	Below QL	Detections above QL
138	37	0	18	19

Total Mercury- Laboratory Blanks Summary				
No. of Blank Samples	Number measured results*	Below DL	Below QL	Detections above QL
4	4	4	0	0

*Laboratory reported daily method blank detections at levels 10-100X below the detection limit.

Analytical precision was assessed in three lab duplicate samples. For the Organochlorines, 3 duplicate samples indicated a Relative Percent Difference (RPD) range of 0-14, 0-12, and 0-29% across the suite of 28 Organochlorines compared in the Duplicate samples. For PCBs, three duplicate samples indicator Relative Percent Difference scores of 0-102%, 0-109%, and 0-37% across the suite of 209 PCB Congeners in that method. With the lighter, less chlorinated PCBs generally showing greater variability than the heavier, more chlorinated congeners. For PBDEs, the three duplicate samples indicated Relative Percent Differences of 1-21%, 10-15%, and 1-21% across the 59 Congeners in the analytical method. These RPD values for the three duplicate samples are shown in Tables 5a-5e. The RPD scores for the Mercury analysis is shown in Table 5d. With the exception of a sediment sample comprised mostly of sand and gravel, the RPD scores were very good, with most less than 5%.

A third type of quality assurance sample is known as Matrix Spike, or Matrix Spike Recovery (MSR) samples. These are Quality Assurance samples where a known amount of the compound(s) be

measured are added to a similar media type or to a sub-sample or split of the environmental sample. The technique assesses method accuracy by evaluating how much of a known sample is measured (recovered) during the sample preparation and analytical process. For trace organic analysis, a recovery of +/- 30-50% of the known, added amount is to be expected. Tables 6a-6c summarized the Spike Recovery data for each of the three organic chemistry classes, Organochlorines, PCBs and PBDES. The percent recoveries of the known, spiked amount was mostly in this range for the PCBs and PBDEs, while the variability in the MSR values for Organochlorines was much more widely variable. Overall, as is typical of trace-level organic chemistry, the MSR data indicates that there is typically some loss of the target compound during extraction and analysis and thus, the methods tend to underestimate, rather than underestimate, the actual environmental concentration. For the Mercury analysis, there were 21 MSR samples that all showed recoveries in the 100-110% range across both tissue and sediments. Data associated with this study were not recovery corrected, either here or in the database.

Data Validation reports from the Analytical Chemistry lab provide laboratory insights into sample-method performance. This notes from the laboratory Data Validation reports are re-printed below. The Data Validation report from the Analytical Lab associated with the Organochlorine results noted the following points;

> 1) "The QC samples were prepared alongside the client samples and were subjected to the same analytical procedures. The client sample data were evaluated in relation to the batch QC sample results. "Sample analyte concentrations are not blank corrected. Sample data should be evaluated with consideration of analyte levels in the procedural blanks.
> 2) "By virtue of the isotope dilution/internal standard quantification procedures, data are recovery corrected for possible losses during extraction and clean up procedures. " All initial calibration, calibration verification, procedural blank, OPR, duplicate and labeled compound recovery specifications were met, with the exception of the following- The recoveries of surrogate L-endrin aldehyde fell below the lower method control limits across the batch, as indicated by the V flags on Form 2 of the report pages. The results are recovery corrected, and the recoveries are sufficient for accurate quantification.
> 3) "All samples were extracted outside the method recommended sample hold time of 1 year from the date of collection. Samples '14' and '16' and the duplicate required an additional instrumental run, as indicated by the suffix 'i' added to the AXYS IDs."

The Data Validation report associated with these PCB results noted the following points;

> "1) The QC samples were prepared alongside the client samples and were subjected to the same analytical procedures, and client sample data were evaluated in relation to the batch QC sample results,
> 2) Sample analyte concentrations are not blank corrected- sample data should be evaluated with consideration of analyte levels in the procedural blanks,
> 3) By virtue of the isotope dilution/internal standard quantification procedures, data are recovery corrected for possible losses during extraction and clean up procedures.

4) All initial calibration, calibration verification, procedural blank, OPR, duplicate and labeled compound
recovery specifications were met, with the exception of the following: The recoveries of surrogates 1L and 3L in sample 33 (AXYS ID L42839-13) fell below the lower method control limits, as indicated by the V flags on the report Form 2.
5) All samples were extracted outside the recommended sample hold time of 1 year from the date of collection.

The Data Validation report associated with these PBDE results noted the following points.

1) "The QC samples were prepared alongside the client samples and were subjected to the same analytical
procedures. The client sample data were evaluated in relation to the batch QC sample results.
2) "Sample analyte concentrations are not blank corrected. Sample data should be evaluated with
consideration of analyte levels in the procedural blanks.
3) "By virtue of the isotope dilution/internal standard quantification procedures, data are recovery
corrected for possible losses during extraction and clean up procedures.
4) "All initial calibration, calibration verification, procedural blank, OPR, duplicate, and labeled compound recovery specifications were met, with the exception of the following:
    1. BDEs 7, 10, and 30 are not reportable in this batch, as indicated by the NQ flags on the report
    pages. These three analytes are not included in the database.
    2. The values for BDEs 12/13, 116, and 128 are underestimated in this batch. The true concentrations may be two to five times higher.
    3. The recoveries of several surrogates in sample 33 (AXYS ID L42839-13), and the recoveries of
    surrogate 209L in samples 4 and 6 (AXYS IDs L42839-3 & -4), fell below the lower method
    control limits, as indicated by the V flags.
5) "All samples were extracted outside the method recommended sample hold time of 1 year from the date of collection.
6) "In the analysis of matrix spikes, good recoveries are expected where the contribution from the unspiked sample is significantly below the spiked amount. Where the contribution from the sample exceeds the spiked amount, the calculated recoveries may be high, or low, or even negative."

Laboratory notations from the Mercury laboratory only noted the poor RPD scores for one sediment sample with a high percent sand and gravel. This triplicate sample was evaluated twice, in triplicate, with similar highly variable results.

Table 5a. Relative Percent Difference values from Duplicate Performance Data for Organochlorines.

Organochlorine Duplicate Analysis Summary			
Duplicates	**Sample 14 RPD (%)**	**Sample 27 RPD (%)**	**Sample 28 RPD (%)**
All OCs (min)	0	0	0
All Ocs (max)	27	12	29
% Lipid	13	ND	4
% Moisture	1	12	1
2,4'-DDD	5	ND	9
2,4'-DDE	3	ND	9
2,4'-DDT	7	ND	5
4,4'-DDD	4	ND	7
4,4'-DDE	14	ND	1
4,4'-DDT	3	ND	5
Aldrin	ND	ND	ND
alpha-Endosulphan	ND	ND	1
beta-Endosulphan	ND	ND	ND
Chlordane, alpha (cis)	2	ND	0
Chlordane, gamma (trans)	1	ND	5
Chlordane, oxy-	3	ND	3
Dieldrin	0	ND	2
Endosulphan Sulphate	3	ND	5
Endrin	7	ND	0
Endrin Aldehyde	ND	ND	14
Endrin Ketone	ND	ND	29
HCH, alpha	27	ND	8
HCH, beta	ND	ND	5
HCH, delta	ND	ND	ND
HCH, gamma	1	ND	7
Heptachlor	ND	ND	ND
Heptachlor Epoxide	3	ND	2
Hexachlorobenzene	24	ND	5
Methoxychlor	ND	ND	ND
Mirex	20	ND	1
Nonachlor, cis-	8	ND	1
Nonachlor, trans-	6	ND	4

Table 5b. Relative Percent Difference values from Duplicate Performance Data for Polychlorinated Biphenyls (PCBs).

PCB Duplicates- Relative Percent Difference			
Compounds	Sample 14 RPD	Sample 27 RPD	Sample 28 RPD
All (min)	0	0	0
All (max)	109	102	34
TOTAL PCBs	4	25	1
Total Monochloro Biphenyls*	3	89	18
Total Dichloro Biphenyls*	10	12	6
Total Trichloro Biphenyls*	5	14	4
Total Tetrachloro Biphenyls*	5	4	0
Total Pentachloro Biphenyls*	8	4	1
Total Hexachloro Biphenyls*	4	17	1
Total Heptachloro Biphenyls*	13	16	1
Total Octachloro Biphenyls*	13	11	6
Total Nonachloro Biphenyls*	9	39	8
Decachloro Biphenyl*	na	nd	9

*Average RPD value per Chlorination category, per duplicate sample.

Table 5c. Relative Percent Difference values from Duplicate Performance Data for Polybrominated Diphenyl Ethers (PBDEs) .

Polybrominated Diphenyl Ether Duplicates- Relative Percent Difference			
	Sample 14 RPD (%)	Sample 27 RPD (%)	Sample 28 RPD (%)
13C12-4,4'-DiBDE	5	15	4
13C12-2,4,4'-TriBDE	5	17	5
13C12-2,2',4,4'-TeBDE	3	15	6
13C12-3,3',4,4'-TeBDE	1	13	7
13C12-2,2',4,4',5-PeBDE	2	14	7
13C12-2,2',4,4',6-PeBDE	1	13	7
13C12-3,3',4,4',5-PeBDE	1	14	7
13C12-2,2',4,4',5,5'-HxBDE	2	10	17
13C12-2,2',4,4',5,6'-HxBDE	2	13	17
13C12-2,2',3,4,4',5',6-HpBDE	2	14	21
13C12-2,2',3,3',4,4',6,6'-OcBDE	2	10	21
13C12-2,2',3,3',4,4',5,5',6,6'-DeBDE	21	15	18
13C12-2,2',3,4,4',6-HxBDE	6	10	16
% Moisture	1	12	1
% Lipid	13	n/a	4

Table 5d. Relative Percent Difference values from Triplicate Performance Data for Total Mercury (THg) .

Daily Triplicate Results Total Mercury- Relative Percent Difference			
DATE OF ANALYSIS	SAMPLE INFO	ANALYTICAL ATTEMPTS	PERCENT DIFFERENCE
1/6/2026	L42839-6	3	2.7%
1/6/2026	L42845-1	3	1.5%
1/6/2026	L42845-9	3	2.2%
1/7/2026	L42845-3	3	4.3%
12/30/2025	MSC284BZ	3	1.8%
12/31/2025	MSC286BZ	3	26.5%*
12/31/2025	MSC286BZ	3	48.6%*

*Sediment sample with high gravel content, triplicate RPD tested twice.

Table 6a. Matrick Spike Recovery Data (in %) from various QA Samples for Organochlorine Pesticides.

Organochlorines Matrix Spikes					
Sample	Sample Type	Analyte Type	Number of Samples	Percent recovery (min)	Percent recovery (max)
Spiked Matrix	OPR	REG	84	95	114
Spiked Matrix	OPR	SURR	84	39	284
Lab Blank	MB	REG	84	82	109
Lab Blank	MB	SURR	81	16.8	121
14	MS	REG	27	na	na
14	OPR	BLNK	27	na	na
19	MS	REG	28	94.7	114
19	MS	SURR	27	35.5	83
27	MS	REG	27	na	na
27	OPR	BLNK	27	na	na
28	MS	REG	27	na	na
28	OPR	BLNK	27	na	na
32	MS	REG	28	98	284
32	MS	SURR	27	22.3	85.2
35	MS	REG	28	90.8	118
35	MS	SURR	27	58	127

Table 6b. Matrix Spike Recovery Data (in %) from various QA Samples for PCBs.

PCB Matrix Spikes				
Sample	Sample Type	Analyte Type	Percent recovery (min)	Percent recovery (max)
Spiked Matrix	OPR	C_UP	48.2	90.4
Spiked Matrix	OPR	REG	78.1	113
Spiked Matrix	OPR	SURR	19.4	98.6
Lab Blank	MB	C_UP	71.8	90.1
Lab Blank	MB	SURR	28.3	99
19	MS	REG	87.8	107
19	MS	C_UP	70.4	92
19	MS	SURR	32.3	98.1
32	MS	REG	88.7	128
32	MS	C_UP	89.9	94.1
32	MS	SURR	56.1	93.3
35	MS	REG	88.2	113
35	MS	C_UP	83.9	89.7
35	MS	SURR	64.5	91.1

Table 6c. Matrix Spike Recovery Data (in %) from various QA Samples for PBDEs.

Polybrominated Diphenyl Ethers- Matrix Spikes				
Sample	Sample Type	Analyte Type	Percent recovery (min)	Percent recovery (max)
Spiked Matrix	OPR	C_UP	71	99.9
Spiked Matrix	OPR	REG	87.1	195
Spiked Matrix	OPR	SURR	49.7	98.5
Lab Blank	MB	C_UP	69.3	128
Lab Blank	MB	SURR	37.7	134
19	MS	C_UP	71.6	74.8
19	MS	SURR	57	91.1
32	MS	C_UP	86.1	86.1
32	MS	SURR	32.7	101
35	MS	C_UP	87.4	87.4
35	MS	SURR	54.6	91

Table 6d. Matrix Spike Recovery Data (in %) from various QA Samples for Total Mercury Analysis.

Total Mercury Results- Certified Reference Materials			
Sample Type	Date of Analysis	CRM USED	% Recovery
Sediment	12/30/2025	IAEA 456	105%
Sediment	12/30/2025	IAEA 456	108%
Sediment	12/30/2025	IAEA 456	112%
Sediment	12/31/2025	IAEA 456	99%
Sediment	12/31/2025	IAEA 456	107%
Sediment	12/31/2025	IAEA 456	103%
Tissue	1/6/2026	IAEA 407	108%
Tissue	1/6/2026	IAEA 407	108%
Tissue	1/6/2026	IAEA 407	109%
Tissue	1/6/2026	IAEA 407	108%
Tissue	1/6/2026	IAEA 407	109%
Tissue	1/6/2026	IAEA 407	110%
Tissue	1/7/2026	IAEA 407	109%
Tissue	1/7/2026	IAEA 407	109%
Tissue	1/7/2026	IAEA 436	106%
Tissue	1/7/2026	IAEA 436	110%
Tissue	1/7/2026	IAEA 407	108%
Tissue	1/7/2026	IAEA 436	108%
Tissue	1/7/2026	IAEA 407	109%
Tissue	1/7/2026	IAEA 436	107%
Tissue	1/7/2026	IAEA 407	108%
Tissue	1/7/2026	IAEA 436	105%

Analytical Chemistry - Environmental Sample Results

An overview of the dataset collected in this Pilot Implementation of a Columbia River Mainstem Monitoring Program is presented below. It summarizes sediment samples from 10 locations (7 sites collected successfully) and 19 Resident Fish Tissue composite samples (between predator and forage fish categories) across 10 fish collection locations in the Booneville Reservoir. This Pilot study and dataset also includes three, 3-5 fish composite tissue samples from adult Chinook fillets and one 3-fish composite sample of Coho fillets. Three, composite whole-body, juvenile hatchery Chinook samples were also evaluated for pollution loads. The total analytical endpoints generated in this study are over ten thousand and are not fully interpreted here. Rather, this report documents the methods, a data quality review, a high-level summary of what was measured and a summary of feasibility and lessons learned. Data for this project can be retrieved from EPA Water Quality Exchange (WQX) or the USGS National Water Information System (NWIS) by searching on the 'Station Number' for these sites, as identified in Appendix B. Data retrieval from the USGS NWIS portal can be found here (https://waterdata.usgs.gov/nwis/qw) or via the US EPA Water Quality Exchange site (https://www.epa.gov/waterdata/water-quality-data). However, full interpretation of the dataset generated here is to come under a separate cover and task.

Polychlorinated biphenyls (PCBs) were analyzed for 209 individual congeners in both the tissue and sediment samples. Total PCBs were calculated for each sample as well as subgroup totals. In all but one tissue sample, the highest two total PCB subgroups were Total Hexachloro Biphenyls and Total Pentachloro Biphenyls. The one outlier tissue sample had Total Tetrachloro Biphenyls and Total Hexachloro Biphenyls as its top two subgroups. In all tissue samples the concentrations of these subgroups accounted for 55 to 77 percent of total PCBs. Individual congeners in all tissue samples with the highest concentrations were either PCB 153 or 129, in 68 and 32 percent of samples respectively. Like the tissue samples the subgroup Total Hexachloro Biphenyls had the highest concentrations in all but one sediment sample. Four of the six sediment samples had the subgroups Total Hexachloro Biphenyls and Total Dichloro Biphenyls accounting for the highest concentrations in these samples and ranging from 36 to 42 percent of total PCBs in those samples. The subgroup Total Monochloro Biphenyls had the highest concentrations of the remaining two samples and combined with either Total Hexachloro Biphenyls or Total Dichloro Biphenyls these concentrations accounted for 28 and 48 percent of total PCBs. In all sediment samples PCB congener 86 had the highest concentrations and accounted for 16 to 30 percent of total PCBs.

In both the tissue and sediment samples there were a total of 28 individual organochlorine pesticide compounds analyzed. However, of these compounds the DDT degradation products accounted for the highest concentrations in both tissue and sediment samples. Of the DDT degradation products in both tissue and sediment samples p,p'-DDE had the highest concentration in every sample, except for one sediment sample where p,p’-DDT had the highest concentration. The percentage of p,p'-DDE of total DDT in all the tissue samples ranged from 71 to 88 percent. In sediment samples where p,p'-DDE was the highest concentration, the percentage of total DDT ranged from 66 to 77 percent. The one sediment sample where p,p’-DDT had the highest concentration, that accounted for 93 percent of total DDT for that sample. Of the non-DDT compounds analyzed in the tissue samples, three compounds, Dieldrin, Hexachlorobenzene, and Nonachlor (trans), had the highest concentrations, accounting for 11, 43, and 46 percent of all samples, respectively. When the non-DDT compounds are summed, the samples where Dieldrin had the highest concentrations, Dieldrin accounted for 24 to 25 percent of the total non-DDT compound concentrations. Hexachlorobenzene ranged from 27 to 56 percent of total non-DDT compound concentrations and Nonachlor (trans) ranged from 23 to 44 percent of total non-DDT compound concentrations. The only non-DDT compound detected in the sediment samples was Hexachlorobenzene and was detected in four of the six samples.

There were 46 primary analytes measured in the PBDE method across 34 different samples of fish tissue and sediment. Overall, this resulted in a detection frequency of 46% across both media. In tissues, the most common congener was the 2,2',4,4'-TetraBDE, also known as BDE-47, and this congener also had the largest concentrations reported in tissues. In sediments, the more fully brominated congeners, like DecaBDE and NonaBDEs were much more common than in tissues, and- along with TetraBDE-, accounted for the largest concentrations reported in sediments. Overall, the magnitude of the sediment concentrations were substantially lower than those measured in tissues by several fold, with regard to the most common and higher concentration PBDEs.

The results for the total mercury measures were largely consistent with previous studies in the Columbia River. Sediment concentrations were generally low, with the highest measured concentration a bit off an outlier at 0.42 ug/kg dw, which was 4 times the next highest reported sediment concentration, and was collected at a location near the city of The Dalles. Overall, total mercury concentrations in fish tissue were an order of magnitude higher, on dry weight basis, than in sediments. As expected, the highest mercury fish tissue concentrations were in the larger, more predatory species such as Northern Pikeminnow and Smallmouth Bass.

Juvenile salmon had the lowest total mercury concentrations from their whole-body composite samples, followed by Coho salmon and Chinook salmon composite fillet tissue samples. Prickly sculpin and Yellow perch had intermediate tissue concentrations between the salmon species and the piscivorous, and generally older, Northern pikeminnow and Smallmouth Bass samples. Only one sample, a composite of smaller, Northern pikeminnow retained as representative of 'forage fish' in the sampling frame, exceeded 300 ug/kg on a wet weight basis.

Table 7. Summary statistics of compounds analyzed in fish and sediment samples collected in the Columbia River from Bonneville Dam to near The Dalles, Oregon.

[Abbreviations: µg/kg, microgram per kilogram; DDT, dichlorodiphenyltrichloroethane; ND, not detected; PBDE, polybrominated diphenyl ether; PCB, polychlorinated biphenyl]

Sample type	Summary statistic	Organochlorine pesticides and pesticide degradation products (not including DDT)	DDT degradation products	PBDE congeners	PCB congeners
All Fish (µg/kg, wet weight)	Min	nd	0.0135	nd	nd
	Max	3.82	36.1	8.24	2.32
	Mean	0.201	2.43	0.077	0.046
Sediment (µg/kg, dry weight)	Min	nd	nd	nd	nd
	Max	0.00699[1]	1.740	0.02690	0.00818
	Mean	0.00588[1]	0.129	0.00176	0.00049

[1]Only compound detected was hexachlorobenzene

Table 8. Summary statistics of compounds analyzed in fish and sediment samples collected in the Columbia River from Bonneville Dam to near The Dalles, Oregon.

[Abbreviations: µg/kg, microgram per kilogram; DDT, dichlorodiphenyltrichloroethane; ND, not detected; PBDE, polybrominated diphenyl ether; PCB, polychlorinated biphenyl]

Contaminant Class		Total concentrations measured (µg/kg wet weight)							
		Chinook (adult)	Chinook (juvenile)	Coho (Adult)[1]	Northern pikeminnow	Prickly sculpin	Smallmouth bass	Yellow perch	Sediment
∑Organochlorine pesticides and pesticide degradation products (not including DDT)	Min	5.78	0.92		1.63	1.26	0.29	0.15	ND
	Max	9.63	1.74	3.15	2.78	3.10	1.49	0.21	0.01 [2]
	Mean	7.18	1.44		2.06	2.01	0.76	0.18	0.01 [2]
∑DDT degradation products	Min	4.96	4.92		23.82	9.08	6.33	2.36	0.02
	Max	12.26	19.24	9.05	26.28	22.28	42.44	2.77	1.88
	Mean	7.99	12.89		21.32	15.50	17.75	2.54	0.34
∑PBDE congeners	Min	0.35	1.15		1.43	1.1	0.44	0.18	0.04
	Max	1.04	7.34	0.47	1.58	9.97	5.12	0.61	0.09
	Mean	0.72	3.93		1.52	3.82	1.58	0.39	0.06
∑PCB congeners	Min	7.29	4.00		6.5	3.48	2.00	0.68	0.02
	Max	15.2	9.8	6.13	7.91	14.55	13.6	0.84	0.05
	Mean	10.57	6.51		7.38	7.69	6.60	0.74	0.04
Total Mercury	Min	61.9	21.7		20.0	32.8	26.2	75.4	3.0 [3]
	Max	89.1	25.0	49.9	359.3	75.1	293.1	103.7	75.7 [3]
	Mean	76.4	23.4		111.9	59.6	154.7	89.6	25.7 [3]

[1]Only one sample collected.

[2]Only compound detected was hexachlorobenzene.

[3] Sediment Mercury on a dry weight basis**.**

Summary

Despite several delays, primarily around permitting constraints, suitable fish collection window and seasons with suitable weather, the study was successful and met nearly all its expected sample targets. With the use of a stratified, random design- that enables extrapolation of results to the entire reservoir- it is expected that some locations will be unsuitable for sampling. This was the case for 3 of the sediment sites that were bedrock or cobbles with little to no fine materials, and at one fish sampling location with insufficient predators for sample analysis. This challenge is commonly encountered in randomized designs and the planning for and use of "oversample sites" was utilized and beneficial in this study. Obtaining a NOAA approved fish collection permit in waters with an abundance of ESA-listed species was not a trivial task, and came with multiple sampling time-frame considerations that added to several scheduling delays. An abundance of additional time and flexibility with alternative gear types, starting with least invasive and moving to more invasive should be expected and planned for in such waters.

This is a Summary Data Report and was not intended to be a data interpretation report. There are numerous considerations that ought to be taken into account when applying this data to other benchmarks or screening values, to other studies, and to historical data that are not addressed here. Important and relevant datasets that should be considered when interpreting this work include but are not limited to: Herger (2016), Lundin (2019) and (2021), Nilsen (2014), Sloan (2010), Schick (2022), West (2017), US EPA (2002) and (2009), A review of the concentrations, as summarized in Table 8, indicates a couple of insights. First, for the majority of pollutants, the sediment mean concentrations were generally 5-10 times lower than the mean concentration in other fish tissues. Of the three organic pollutant classes, the sum of DDT compounds dominated measured concentrations of the other two classes, regardless of species or media type, and despite much fewer compounds (ie. 6) summed than with the PCBs or Organochlorine sums. However, relative toxicity can vary between these contaminant groups by more than an order of magnitude. This report does not evaluate relative toxicity. Northern pikeminnow and smallmouth bass tended to have much higher tissue concentrations, especially for DDT, than the other species. This is somewhat expected from the higher piscivorous diet of these species. Of note is the relatively comparable r concentrations of DDT and PBDEs in juvenile salmonids as to other species sampled. These juvenile salmon are around a year old and yet carry a pollution load in DDT and PBDEs as juveniles on par with that of much older, resident fish in the reservoir. Lastly, in the juvenile to adult salmon comparisons show growth dilution of tissue concentrations for DDT and PBDEs, but concentration increases with age for PCBs and Organochlorines. Adult Chinook composite samples tended to be higher than the single coho composite sample, which might be expected as adult Chinook salmon are generally older, have higher fat content, and spend more time in the ocean and typically migrate farther than coho salmon. Overall, these data suggest differential exposure and tissue accumulation during the life cycle of these fish between these four (treating DDT as its own category) chemical suites.

Lastly, the multi-party collaboration in this study was sizable, complicated, and ultimately successful. In this study, the Yakama Nation lead as the Project Manager, USGS provided technical and analytical leadership, contracting and support, the Washington Department of Fish and Wildlife provided fish collection expertise and equipment, the US Army Corp of Engineers- and the Bonneville Fish Passage Center was a supportive partner, as were the commercial and subsistence Yakama Nation Tribal fishers who caught and supplied the fish from their traditional fish harvest methods. This Pilot is an example of successful, collaborative science and monitoring to generate data from a large river, that is both difficult and expensive to characterize and falls at a jurisdictional intersection between States, Tribes and Federal Agencies.

Lessons Learned

As this study was intended to be a Pilot of a larger, Mainstem Columbia Monitoring Program, some lessons learned from the Pilot effort are shared here. First, it is important to not underestimate the permitting process, requirements, and the constraints, in time and methods, that will likely come with a fish collection permit granted in waters with an abundance of ESA listed species. Early planning and additional time during the collection windows should be expected. Likewise, contracting with the participating agencies and the analytical laboratories often takes longer than expected, can be a bottleneck in project progression, and requires particular skill sets, experience, and capacity (in the contracting and in the analysis request,) . It is also important to note that the timing of official awards and approval notifications may be needed prior to agencies proceeding with contracting. Sub-awards also take time, and contracting delay is cumulative and not a parallel process. Therefore, we recommend advanced contract planning, clearly communicating contracting needs and timeline impacts, and reducing the number of contracting steps and entities. Additionally, for monitoring grant awards, we strongly encourage awarding agencies to work with their legislative and legal entities to lengthen the two year grant period. Two years is an insufficient time frame for complicated-interagency coordination and work that involves contracting, QAPP development and approval, sampling, analysis, data QA/QC, data analysis, and reporting and interpretation. Also, inflation driven laboratory price increases, particularly in 2023 and 2024, resulted in fewer samples being analyzed than originally expected.

Regarding the field work, wading and boat access to many sampling locations can be challenging to impossible, given the water level (ie. depth), the extent of and density macrophyte beds, and wind conditions on the Reservoir. Given this, flexibility, such as 'oversample sites' as was utilized here, is mandatory in a design. In addition to the 'upstream and downstream 500 meter' instructions from a given point, an additional set of latitudes and longitudes calculated and mapping beforehand designating the upstream and downstream extent of each fish collection frame would be helpful to the sampling team. The use of a second shuttle boat focused on shuttling samples to and from the collection team is recommended to enable fish dissection and processing to start sooner and minimize holding times before processing the samples (which did happened daily but often into the early evening hours). Hook and line and possibly night shocking could be the most effective method to capture predators. Obviously, daytime work is preferred to night work as it is logistically easier, cheaper, and safer, but greater catch efficiencies are usually encountered with night collection. A combination of first hook and line methods for the selected sites for predators, then the following day with electrofishing the same sites, as needed, to complete the target catch is recommended. Data from this survey cannot be used for catch per unit effort (CPUE), as each site was electrofished multiple times essentially performing a depletion as well as exposing the remaining fish to the electrofishing boat which resulted in them no longer being naïve to the gear and method. However, if desired and planned, methods could be modified to generate a CPUE for informing fisheries managers about forage and predatory relative abundance.

References

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Appendix A. Overview of Field Operations

This section describes the daily field activities. Included are discussion of field-crew configuration and responsibilities, boat operations, the flow of daily operations, collection permits, and general safety considerations.

A. Crew Configuration and Responsibilities

Field operations may require a two-or four-person sampling crew. In the field, each crew is supervised by a crew leader, who is responsible for daily operational planning, data quality, and safety. There is one dedicated boat operator.

B. Boat Operations

Each crew requires a boat for sampling. Care must be taken to maintain the boats in good order.

The boat trip from the ramp to the sample site may be many miles and may involve potential hazards. All boats should be equipped with a high-quality dash-mounted GPS/sonar unit with preloaded basemaps. Site location (latitude, longitude) data should also be loaded into the GPS units as waypoints. Crews should also carry navigation charts or an atlas. As part of pre-visit activities, crews should plan their route to make sure they use the closest suitable ramp, and that they are aware of any hazards, including rocks, rapids, and shoals. Also, crew must be aware of hazards associated with water level fluctuations including difficulties of trailering the boat and parking of vehicles out of the inundation zone.

C. Flow of Daily Operations

After navigating to the sample site, the crew leader evaluates whether the site is safe to sample under the existing conditions (sampleability may be apparent at the boat ramp). If the site is not safe to sample or moved per the constraints set forth in the protocol, the crew will select the nearest oversample site and proceed with the protocol described here. If the site is safe to sample, the crew will measure water temperature and take secchi disk depths within 30 m from shore. Then the crew will decide based on information known about this section of river and the time of year, water temperature, etc. which type of alternative fishing procedures are appropriate, these include minnow traps, hoop nets, baited setlines and hook and line.

Next, two baited setlines will be set parallel to shore within 30 m from the shoreline at the beginning and end of the 1000 m shoreline reach. If the number of fish captured by the other gears is insufficient or it's determined that hook and line is considered the more efficient method), the crew will conduct "hook and line" sampling within the boundary of the sampling site. Lastly, if needed (e.g., if all the other fishing gears are ineffectual), electrofishing is conducted over the 1000-m long shoreline reach (measured along the shoreline within 30 m offshore and follows along the shoreline for 500 m upstream and downstream). If needed, electrofishing may also be conducted at night if conditions

permit, as nighttime electrofishing may increase the chances of capturing target species. Electrofishing will be conducted only after prior approval from the NOAA.

Note: If used, minnow traps should be deployed at positions that are approximately 125, 250, and 375 m along the 500 m shoreline reach upstream and downstream depending on how many may be needed to collect fish. These are placed either directly adjacent to shore if riprap or rock feature is the shoreline type at a depth of approximately 2 m or in a shallow water area at a depth of approximately 2 m if the shoreline has a low gradient slope. Next, three hoop nets are set at positions that are approximately 125, 250, and 375 m along the length of the 1000 m shoreline reach within 30 m from shore. The exact placement of the traps and nets can be altered (e.g., if the location is unsafe or unsuitable for the gear) but traps and nets should not be set close enough so as to interfere with the fishability of the gear.

D. Collection Permits

States require collecting permits for fish sampling. Federal permits are also required. In some cases, Tribal permits may be required. Copies of the permits should be carried on boats when sampling. Crews should closely follow the specifications of the permit(s). These specifications may include notification of the permitting agency prior to field sampling, and submission of an annual report listing the fish collected and their disposition.

E. Site Location Adjustment

There can be situations where the actual “X” site is sampleable, but the survey reach used for the collection of fish (a 1000 m reach measured along the shoreline – 500 m upstream of X and 500 m downstream) cannot be sampled. Reasons for not sampling are either the presence of a confluence with a major tributary or a safety concern such as proximity to a dam. In these cases, if the 1000 m sample reach can be shifted so that the X-site is still in the reach, then the site can still be sampled. Otherwise, the site should be found ‘unsampleable’. The maximum distance that the site can be shifted is 500 m.

F. Site Verification: Determining Sampleability During the Sampling Visit

Upon arrival at a site the crew will verify the site location and verify that the site meets the protocols of sampleability. Information relevant to the site verification or conditions on the day of sampling is entered on the Site Verification Form (Figure 2). Fill out the header information: Site #, site name, date, crew personnel, coordinates for site from Table 1 (see Figure 3 for map of locations); the crews will use GPS to locate the site. The coordinates from Table 1 will be recorded as the GIS coordinates for the site. The acceptable tolerance goal is that the sampling station be established within the accuracy expected from a properly functioning GPS unit of the caliber that will be used for the study. The actual latitude/longitude coordinates of the sampling site as indicated on the GPS unit, are also recorded on the data sheet.

Fish Retention Protocols

Specific procedures for the deployment of fishing gear and for boat electrofishing can be found in Appendices A1-A5.

Fish Retention Procedures – [Note: Original QAPP called for a target individual fish-composite sample goal of 10 similar species-age fish for a composite sample. Upon collection day, and logistical and time considerations, this was rarely achievable. Instead, the fish tissue composite goal was modified to 5-fish composites of similar size fish of the same species.] Sample sets targeting five fish, same species, in small (forage fish) category- see below; and five fish, same species, in Large (predatory fish) category- see below across ALL gear types, were generated from collected tissues.

The collection of five individuals of the same species from across all gear types set at a given sampling "Site", in the forage and predatory categories, is the goal. ["Site" here is defined as the general reservoir location where multiple other samples and gear types might be clustered around. (see Table 1 above)]. No more than 10 individuals per species, from all traps per site is required; additional individuals should be noted as caught, length measured/ estimated, and released. Only fish retained for the composited fish samples need to be handled cleanly, other fish handling can be routine. Combine fish of like species from multiple traps, if needed, in each compositing bin or live well until-10 of that species is collected (from 1 or more traps/ gear types). A "clean hands" person wearing nitrile gloves, and a "dirty hands" personnel should be designated.

1) Clean or Dirty Hands. Fish less than <10 cm can be noted by species, tallied, and discarded immediately.
2) Clean Hands. Fish of proper species and size range (below) to meet the group of 10 shall be retained in clean buckets (4-5 total) per each collection trip (ie. multiple gear types and replicates). Clean buckets with lids will be filled with site water and used to store fish until it is obvious which species will make-up the 10 fish, single species composites. As the gear is hauled, clean hands will place candidate species-size fish into species specific buckets.
3) Clean or Dirty Hands. After those 2 species-size groups to be formed becomes obvious, usually halfway or more through hauling all the gear, other fish can be measured and released immediately.
4) All other fish, outside of the 2, 5-fish chosen species composites, need to be measured (total length), tallied, and can be released immediately thereafter.

Collection categories

The check lists below apply for all gear types. Lethal gear types should be hauled first (ie. long line), and non-lethal gear (traps and pots) hauled second, to minimize unnecessary mortalities.

Resident Forage Fish Category- 10 whole fish per species (<30 cm ± 3cm total length) from the following species priority list should be retained, across all gear types fished at a given "Site". The goal is to collect 10 individuals of a given species per sample site. The priority for the collection and retention of target species in the "Resident Forage Fish" category is:

1) Largescale sucker
2) Peamouth
3) Sculpin
4) Carp
5) Red Side Shiner
6) Speckled Dace
7) Chiselmouth

Resident Predatory Fish Category- 10 whole fish per species (>20 cm ± 3cm total length), to be filleted later at the dock, from the following species priority list should be retained, across all gear types and traps at a given "Site". The goal is to collect 10 individuals of a given species per sample site. The priority for the collection and retention of target species in the "Predator Fish" category is:

1) Smallmouth Bass
2) Northern Pikeminnow
3) Walleye
4) Largemouth Bass

Note: Biggest and Smallest of the Selected Species rule**-** As the "selected species type" per category becomes clear during collection at a site, additional fish beyond the 10 already collected in a clean bucket should only be kept IF the new fish is obviously larger or smaller (but still > the minimum of 10 cm) than any of the 10 fish in the species-size bucket so far; by visual assessment (measuring and remeasuring not mandatory here). If the biggest or smallest of the chosen species category is encountered after 10 individuals are retained, the biggest/ or smallest fish should be retained and an intermediately sized fish in the bucket then discarded. [The goal is to have the 10 fish in the bucket represent the entire range of total lengths encountered across all gear types; (ie. to maximize variability in the species-category defined).]

Sample Processing

Sediment

To improve the chances of getting the most detections and higher concentrations within the sediment, we ideally want to collect fine grain sediment that is smaller than sand, i.e., silts and clays which typically have higher amounts of organics. If the water depth at a site is shallow, hand collection techniques are possible with either a hand scoop or hand operated dredge device. When using dredges, large gravels and rocks are removed from the samples if possible before transferring the

sediment into the collection container. If only large gravel substrates or larger are collected, then the sample is considered not sampled. The sampling team must maintain sample integrity from the time of collection to the shipment and arrival at the laboratory. Sample integrity is maintained by taking precautions to prevent loss of contaminants that might be present in the sample and avoiding possible introduction of contaminants to the sample during handling. Once a sample is collected, sample integrity is maintained through controlled sample handling, storage, and preservation procedures.

Sampling Period

There may be no ideal time period where all fish desired to be sampled are easily collected and also avoid sampling issues such as high flows, presence of endangered species or high water temperatures. It's possible that either late June to August might allow for easier collection of fish, however, water temperatures may become too high to allow for electrofishing. On the other hand, sampling between late-September and mid-October may avoid the high flow and temperature issues, yet may be more difficult to collect the appropriate fish species.

Field Recordkeeping

One Fish Field Data Form will be completed for each sampling site (Appendix B). All fish collected will be tallied by species, total length category noted/ measured, recorded, before being discarded. Data recorded for this form will be entered on either hardcopy data forms or input into handheld computers. Data will be backed-up daily, either by Xeroxing of hardcopy data sheets or download of handheld computer files to another computer. Also, a field logbook to document any other data that may be useful in evaluating the quality of the data will be maintained by the crew.

General Safety Considerations for Field Operations

Field work on large rivers is inherently hazardous and involves significant risks to crew safety and health. Additional resources include the American Red Cross and Handal (1992), Ohio EPA (1990), USCG (1987), and USEPA (1986). Web sites with useful safety information include www.cdc.gov/niosh (occupational safety), www.nws.noaa.gov/safety (weather safety), www.uscgboating.org (boating safety), and www.firstaidguide.net (includes insect bite information). Personnel on field crews should be in sound physical condition, be able to swim, and have a physical exam annually or in accordance with their agency policy. Crew members with "MedicAlert" health conditions (e.g., severe allergies, diabetes, susceptibility to seizures) should make crew leaders and other crew members aware of their condition, the symptoms, and the actions required in a health emergency.

During field activities, crews may observe apparent violations of environmental regulations, may discover improperly disposed hazardous materials, or may observe or cause an accidental spill or release of hazardous materials. In such cases, it is important that the proper actions be taken and that field personnel do not become exposed to harmful substances. Know the location of the nearest hospital, and how to access emergency services such as State Patrol and 911.

Fish Collection Procedures

(see Standard Operating Procedures from WDFW and USGS for- a) hook and line, b) Hoop Net deployment, and c) Electroshocking Boat in the Revised QAPP for this project and/or under separate attachment.)

Fish Retention Procedures

Goal: -5 fish, same species, in small (forage fish) category- see below

-5 fish, same species, in Large (predatory fish) category- see below

-across ALL gear types.

Five individuals of the same species from across all gear types set at a given sampling "Site", in the forage and predatory categories, is the goal. ["Site" here is defined as the general reservoir location were multiple other samples and gear types might be clustered around.] No more than 10 individuals per species, from all traps per site, is required; additional individuals should be noted as caught, length measured/ estimated, and released. Only fish retained for the 10 fish composites need to be handled cleanly, other fish handling can be routine. Combine fish of like species from multiple traps, if needed, in a given compositing bin or live well until- 10 of that species is collected (from 1 or more traps/ gear types). A "clean hands" person wearing nitrile gloves, and a "dirty hands" personnel should be designated.

5) Clean or Dirty Hands. Fish less than <5 cm can be noted by species, tallied, and discarded immediately.
6) Clean Hands. Fish of proper species and size range (see below) to meet the group of 10 shall be retained in clean buckets (4-5 total) per each collection trip (ie. from multiple gear types and replicates). Clean buckets with lids will be filled with site water and used to store fish until it is obvious which species will make-up the 10 fish, single species composites. As the gear is hauled, clean hands will place candidate species-size fish into species specific buckets.
7) Clean or Dirty Hands. After those 2 species-size groups to be formed becomes obvious, usually halfway or more through hauling all the gear, other fish can be measured and released immediately.
8) All other fish, outside of the 2, 10-fish chosen species composites, need to be measured (total length), tallied, and can be released immediately thereafter.

Gear Type: This check list below applies for all gear types. Lethal gear types should be hauled first (ie. long line), and non-lethal gear (traps and pots) hauled second, to minimize unnecessary mortalities.

Resident Forage Fish Category- 10 whole fish (<30 cm total length) from the following species priority list should be retained, across all traps at a given "Site". Priority for target species in the "Resident Forage Fish" category is-

8) Largescale sucker
9) Peamouth
10) Sculpin
11) Carp
12) Red Side Shiner
13) Speckled Dace
14) Chiselmouth

Resident Predatory Fish Category- 10 whole fish (>20 cm total length), to be filleted later at the dock, from the following species priority list should be retained, across all gear types and traps at a given "Site". Note: while predatory fish >30 is the target, predators on the target list >20 should be temporarily retained until larger fish are available. Predator Guild list of priority species-

5) Smallmouth Bass
6) Northern Pikeminnow
7) Walleye
8) Largemouth Bass

Note: Biggest and Smallest of the Selected Species rule- As the "selected species type" per category becomes clear during collection at a site, additional fish beyond the 10 already collected in a clean bucket should only be kept IF the new fish is obviously larger or smaller (but still > the minimum of 10 cm) than any of the 10 fish in the species-size bucket so far; by visual assessment (measuring and remeasuring not mandatory here). If the biggest or smallest of the chosen species category is encountered after 10 individuals are retained, the biggest/ or smallest fish should be retained and an intermediately sized fish in the bucket then discarded. [The goal is to have the 10 fish in the bucket represent the entire range of total lengths encountered across all gear types; ie. to maximize variability in the species-category defined.]

All fish collected- will be tallied by species, total length category noted/ measured, recorded, before being discarded.

Retained Fish: will be transferred to the dock and the fish processing location were additional measurement (ie. weights) and sub-samples will be collected. See Fish Dissecting Field Sheet.

Appendix B. Laboratory Sample List with USGS S Station Numbers identified.

Sample Integer (SINT)	User Code	Agency Code	Station Number	Start Date	Medium	Project
1	OR	USGS	14128600	202310171200	BA	MCT
2	OR	USGS	454123121261400	202310181200	BA	MCT
3	OR	USGS	454143121183900	202310191200	BA	MCT
4	OR	USGS	454143121183900	202310191210	BA	MCT
5	OR	USGS	454256121370100	202310171200	BA	MCT
6	OR	USGS	454256121370100	202310171210	BA	MCT
7	OR	USGS	454338121323900	202310181200	BA	MCT
8	OR	USGS	454338121323900	202310181210	BA	MCT
9	OR	USGS	454148121273100	202310181200	BA	MCT
10	OR	USGS	454148121273100	202310181210	BA	MCT
11	OR	USGS	453847121121400	202310201200	BA	MCT
12	OR	USGS	453952121125800	202310201200	BA	MCT
13	OR	USGS	14105700	202310201200	BA	MCT
14	OR	USGS	14105700	202310201210	BA	MCT
14D	OR	USGS	14105700	202310201211	BA	MCT
15	OR	USGS	454212121453700	202310171200	BA	MCT
16	OR	USGS	454212121453700	202310171210	BA	MCT
15	OR	USGS	454212121453700	202310171200	BA	MCT
16	OR	USGS	454212121453700	202310171210	BA	MCT
17	OR	USGS	454038121531400	202308281200	ST	MCT
19	OR	USGS	454212121453700	202308281200	ST	MCT
21	OR	USGS	454300121352100	202308311200	ST	MCT
22	OR	USGS	454136121250300	202308311200	ST	MCT
26	OR	USGS	453606121101000	202308281200	ST	MCT
27	OR	USGS	454143121174000	202308311200	ST	MCT
27D	OR	USGS	454143121174000	202308311201	BA	MCT
28	OR	USGS	453606121101000	202306281200	BA	MCT
28D	OR	USGS	453606121101000	202306281201	BA	MCT
29	OR	USGS	453606121101000	202309011200	BA	MCT
30	OR	USGS	453606121101000	202309011210	BA	MCT
31	OR	USGS	453811121574500	202305141200	BA	MCT
32	OR	USGS	453811121574500	202306031200	BA	MCT
33	OR	USGS	453811121574500	202306171200	BA	MCT
34	OR	USGS	453606121101000	202309211200	BA	MCT
35	OR	USGS	453606121101000	202309211210	BA	MCT
37	OR	USGS	14128600	202404171200	BA	MCT
39	OR	USGS	453847121121400	202404181200	BA	MCT

40	OR	USGS	454123121261400	202404171200	BA	MCT
42	OR	USGS	454123121261400	202404171210	BA	MCT

*Multiple samples sometimes submitted from the same Station Number (e.g. The Dalles boat ramp)

BA- biological sample of tissue, ST-sediment sample. MCT-Mid-Columbia Toxics, project code with data uploaded to USGS NWIS and EPA WQX.

Appendix C . Analytical Chemistry Method Details

Organochlorines

The Organochlorine results were generated extracted and cleaned up according to SGS AXYS Method MLA-013: ***Analytical Method for The Determination of: Polybrominated Diphenyl Ethers, PCB Congeners, Chlorinated Pesticides, Technical Toxaphene, Toxaphene Congeners/Parlars and Polychlorinated Dibenzodioxins and Furans using Co-Extraction Techniques,*** and chemical analysis procedures were in accordance with **SGS AXYS Method MLA-228:** ***Analytical Procedures for Organochlorine Pesticides by GC-MS/MS***.

"This narrative describes the analysis of fourteen tissue samples for organochlorine pesticides using atmospheric pressure chemical ionization Gas Chromatography with tandem quadrupole Mass Spectrometry (APGC-MS/MS).

SAMPLE RECEIPT AND STORAGE
The samples were received at SGS Wilmington (North Carolina, USA) on March 5th 2025. Sample temperatures were -25°C on receipt, and the samples were stored at -20°C in the dark prior to homogenization and extraction.

SAMPLE PREPARATION AND ANALYSIS
Samples were homogenized and initially extracted at SGS Wilmington, after which the raw extracts were shipped to SGS AXYS (Sidney, BC, Canada) for cleanup, lipids determination, instrumental analysis, and reporting of data.

The samples were extracted and cleaned up according to SGS AXYS Method MLA-013: ***Analytical Method for The Determination of: Polybrominated Diphenyl Ethers, PCB Congeners, Chlorinated Pesticides, Technical Toxaphene, Toxaphene Congeners/Parlars and Polychlorinated Dibenzodioxins and Furans using Co-Extraction Techniques***.

Analysis procedures were in accordance with **SGS AXYS Method MLA-228:** ***Analytical Procedures for Organochlorine Pesticides by GC-MS/MS***.

The samples and QC samples (a procedural blank, a reference sample called Ongoing Precision and Recovery (OPR), a sample duplicate and a sample matrix spike) were analyzed in a batch named WG93606.

Canola oil was used as the matrix for the preparation of the procedural blank and the OPR. Sample '14' (SGS AXYS ID: L42839-9) was analyzed in duplicate and assigned SGS AXYS ID WG93606-103. Sample '32' (SGS AXYS ID: L42839-12) was used as the matrix for the matrix spike (MS) and assigned SGS AXYS ID WG93606-104.

An accurately weighed sub-sample (approximately 10g wet weight) of each sample was spiked with 13C-labeled PBDE, PCB and Pesticide quantification standards and Soxhlet-extracted with dichloromethane (DCM). The resulting extract was spiked with 13C-labeled cleanup standards, subsampled for lipid determination, and split into two unequal portions: a 4/5 portion for PBDE/PCB analyses and a 1/5 portion for Pesticide analysis. This narrative describes the analysis of the 1/5 portion for Pesticide analysis. The extract was chromatographically cleaned up on Biobead (gel permeation, size exclusion) and Florisil chromatographic columns. The resulting extract was reduced in volume and fortified with 13C-labeled recovery (internal) standards prior to instrumental analysis. The final extract volume was 40µL; 1µL was injected onto the instrument.

CALCULATION
Target analyte concentrations were determined by isotope dilution or internal standard quantification

procedures using Waters MassLynx software.

Sample specific detection limits (SDLs) were calculated for each target analyte and used as the detection qualifier. If the software selected an unrepresentative area for the detection limit calculation, the data validation chemists made corrections.

Because of instrument variability and lab background levels, it is SGS AXYS' policy to report detection limits no lower than 0.01 ng absolute (0.0058 ng/g on a 10 g sample and prorated for a final extract volume of 40µL and extract splitting for lipid determination and 1/5th extract for analysis).

The quantification procedures accounted for the splitting of samples. The final results are in terms of the whole sample extracted.

REPORTING CONVENTIONS

For internal tracking, SGS AXYS assigned the US Fish and Wildlife Service contract number 9946. AXYS logged the samples under unique laboratory identifiers of the form L42839-X, where X is a numeral; all data reports refer to both the client and AXYS IDs.

If a sample extract is analyzed more than once ("additional work"), each additional instrumental run is distinguished by a suffix added to the AXYS ID. The one suffix for identifying additional work used in this data package is:

- i = instrumental reanalysis

The following laboratory qualifiers are used on the report pages:

- J = the concentration is below the limit of quantification
- K = a peak was detected that did not meet all the criteria for positive identification as the
- target analyte; the reported value is the estimated maximum possible concentration.
- U = not detected
- V = the recovery of the labeled compound fall outside the method control limit

On the report pages, results are reported to three significant figures, in units of nanograms per gram (ng/g) on a wet weight basis.

The 9946_2 EDD uses data qualifier flags J and K, and reports the results in parts per million (ppm)."

PCBs

Analysis of the PCBs followed an analogous EPA 1668C method of "SGS AXYS Method MLA-210: Analytical Method for the determination of 209 PCB Congeners by GC-MS/MS". This method has equal or superior detection limits and performance than EPA 1668C. A summary of this method provided by laboratory itself states-

"This narrative describes the analysis of fourteen tissue samples for polychlorinated biphenyl (PCB) congeners using atmospheric pressure chemical ionization Gas Chromatography with tandem quadrupole Mass Spectrometry (APGC-MS/MS).

SAMPLE RECEIPT AND STORAGE

The samples were received at SGS AXYS on March 5th 2025. Sample temperatures were -25°C on receipt, and the samples were stored at -20°C in the dark prior to homogenization and extraction.

SAMPLE PREPARATION AND ANALYSIS

The samples were extracted and cleaned up according to SGS AXYS Method MLA-013: ***Analytical Method for The Determination of: Polybrominated Diphenyl Ethers, PCB Congeners, Chlorinated Pesticides, Technical Toxaphene, Toxaphene Congeners/Parlars and Polychlorinated Dibenzodioxins and Furans using Co-Extraction Techniques***.

Analysis procedures were in general accordance with SGS AXYS Method MLA-210***: Analytical Method for the determination of 209 PCB Congeners by GC-MS/MS***.

The samples and associated QC were analyzed in a batch named WG93606. The batch contained a procedural blank, a lab generated reference sample known as Ongoing Precision and Recovery (OPR), a sample duplicate and a matrix spike testing sample. The procedural blank and OPR were prepared using canola oil as the matrix. Sample '14' (SGS AXYS ID: L42839-9) was analyzed in duplicate and assigned SGS AXYS ID WG93606-103. Sample '32' (SGS AXYS ID: L42839-12) was used as the matrix for the matrix spike (MS) and assigned SGS AXYS ID WG93606-104.

For each sample, an accurately weighed subsample of approximately 10g was spiked with 13C-labeled PBDE, PCB and pesticide quantification standards, and then extracted in a Soxhlet apparatus using dichloromethane (DCM). The resulting extract was spiked with 13C-labeled cleanup standards and a small portion of the extract was split for lipid determination. After this, the extract was split into two unequal portions: a 4/5 portion for PBDE and PCB analysis and a 1/5 portion for Pesticide analysis. This narrative describes the analysis of the 4/5 portion for PCBs. The extract was cleaned up using acid/base Silica and by GO-EHT Automated Cleanup System using Silver Nitrate Silica, Sulfuric Acid Silica, Magnesium Silica (Florisil) and Zirconium Dioxide chromatographic columns. The final extract was concentrated and spiked with 13C-labeled recovery (internal) standards prior to instrumental analysis (prior to the addition of the recovery standards. The final extract volume was 20µL; 1µL of which was injected onto the APGC-MS/MS.

CALCULATION

Target analyte concentrations were determined by isotope dilution or internal standard quantification procedures using Waters MassLynx software.

Sample specific detection limits (SDLs) were calculated for each target analyte and used as the detection qualifier. If the software selected an unrepresentative area for the detection limit calculation, the data validation chemists made corrections.

To account for instrument variability and lab background levels, it is SGS AXYS's policy to report detection limits no lower than 0.2 pg absolute (e.g. 0.029 pg/g on a 10 g sample size and prorated for 4/5th splitting and the removal of extract for lipid analysis). The reported detection limit is the greater of the SDL and the 0.2 pg absolute reporting limit. This is reflected on the analysis report forms.

Homologue totals were obtained by summing the concentration of all detected congeners at each level of chlorination. Toxic Equivalents (TEQs) were calculated using WHO 2005 Toxic Equivalent Factors (TEFs). Congener peaks that did not meet the method ion abundance ratio criteria were not included in the homologue totals or the TEQ calculations.

The quantification procedures accounted for the splitting of samples. The final results are in terms of the whole sample extracted.

REPORTING CONVENTIONS
For internal tracking, SGS AXYS assigned the US Fish and Wildlife Service contract number 9946. AXYS logged the samples under unique laboratory identifiers of the form L42839-X, where X is a numeral; all data reports refer to both the client and AXYS IDs.

The following laboratory qualifiers are used on the report pages:

- C/Cx = indicates the co-elution of two or more PCB congeners. The result for the co-elution is reported once only, against the congener flagged 'C', which is the congener with the lowest IUPAC number 'x'. The remaining congeners in the co-elution are flagged 'Cx'
- J = the concentration is below the limit of quantification
- K = a peak was detected that did not meet all the criteria for positive identification as the target analyte; the reported value is the estimated maximum possible concentration.
- U = not detected
- V = the recovery of the labeled compound fall outside the method control limit

On the report pages, results are reported to three significant figures, in units of picograms per gram (pg/g) on a wet weight basis.

The 9946_2 EDD uses data qualifier flags J and K, and reports the results in parts per million (ppm)."

PBDEs

The samples were extracted and cleaned up according to SGS AXYS Method MLA-013: ***Analytical Method for The Determination of: Polybrominated Diphenyl Ethers, PCB Congeners, Chlorinated Pesticides, Technical Toxaphene, Toxaphene Congeners/Parlars and Polychlorinated Dibenzodioxins and Furans using Co-Extraction Techniques***. Analysis procedures were in general accordance with '**USEPA Method 1614A: *Brominated Diphenyl Ether Congeners in Soil, Sediment and Tissue by HRGC/HRMS***' as documented in SGS AXYS
Method MLA-033.

"This narrative describes the analysis of fourteen tissue samples for polybrominated diphenylethers (PBDE) using gas chromatography / high resolution mass spectrometry (GC/HRMS).

SAMPLE RECEIPT AND STORAGE
The samples were received at SGS Wilmington (North Carolina, USA) on March 5th 2025. Sample temperatures were -25°C on receipt, and the samples were stored at -20°C in the dark prior to homogenization and extraction.

SAMPLE PREPARATION AND ANALYSIS
Samples were homogenized and initially extracted at SGS Wilmington, after which the raw extracts were shipped to SGS AXYS (Sidney, BC, Canada) for cleanup, lipids determination, instrumental analysis, and reporting of data.

The samples were extracted and cleaned up according to SGS AXYS Method MLA-013: ***Analytical Method for The Determination of: Polybrominated Diphenyl Ethers, PCB Congeners, Chlorinated Pesticides, Technical Toxaphene, Toxaphene Congeners/Parlars and Polychlorinated Dibenzodioxins and Furans using Co-Extraction Techniques***.

Analysis procedures were in general accordance with '**USEPA Method 1614A: *Brominated Diphenyl Ether Congeners in Soil, Sediment and Tissue by HRGC/HRMS***' as documented in SGS AXYS

Method MLA-033.

The samples and QC samples (a procedural blank, a reference sample called Ongoing Precision and Recovery (OPR), a sample duplicate and a sample matrix spike) were analyzed in a batch named WG93606.

Canola oil was used as the matrix for the procedural blank and OPR (SGS AXYS ID: WG93606-101 and -102, respectively). Sample '14' (SGS AXYS ID: L42839-9) was used as the matrix for the sample duplicate (SGS AXYS ID: WG93415-103) and sample '32' (SGS AXYS ID: L42839-12) was used as the matrix for the matrix spike (MS) (SGS AXYS ID: WG93606-104).

An accurately weighed sub-sample (approximately 10g wet weight) of each sample was spiked with ^{13}C-labeled PBDE, PCB and Pesticide quantification standards and Soxhlet-extracted with dichloromethane (DCM). The resulting extract was spiked with ^{13}C-labeled cleanup standards, subsampled for lipid determination, and split into two unequal portions: a 4/5 portion for PBDE/PCB analyses and a 1/5 portion for Pesticide analysis. This narrative describes the analysis of the 4/5 portion for PBDEs. The extract was cleaned up using the standard chromatographic columns listed on the workup sheets. The cleaned extract was reduced in volume and spiked with ^{13}C-labeled recovery (internal) standards, for a final volume of 20µL, 1µL of which was injected onto the GC-MS.

CALCULATION
Target analyte concentrations were determined by isotope dilution or internal standard quantification procedures using Micromass OPUSQuan software.

Sample specific detection limits (SDLs) were determined from analysis data following the same procedures used to convert target peak responses to concentrations. The SDLs were calculated for each target analyte and used as the detection qualifier.

To account for instrument variability and background levels, it is SGS AXYS policy to report detection limits no lower than 1.0 pg absolute (i.e. 0.144 pg/g on a 10 g sample size and prorated for lipid analysis sub-sampling and 4/5th splitting). The reported detection limit is the greater of the SDL and the 1.0 pg absolute reporting limit.

The sub-sampling of the extracts for lipid analysis and the 4/5th extract splitting is accounted for in the quantification procedures, such that the final results are in terms of whole sample extracted.

REPORTING CONVENTIONS
For internal tracking, SGS AXYS assigned the US Fish and Wildlife Service contract number 9946. AXYS logged the samples under unique laboratory identifiers of the form L42839-X, where X is a numeral; all data reports refer to both the client and AXYS IDs.

The following laboratory qualifiers are used on the report pages:

- C/Cx = indicates the co-elution of two or more BDE congeners. The result for the co-elution is reported once only, against the congener flagged 'C', which is the congener with the lowest IUPAC number 'x'. The remaining congeners in the co-elution are flagged 'Cx'
- J = the concentration is below the limit of quantification
- K = a peak was detected that did not meet all the criteria for positive identification as the
- target analyte; the reported value is the estimated maximum possible concentration.
- MAX = Estimated maximum values. BDE congeners 206, 207, & 208 are always flagged MAX unless they are non-detect or flagged K.
- NQ = not quantified
- U = not detected
- V = the recovery of the labeled compound fall outside the method control limit

On the report pages, results are reported to three significant figures, in units of picograms per gram (pg/g)

on a wet weight basis.

The 9946_2 EDD uses data qualifier flags J, K, and MAX, and it reports the results in parts per million (ppm). Unreportable analytes, which appear on the report pages with an ‘NQ” flag, are excluded from the EDD.