NEWSS salmon recovery projects

eDNA research

Submitted by

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Executive Summary

This project is a partnership between UNBC and the Nechako Environment and Water Stewardship Society (NEWSS), funded through the BC Salmon Restoration and Innovation Fund. Each summer a field crew from UNBC hired and trained students from School District 91, combining educational training with scientific data collection and field research experience. The following report describes the activities that took place in 2025 and provides a summary of the results of the assessment of environmental DNA (eDNA), and in some cases minnow trapping data, for the presence of four key salmonid species, Chinook salmon (*Oncorhynchus tshawytscha*), Sockeye/Kokanee salmon (*O. nerka*), Coho salmon (*O. kisutch*) and Rainbow trout (*O. mykiss*) over the span of the project, 2023-2025. For a subsample of locations, results from surveys prior to 2023 (2020-2022) are included for comparison. Chinook salmon show consistent and widespread use of the creeks across the study area in core Nechako areas, from McPhee Creek (Prince George) to Twin Creek (Upper Nechako, below the Kenney Dam). This provides evidence of the use of this area for rearing by juvenile Chinook (presumably from the Middle Fraser, Summer 5-2 conservation unit, which spawn in this area). Similar results are seen for two separate Middle Fraser, Spring 5-2 runs which spawn in the Endako and Chilako rivers, respectively. Sockeye are detected less frequently than Chinook and results may be confounded by earlier spawning runs moving through the study area and the presence of kokanee populations in some creek drainages. The detection of Sockeye in mid-June samples in creeks along the Nechako, however, does provide evidence of juvenile Sockeye within the core Nechako area. Coho are detected the least often in the survey, however, these results more than double the previous observations, and due to the winter timing of the Interior Fraser Coho spawning runs in this area, provide evidence of rearing within the core Nechako area. Coho are not detected in either the Endako or Chilako sites. Creeks around the town of Vanderhoof (Moss, Clear, Murray and Knight) were the subject of the most intensive surveys, both in terms of number of sites per creek and years surveyed. They served both as a learning lab for the high school students, and as the location of key sites associated with past and ongoing restoration efforts. These results show that species use varied by location along creeks, with Chinook being found at sites closest to the confluence with the Nechako, while Rainbow trout and other minnow species are found in more upstream sites. To study passage of Chinook through beaver dams, we used eDNA to examine a natural beaver dam complex in the lower reach of Murray Creek. These surveys show a dynamic relationship between dam height and Chinook passage. Chinook were found throughout the complex in the earlier years, while in the later years, correlated with increasing dam height, Chinook were no longer detected in the uppermost pond(s). In 2025, we initiated a metagenomic project at sites within Vanderhoof creeks that were assessed for both fish and invertebrate biodiversity in June/July and August/September timepoints from 2023-2025. This provides baseline data for ongoing monitoring of biodiversity at NEWSS restoration sites.

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Appendix 3. Summary of Minnow Trapping, Vanderhoof Area, 2025

Methodology

Hiring summer field crew

Our work with School District 91 (SD 91) began in 2020. Beginning in 2020 and culminating in 2025 we have hired summer students from Nechako Valley Secondary School (NVSS) each summer to assist us with our field and laboratory work. Each year we would work with Casey Litton (NVSS) and Darren Carpenter School District 91(SD 91) to have our job posting for summer positions posted in late April of each year. We would then conduct interviews at NVSS either online or in person, along with Rebecca Morin and one additional NVSS teacher. Rebecca Morin also provided support to each selected student in completing all required employment documentation. During the three years of this project, we hired a total of nine students (three each year).

In cooperation with NVSS, David Peterson's classroom was converted into an operational eDNA laboratory to support monitoring and student training. An initial orientation session was held with NVSS staff and summer students, led by Brent Murray, Barry Booth, or Jesi Lauzon, alongside Casey Litton (NVSS). Students were introduced to project objectives, study design, and eDNA sampling protocols. Field training included hands-on use of the Halltech eDNA OSMO sampler. Upon returning from the field, students processed and stored samples and completed filter sterilization procedures. Annual project delivery was carried out collaboratively with NVSS staff whereby they provided us with access to School District 91 equipment and lab space. Further training of the students was effectively 'on the job training'. During this time students were continually exposed to new facets of the job (e.g., how to place minnow traps, handle fish, record field data) as the summer progressed.

Creeks sampled

From 2020-2025 we sampled 41 different creeks (Table 1). Table 1 lists the named creeks, relative sizes of their drainage and likely impacts. Over this time period, we have sampled 7-11 creeks on a consistent basis since 2020. Starting in 2023, we greatly expanded the number of creeks to encompasses the range of creeks along the Nechako downstream of the Kenney Dam to the City of Prince George. We also included sites within the Endako River and Chilako River watersheds.

Table 1. Creeks sampled over the course of the project 2020-2025. [1]Watershed size and stream order were taken from or estimated using iMapBC online web tools (April 2026). [2]Potential impacts of human activity were estimated from analysis of iMapBC and Google Earth orthophotos.

				Potential Impacts[2]			
Sampling area	Creek (River)	Watershed Size[1]	Order[1]	Agriculture	Forestry	Rail Culvert	Other impacts
Upper Nechako							
	Twin	1064 *	3	None	High	No	
	Cutoff	8071	4	Moderate	High	No	Culverts on Kenny Dam FSR
	Swanson	22449	5	Low-Mod	High	No	
	Targe	34684	5	Low	High	No	
	Greer	40947	6	Low	High	No	
	Tahultzu	8951	4	Moderate	High	No	
	Smith	22805	5	Moderate	High	No	
Nautley - Vanderhoof							
	"Dog" unnamed	1033 **	4	Low	High	No	Perched culverts on Dog Creek FSR
	Tatsutnai	6819	4	Low	High	No	
	Nine Mile	6609	5	Moderate	High	No	Perched culvert on Settlement Road
	Kluk	3191	4	Low	High	No	
	Halsey	938	2	High	Moderate	No	
	Engen	9829	5	High	Low	No	
Vanderhoof							
	Moss	2061	3	High	Low	No	
	Clear	8413	4	High	Moderate	No	Culverts on Highway 27 and Braeside Road
	Stoney	58551	6	High	High	No	
	Murray	11979	4	High	High	No	
	Knight	6118	4	High	Moderate	No	Misaligned culvert on Sacknner Road
Vanderhoof - Stuart							
	Chilco	7304	5	High	Low	No	
	Sinkut River	57130	6	Moderate	High	No	
	Hulatt	7875	4	High	Moderate	Yes	
	Cluculz	68408	4	Moderate	High	No	Lake shore development - Cluculz Lake
Stuart - Prince George							
	Hutchison	5124	4	Low	High	Yes	
	Zelkwas	4277	4	High	Moderate	Yes	
	Breeze	6599	4	High	High	No	
	Tachintelachick	20827	6	Moderate	High	Yes	
	Sweden	4955	4	Moderate	High	Yes	
	McPhee	8273	4	Moderate	Moderate	No	
Chilako River							
	Chilako River	363444	6	High	High	No	
	Beaverley	21686	5	High	High	No	
	Dahl	20810	5	Low	High	No	Lake shore development - Norman Lake
	CK 37 Unknown	2186	3	Low	High	High	
	Gregg	11400	4	Low	High	No	Ranch near confluence with Chilako
	CK 53 Unknown	7599	4	Moderate	High	No	
	Butcherflats	33913	5	Moderate	High	No	
Fraser Lake	Ormond	24810	5	Moderate	High	No	
Endako River							
	Endako River	106,000 **	5	Moderate	High	No	Endako Mine
	Watkins	956 **	4	Low	High	No	Endako Mine, Highway 16
	Tchesinkut	34361	5	Moderate	High	No	
	Shovel	5394	5	Low	High	No	Extensive fires in past years
	Sauls	2331	3	Low	High	Yes	Highway 16, Government Road, channelization

* from https://www.newssociety.org/

** estimated from iMapBc data

1 unless noted, Watershed Size and Stream Order from iMapBC

2 Potential Impacts: magniitude of impacts derived from visual estimates from iMaps and Google Earth orthophotos.
Rail culverts: yes = stream flows through culvert at a rail crossing near sampling sites, no = creek is not affected by culvert

Sampling in 2025

In 2025, we took water/eDNA samples from 38 different creeks/water bodies. These are presented in Table 2. As in previous years, the number of sites per creek varied from 1 to over 10, with most sites being sampled 2 or 3 times in 2025 (Table 2).

Additional features/highlights of our sampling are as follows:

- We continued to work with NEWSS by sampling in conjunction/in proximity to the previous and current restoration projects. This includes a number of Beaver Dam Analogs (BDA) constructed in the fall of 2024 (This Report, NEWSS 2026). Site KN02 is this just below the two BDAs on Knight Creek while KN03 and KN04 are located in the resulting ponds. Similarly, MU08 is located in the pond created by a BDA on Murray Creek, while MU07 is located downstream.

- We continued our riverboat sampling collecting water samples from 19 creeks in 2025. Due to water level restrictions, filter samples were collected only at June (T1) and August (T3) time points.
- We also continued our examination of how salmon are utilizing a developing beaver dam complex on the bottom 1 km section of Murray Creek. This was the fifth year of sampling of this beaver dam complex.

Table 2. 2025 eDNA sample locations and dates for post freshet sampling (T1,) midsummer (T2) and late summer/fall (T3). "-" indicates not sampled. "*dam open*" indicates the dam is compromised – meaning flowing water through/around structure and no upstream impoundment. "No Water" indicates no surface flow observed at this time point – not sampled.

Location/Area	**Site #**	**T1**	**T2**	**T3/T4**
Vanderhoof Beaver Dam Survey (Murray Creek)	BD01 (MU00)	-	-	18-Oct
	BD 02	-	-	18-Oct
	BD03	-	-	***dam open***
	BD04	-	-	***dam open***
	BD 05	-	-	18-Oct
	BD 06	-	-	18-Oct
	BD 07	-	-	18-Oct
	BD 07.2	-	-	***dam open***
	BD 08	-	-	18-Oct
	BD 09	-	-	18-Oct
	BD 10	-	-	18-Oct
	BD11 (MU01)	-	-	***dam open***
	BD 12	-	-	18-Oct
Murray Creek	MU 00	-	-	-
	MU 01	26-Jun	23-July	26-Aug
	MU 02	25-Jun	23-July	27-Aug
	MU 03	25-Jun	23-July	27-Aug
	MU 04	25-Jun	25-July	-
	MU 04.1	-	-	-
	MU 05	-	-	26-Aug
	MU 06	-	25-July	26-Aug
	MU 07	25-Jun	25-July	27-Aug
	MU 08	25-Jun	-	27-Aug
	MU 09	26-Jun	25-July	27-Aug
	MU 10	26-Jun	25-July	***No water***

Table 2 continued. 2025 eDNA sample locations and dates.

Knight Creek	KN 01	5-Jun	23-July	26-Aug
	KN 02	5-Jun	23-July	26-Aug
	KN 03	5-Jun	***No water***	***No water***
	KN 04	5-Jun	***No water***	***No water***
Moss Creek	MS 0.9	9-Jul	7-Aug	-
	MS 1.0	9-Jul	7-Aug	23-Sep
	MS 1.5	-	-	-
Clear Creek	CL 0.9	9-Jul	7-Aug	-
	CL 01	9-Jul	7-Aug	23-Sep
	CL 02	9 Jul		23-Sep
	CL 03	9-Jul	8-Aug	23-Sep
	CL 04	-	8-Aug	23-Sep
Eden Creek	EN 01	9-Jul	8-Aug	23-Sep
Ft Fraser				
Unnamed "Dog" Creek	DC 01	2-Jul	08-Aug	10-Sep
Tatsutnai Creek	TC 01	2-Jul	08-Aug	10-Sep
Nine Mile Creek	9M 01	2-Jul	08-Aug	10-Sep
Ormond Creek (Fraser Lake)	OR 01	2-Jul	08-Aug	10-Sep
Upper Nechako				
Twin Creek	TW 01	-	20-Aug	20-Sep
	TW 02	3-Jul	-	-
	TW03	3-Jul	20-Aug	***No water***
Swanson Creek	SW 01	3-Jul	20-Aug	20-Sep
	SW 02	3-Jul	20-Aug	20-Sep
Greer Creek	GR 01	3-Jul	20-Aug	20-Sep
	GR 03	3-Jul	20-Aug	-
	GR 04	3-Jul	20-Aug	20-Sep
	GR 5.9	-	20-Aug	-
	GR 06	3-Jul	20-Aug	20-Sep
Cutoff Creek	CT 01	3-Jul	20-Aug	***No water***
	CT 02	3-Jul	20-Aug	20-Sep

Table 2 continued. 2025 eDNA sample locations and dates.

Endako River watershed

Endako from HWY	ED 13	10-Jul	26-Aug	10-Sep
"	ED 18	-	-	-
"	ED 22	10-Jul	26-Aug	10-Sep
"	ED 26	-	-	-
"	ED 66	-	-	-
"	ED 66.1	10-Jul	26-Aug	10-Sep
Tchesinkut Creek	TH 01	10-Jul	26-Aug	10-Sep
Watkins Creek	WA 01	10-Jul	26-Aug	***No water***

Chilako River watershed

Beaverley Creek	BE 01	2-Jul	-	28-Aug
Butcherflats	BU 01	11-Jun	6-Aug	17-Sep
Dahl Creek	DH01	11-Jun	6-Aug	28-Aug
Chilako River	CK 16 @ HWY 16	11-Jun	6-Aug	28-Aug
"	CK 37	11-Jun	6-Aug	28-Aug
"	CK 53	11-Jun	6-Aug	17-Sep
"	CK 102	11-Jun	-	17-Sep
"	CK 116	11-Jun	-	28-Aug
Unnamed tributary 1	CK 37 UNK	11-Jun	6-Aug	28-Aug
Unnamed tributary 2	CK 53 -UNK	11-Jun	6-Aug	17-Sep

Nechako main by river boat

Breeze Creek	BZ 00	16-Jun	-	21-Aug
Chilako River	CK 00	16-Jun	-	21-Aug
Cluculz Creek	CZ 00	16-Jun	-	21-Aug
Cutoff Creek	CT 00	17-Jun	-	22-Aug
Engen Creek	EG 00	***No water***	-	***No water***
Halsey Creek	HY 00	17-Jun	-	21-Aug
Hulatt Creek	HL 00	16 Jun	-	-
Hutchison Creek	HN 00	16-Jun	-	21-Aug
Kluk Creek	KK 00	17-Jun	-	22-Aug
McPhee Creek	MF 00	16-Jun	-	***No water***
Nine Mile Creek	9M 00	17-Jun	-	22-Aug
Sinkut River	SU 00	16-Jun	-	21-Aug
Smith Creek	SM 00	17-Jun	-	22-Aug

Sweden Creek	SN 00	16-Jun	-	21-Aug
Tahultzu Creek	TU 00	17-Jun	-	22-Aug
Targe Creek	TE 00	17-Jun	-	22-Aug
Tachintelachick Creek	TK 00	16-Jun	-	21-Aug
Tatsutnai Creek	TC 00	17-Jun	-	22-Aug
Zelkwas Creek	ZK 00	16-Jun	-	21-Aug

Analysis of environmental DNA

As in previous years, environmental DNA (eDNA) was extracted at a dedicated lab at UNBC supervised by Brent Murray. Each site was sampled in duplicate. Each replicate filter was obtained by filtering between 1-2 litres of water per site, depending on the water quality. Filters were placed in a penny envelope and stored in a silica desiccant bead package at 4°C until the extraction of eDNA. Following our established protocol, eDNA was extracted in two 50 µl elutions (see 2023 report). A comparison of signal between elutions allows for an initial assessment of the presence of inhibitors which are generally greater in the first elution. Both elutions were assessed for the presence of the four target species through the established duplexed tagman assays (Chinook/Rainbow and Coho/Sockeye-Kokanee:) using droplet digital PCR (Murray and Booth 2023). The presence of a target species was confirmed though the presence of 4 or more positive ddPCR droplets in all replicates at a site. Samples with 2-3 positives droplets in the initial survey, below the minimum threshold for acceptance, were reanalysed. If positive droplets were found in the reanalysis, species presence was confirmed and reported at the 4-10 droplet level. If not, samples were considered "not detected" for that species.

A relative signal strength was assessed by summing the copy number estimation in both elutions (this represents 1/10 of the eDNA volume recovered) and averaging between the replicates. Results are reported by showing the data on a relative scale: 4-10 copies (Weak signal); 10-20 copies; 20-50 copies (Good signal); 50-100 copies; > 100 copies (Strong signal); and > 1000 copies (Very Strong signal).

Samples that showed evidence of strong ddPCR inhibitors or were negative (not detected) for all fish species were re-examined with an inhibitor control dual assay and/or further purified with a zymogene purification column. The inhibitor control dual assay includes an assay for an added Lambda DNA (exogenous control) and for plant material (eplant endogenous control). Both are expected to yield a strong to very strong result, with lower to no signal indicating the presence of ddPCR inhibitors in the eDNA extraction. Samples with inhibitors present in all elutions were further purified and retested.

Results and Discussion

eDNA Survey Results 2023-2025

Controls - 2025: All 28 extraction controls (a clean filter processed through each extraction process) showed no evidence of contamination (i.e., all samples showed "no detection" of the 4 target species). Similarly, most non-template controls (NTC) associated with the ddPCR runs showed "no detection" of the target species, except for one Chin/Rain plate which showed low levels Rainbow detection indicating possible cross contamination during reactions setup. These samples were retested and associated NTC was negative. Field/filtering controls were collected at the start and end of each filtering day. These involved the filtering of 1 L of distilled water using the same OSMOS backpack sampling unit used to collect the water filter samples at each site. Of the 27 sampling days (54 field controls), two showed evidence of low levels of Rainbow trout (4-10 droplet range) in the first sampling period and two showed evidence of low level of sockeye salmon in the third time period. These show evidence of low levels of eDNA in the environment during those sampling days. Indeed, the two days in the third time point were associated with river boat sampling at same time as the large sockeye spawning run in 2025. For all samples associated with these days, the baseline for positive detection was increased to that observed in the field controls.

Yearly eDNA Surveys: The results of the eDNA survey for 2023, 2024, and 2025 are shown as a quantitative estimation of the number of copies of the species-specific eDNA detected in 1/10 of the eDNA extracted (Tables 2, 3, 4). Note samples with detections between 4-10 copies, may be based on 1/5 of extraction volume – due to retesting of samples with 2-3 drops in initial survey. Positive detections range from 4 copies to over 1000 copies in the volume tested. At some sites, sampling was not performed due to lack of surface flow or was conducted on isolated pools in the creek bed.

Table 3. eDNA results from 2023. Results are shown by quantitative signal (see legend) for three time points June-July (T1), July-August (T2) and August-Sept (T3). See Table 2 for site codes.

Site	Site code	June SD301 Chin	Rain	Coho	Sock	July SD302 Chin	Rain	Coho	Sock	Aug SD303 Chin	Rain	Coho	Sock
Chilako													
Chilako Main	CK00			-	-					-		-	-
Beaverley	BE02					-		-	-				
Gregg	GG05					-		-	-				
Dahl	DH01							-	-				
Chehischic	CH08					-		-	-				
Unknown trib	CK53-u1							-	-				
Unknown trib	CK53-u2					-		-	-				
Butcherflats	BU01							-	-				
Nechako PG - Vanderhoof													
Sweden	SN00			-									-
Tachintelachick	TK00			-	-								
Breeze	BZ00			-	-								-
Zelkwas	ZK00			-	-								-
Hutchison	HN00			-								-	
Cluculz	CZ00			-								-	
Hulatt	HL00												-
Sinkut	SU00			-	-								-
Vanderhoof													
Knight	***KN01****		-	-	-								
Murray	***MU01***			-				-	-	-			-
	MU02	-		-	-	-		-	-	-		-	-
	MU03	-		-	-	-		-	-	-			-
West arm	***MU04***	-		-	-	-		-	-	-		-	-
	MU4.1*					-		-	-	-		-	-
	MU05	-		-	-	-		-	-			-	-
	MU06	-		-	-	-		-	-	-		-	-
East arm	MU07*					-	-	-	-	-	-	-	-
	MU09*					-	-	-	-	-	-	-	-
Clear	***CL01***			-	-			-	-			-	-
	CL02	-		-	-	-		-	-	-		-	-
Eden (CL trib)	EN01*	-		-	-	-		-	-	-			-
	CL03*	-		-	-	-		-	-	-			-
	CL04*	-		-	-	-		-	-	-			-
Moss	***MS01***				-			-	-				-
Vanderhoof - Fort Fraser													
Engen	EG00	-	-	-	-								
Halsey	HY00												-
Kluk	KK00			-								-	-
Nine Mile	9M00			-									-
	9M01	-		-	-	-		-	-			-	-
Tatsutnai	TC00												-
	TC01			-	-			-	-			-	-
Dog	***DC01***			-				-	-			-	-
Fraser Lake													
Ormond	***OR01***			-	-			-	-			-	-
Endako													
Endako	ED13					-	-	-	-				
	ED18					-	-	-	-				
	ED22							-	-				
	ED26						-	-	-				
Tchesinkut (ED trib)	TH01							-	-				
	ED52			-	-								
	ED54			-	-								
Shovel (ED trib)	SH00			-	-								
	SH01			-	-								
	ED55	-		-	-								
	ED66					-		-	-				
	ED66.1					-		-	-				
Upper Nechako													
Smith	SM00		-	-	-								
Tahultzu	TU00		-	-							-	-	-
Greer	***GR01***			-				-				-	
	GR03			-	-			-	-			-	-
Targe	TE00		-	-	-			-					
Swanson	***SW01***			-				-				-	
	SW02			-	-			-	-			-	-
Cutoff	CT01										-		-
	CT02												-
Twin	TW00			-	-			-					
	TW01			-				-	-			-	-

	Positive drop.	
-	0	Not detected
	4-10	Weak
	10-20	
	20-50	Good
	50-100	
	sup. 100	Strong
	> 1000	Very Strong
	NA	Not filtered

Not sampled due to lack of surface flow

Table 4. eDNA results from 2024. Results are shown by quantitative signal (see legend) for three time points June-July (T1), July-August (T2) and August-Sept (T3). See Table 2 for site codes.

Site	Site code	June/July SD401 Chin	SD401 Rain	SD401 Coho	SD401 Sock	July/Aug SD402 Chin	SD402 Rain	SD402 Coho	SD402 Sock	Aug/Oct SD403 Chin	SD403 Rain	SD403 Coho	SD403 Sock
Chilako													
Chilako Main	CK00			-	-						-	-	-
Beaverley	BE01			-	-			-	-			-	-
	BE02	-		-	-	-		-	-			-	-
	CK16			-	-			-	-			-	-
Dahl	DH01			-	-			-	-			-	-
	DH08			-	-	-		-	-			-	-
	CK37			-	-	-		-	-			-	-
Unk tribe 1	CK37-u			-	-			-	-			-	-
Gregg	GG05	-		-	-	-		-	-	-		-	-
	CK53			-	-			-	-			-	
Unk tribe 2	CK53-u1			-	-			-	-			-	-
Butcherflats	BU01			-	-			-	-			-	-
	CK102			-	-			-	-			-	-
	CK116			-	-			-	-	-		-	
Nechako PG - Vanderhoof													
McPhee	MF00			-								-	-
Sweden	SN00			-	-							-	-
Tachintelachick	TK00	-		-	-							-	-
Breeze	BZ00			-	-							-	-
Zelkwas	ZK00			-	-							-	-
Hutchison	HN00			-	-							-	-
Cluculz	CZ00			-	-							-	
Hulatt	HL00												
Sinkut	SU00			-	-					-	-	-	-
Vanderhoof													
Chilco	*CI00*		-	-	-								
Knight	***KN01****			-	-	**	**	-	-				
Murray	MU00			-	-			-					
	MU01	-		-	-	-	-	-	-	-	-	-	-
	MU02			-	-	-	-	-	-	-		-	-
	MU03	-		-	-	-		-	-	-		-	-
West arm	***MU04***	-		-	-	-		-	-	-		-	-
	MU4.1*	-		-	-	-				-		-	-
	MU05	-		-	-	-		-	-	-		-	-
	MU06	-		-	-	-		-	-	-		-	-
East arm	MU07*	-	-	-	-	-	-	-	-				
	MU08*	-											
	MU09*	-	-	-	-	-	-	-	-	-	-	-	-
Clear	CL0.9			-	-			-	-			-	-
	CL01			-	-			-	-			-	-
Eden (CL trib)	EN01*	-	-	-	-	-		-	-	-		-	-
	CL03*	-		-	-	-		-	-	-	-	-	-
	CL04*	-	-	-	-	-		-	-	-		-	-
Moss	MS0.9			-	-			-	-			-	-
	MS01			-	-			-	-			-	-
	MS1.5							-	-			-	-
Vanderhoof - Fort Fraser													
Engen	EG00		-	-						-	-	-	-
Halsey	HY00			-	-							-	-
Kluk	KK00			-	-							-	-
Nine Mile	9M00			-	-							-	-
	9M01	-		-	-	-		-	-	-		-	-
Tatsutnai	TC00			-	-							-	-
	TC01			-	-			-	-			-	-
Dog	***DC01***			-	-			-	-			-	-
	DC01.1			-	-								
Fraser Lake													
Ormond	***OR1***			-	-			-	-			-	-
Endako													
Endako	ED13			-				-	-			-	-
	ED18			-	-		-	-	-			-	-
	ED22		-	-	-			-	-			-	-
	ED26			-	-	-		-	-			-	-
Watkins (ED trib)	WA01					-		-	-				
Tchesinkut (ED trib)	TH01							-	-			-	-
	ED66			-	-	-	-	-	-			-	-
	ED66.1			-	-	-	-	-				-	-
Burns Lake - Saul	SL02*	-		-	-								
	SL04*	-		-	-	-		-	-	-		-	-
Upper Nechako													
Smith	SM00		-	-						-	-	-	-
Tahultzu	TU00			-						-	-	-	-
Greer	***GR01***			-	-			-				-	-
	GR03			-	-			-	-			-	-
	GR04			-	-			-	-			-	-
	GR05					-		-	-			-	-
	GR06									-		-	-
Targe	TE00		-	-	-					-	-	-	-
Swanson	***SW01***			-	-			-	-			-	-
	SW02			-	-			-	-			-	-
Cutoff	CT00			-						-	-	-	-
	CT02									-		-	-
	CT04									-		-	-
Twin	***TW01***			-	-			-	-			-	-

		Positive: droplet/copy #	
0	-	0	Not detected
1		4-10	Weak
2		10-20	
3		20-50	Good
		50-100	
		sup. 100	Strong
		> 1000	Very Strong
		NA	Not Sampled

	Not sampled due to lack of surface flow
-	No Flow - isolated pool(s) sampled - not detected
**	salvage site

Table 5. eDNA results from 2025. Results are shown by quantitative signal (see legend) for three time points June-July (T1), July-August (T2) and August-Sept (T3). See Table 2 for site codes.

Site	Site code	June/July SD501 Chin	Rain	Coho	Sock	July/Aug SD502 Chin	Rain	Coho	Sock	Aug/Oct SD503 Chin	Rain	Coho	Sock
Chilako													
Chilako Main	CK00			-	-							-	-
Beaverley	BE01			-	-							-	-
	CK16	-		-	-			-	-			-	-
Dahl	DH01	-		-	-			-	-			-	-
	CK37			-	-			-	-			-	-
Unk tribe 1	CK37-u	-		-	-			-	-			-	-
	CK53			-	-			-	-			-	-
Unk tribe 2	CK53-u1			-	-			-	-			-	-
Butcherflats	BU01			-	-			-	-			-	-
	CK102			-	-							-	-
	CK116			-	-							-	-
Nechako PG - Vanderhoof													
McPhee	MF00			-									
Sweden	SN00				-								
Tachintelachick	TK00			-	-					-		-	-
Breeze	BZ00				-								
Zelkwas	ZK00			-								-	
Hutchison	HN00				-								
Cluculz	CZ00			-	-							-	
Hulatt	HL00												
Sinkut	SU00			-	-						-	-	
Vanderhoof													
Knight	KN00		-	-	-							-	
	KN01*			-	-	-	-	-	-	-	-	-	-
	KN02			-	-	-	-	-	-	-		-	-
	KN03			-	-								
	KN04			-	-								
Murray	MU00												
	MU01			-	-			-	-			-	-
	MU02	-		-	-	-		-	-	-		-	-
	MU03	-	-	-	-	-	-	-	-	-	-	-	-
West arm	***MU04***	-	-	-	-	-	-	-	-				
	MU05									-		-	-
	MU06					-		-	-	-		-	-
East arm	MU07*	-	-	-	-	-	-	-	-	-		-	-
	MU08*	-	-	-	-					-	-	-	-
	MU09*	-	-	-	-	-	-	-	-	-	-	-	-
	MU10*	-		-	-	-	-	-	-				
Clear	CL0.9			-	-			-	-				
	CL01			-	-			-	-			-	-
	CL02	-		-	-			-	-			-	-
Eden (CL trib)	EN01*	-		-	-	-		-	-	-		-	-
	CL03*	-		-	-	-		-	-			-	-
	CL04*					-		-	-			-	-
Moss	MS0.9			-	-			-	-				
	MS01			-	-			-	-			-	-
Vanderhoof - Fort Fraser													
Engen	EG00												
Halsey	HY00			-	-							-	-
Kluk	KK00			-	-							-	-
Nine Mile	9M00			-	-							-	-
	9M01	-		-	-	-		-	-	-		-	-
Tatsutnai	TC00											-	-
	TC01			-	-			-	-			-	-
Dog	DC00			-	-								
	DC01	-		-	-			-	-			-	-
Fraser Lake													
Ormond	***OR01***			-	-			-	-			-	-
Endako													
Endako	ED13			-	-		-	-	-		-	-	-
	ED22			-	-			-	-			-	-
Watkins (ED trib)	WA01	-	-	-		-	-	-	-				
Tchesinkut (ED trib)	TH01			-	-			-	-			-	
	ED66.1	-		-	-	-		-	-	-		-	-
Upper Nechako													
Smith	SM00		-	-	-					-	-	-	-
Tahultzu	TU00		-								-		
Greer	***GR01***			-	-			-	-			-	
	GR03			-	-			-	-				
	GR04			-	-			-	-			-	-
	GR05.9					-		-	-				
	GR06	-		-	-	-		-	-	-		-	-
Targe	TE00			-						-	-	-	
Swanson	***SW01***			-	-			-	-		-	-	-
	SW02			-	-			-	-			-	-
Cutoff	CT00		-	-	-					-	-	-	
	CT01	-	-			-	-	-	-				
	CT02	-	-	-	-	-	-	-	-	-	-	-	-
Twin	***TW01***							-	-			-	-
	TW02	-		-	-								
	TW03	-	-	-	-	-	-	-	-				

	Positive: droplet/copy #	
-	0	Not detected
	4-10	Weak
	10-20	
	20-50	Good
	50-100	
	sup. 100	Strong
	> 1000	Very Strong
	NA	Not Sampled

Not sampled due to lack of surface flow

Results by Species

Chinook salmon: Chinook salmon spawning in the study area are part of two Designatable/Conservation units (DU/CU) assessed as threatened stocks by COSEWIC: Middle Fraser Stream Spring 5-2 and Middle Fraser Stream Summer 502 (COSEWIC 2018, DF0 2021: Pacific Salmon Foundation, 2025). Runs in the Stuart-Nechako watershed represent the furthest Northwest migrations of spawning fish of these CU within the Fraser River Basin. Results from 2023-2025 show the widespread use of the lower sections of streams connecting with the mainstem of the Nechako River by Chinook salmon at most time periods. These results are similar to those found in our eDNA surveys since 2020, summarized by creek and species detected (Appendix 1). Chinook were also noted at many locations in the Chilako and Endako systems. Our results provide strong evidence for the widespread habitat use of the study area by juvenile Chinook salmon. The eDNA detections found in this study doubles the number of creeks along the Nechako, from ~20 to 38, that are found to contain Chinook salmon. The timing of detections (June to July) as well as the confirmation by minnow trapping evidence (2023 report) show that sites within 1 km of the confluence with the Nechako are important for the rearing of juvenile Chinook. As juvenile Chinook can spend up to two years in this area, this study highlights the importance of this environment for ongoing conservation of this stock.

Upper Nechako: The upper Nechako is a known spawning area for Chinook. Chinook spawning in the Nechako River are classified as part of the Middle Fraser, Stream Summer 5-2 conservation unit (Pacific Salmon Foundation, 2025, DFO 2021). Spawning occurs from late August to early October, with maximum numbers around mid-September. Spawning locations in Upper Nechako about are fairly evenly distributed (Figure 1 and 2) with large numbers of spawning Chinook being noted about 20 km downstream of the Kenney Dam (NFCP Technical Committee, 2023). From 2023 to 2025 we sampled 7 creeks within this area (Targe, Tahultzu, Smith, Twin, Cutoff, Swanson and Greer). Of these we sampled multiple locations of four of these creeks (Twin, Cutoff, Swanson and Greer). Three creeks (Twin, Swanson and Greer) were consistently sampled since 2020. In 2021 and 2022 July sampling (T2) could not be conducted due to forest fires in the area.

Figure 1: Sampling sites in the Upper Nechako – Canyon Dam to Swanson Creek. The map shown is modified from iMapBC (B.C. Conservation Data Centre, 2026). Reported Chinook observations and Major spawning location reported on iMapBC (April 2026) are shown as well as the relative results of the eDNA survey.

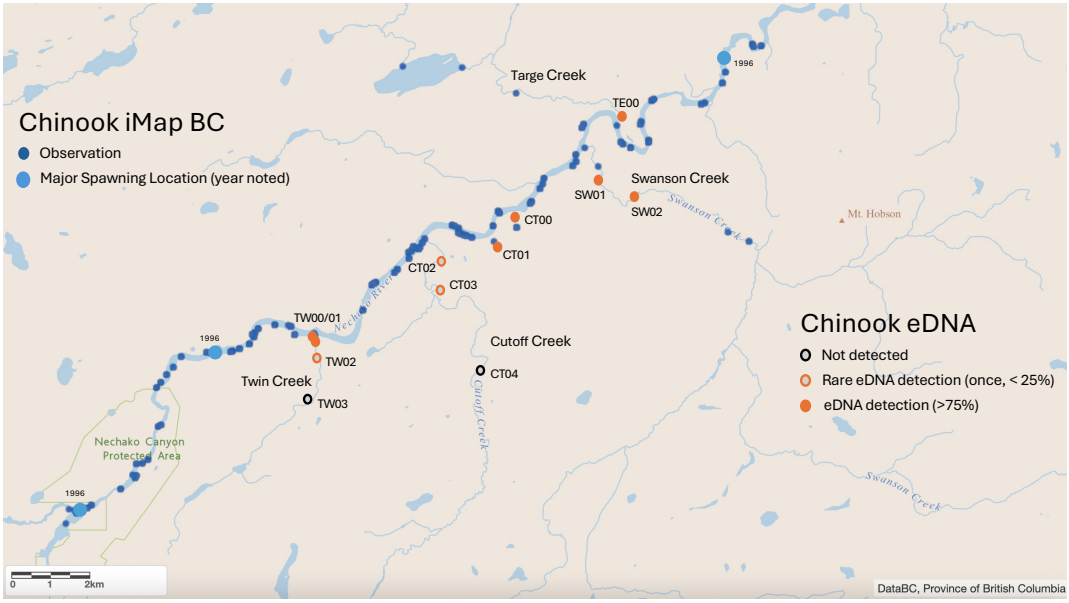


Figure 2: Sampling sites in the Upper Nechako – Greer Creek to Smith Creek. The map shown is modified from iMapBC. Reported Chinook observations and Major spawning location reported on iMapBC (April 2026) are shown as well as the relative results of the eDNA survey.



Twin Creek: Twin Creek is the uppermost and smallest creek in the Upper Nechako with two areas for easy access: one on the Kenney Dam Forest Service Road (KD-FSR) and one on a service road leading to cabins along the Nechako River. Up to 4 sites were sampled on Twin Creek. Two are located close to the Nechako (TW00 and TW01). Both of these sites showed 100% consistently with Chinook detection - TW01 was sampled 15 times over 6 years (average 2.5/year). Site TW02 was located approximately 700 m from the Nechako with Chinook only detected once in 4 sampling days. Site TW03 is located in a beaver pond adjacent to the Forest service road. In June sampling (T1) in 2021, 22 and 25 Chinook were not detected at this site. By August this site is usually dry.

Cutoff Creek: Cutoff Creek is a relatively small low gradient creek. The lower reaches, where most of our sampling was done flows through an extensive wetland complex that is punctuated by numerous beaver dams. Much of the lower section of the creek (downstream of the KD-FSR) are also used for grazing cattle. Due to inconsistent/low flows, it was not consistently sampled over the time span of the project, with no samples collected in 2021. As with Twin Creek Chinook detections are more common at lower sites, CT00 (2 out 4 (2/4) sampling time point) and CT01 (6/7), while upper sites CT02 (1/5), CT03 (1/1) and CT04 (0/1) show fewer detections.

Swanson Creek: Swanson Creek is an intermediate sized creek (~22,500 ha watershed). Two sites were consistently sampled over 6 years. SW01 is located on the former HSP Ranch, while SW02 is on a run, running parallel to the KD-FSR. Except for June 2022 (T1), Chinook were detected at these sites. In some years the lower end of the creek has been noted to run subsurface at later timepoints, disrupting the continuity with the mainstem. Note, starting the fall of 2023, Chinook fry from the SCWA hatchery were added to this creek at the KD-FSR bridge just downstream SW02.

Greer Creek: Greer Creek is the largest creek in this area (41,037 ha) and was progressively sampled at further upstream sites over the time of the study. Detection of Chinook were somewhat inconsistent over time. In earlier studies (2020-2022), Chinook were not detected at the lowest site [GR01 (0/3)] during the first sampling period (June). In contrast, Chinook were at detected GR01 at later time points (4/4), as well as at sites further upstream, GR03 (1/1), in 2020-2022. From 2023 to 2025, Chinook were consistently detected at GR01 and GR03 from at all times points, as well as at GR04 In 2024/25. As noted for Swanson, starting the fall of 2023, Chinook fry from the SCWA hatchery were added to this creek at GR01. High quantitative levels of eDNA detection for Chinook after 2023 may be associated with fish enhancement efforts, although the effects of yearly variations

in returns and season variation in stream flow on eDNA detection are confounding factors. Consistent, high quantitative eDNA detections in most upper Nechako sites are also noted prior to recent enhancement for 2023 (Table 3). The effects of the Big Bar slide (2019) and/or high spring flows on lower juvenile detections in the upper Nechako might also be additional factors for 2020 and 2021. In any case the detection of Chinook from the confluence to over 15 km upstream illustrates in importance of this stream for rearing of Chinook in the upper Nechako.

Targe Creek: Sampling of Targe Creek (as well as for Smith and Tahultzu) only began in 2023 as part of the river boat survey of sites near the mainstem. Targe Creek has the second largest watershed in this zone and drains a lake system containing kokanee populations (Angler's Atlas 2026). Chinook are detected at this lower site the June sampling period for all 3 years. Reduced flow by the August time point (T3) however, made sampling not possible in 2023 (no flow), with isolated pools in 2024 and low flow between pool in 2025. Chinook were not detected in the T3, 2024 or 2025 samples.

Tahultzu Creek: This relatively small creek had consistent Chinook detections at all time points from 2023 – 2025. Consistent flows may be maintained by the presence of a beaver dam just upstream of the sampling site.

Smith Creek: Smith Creek is similar to Swanson in watershed area and drains an area Northwest of the Nechako. Similar to Targe Creek, Chinook were detected in the June sampling of 2023 – 25, however by August flow had drastically reduced with no flow 2023, isolated pools in 2024 and low flow between pools in 2025. Chinook were not detected in the T3, 2024 or 2025 samples.

Nautley River to Vanderhoof: This section represents a second important spawning area for Chinook, with mainstem spawning locations identified from downstream of the confluence with the Nautley River to just upstream of the town of Vanderhoof. Spawning run timing is similar to the upper Nechako, peaking in mid-September, with spawning locations distributed from Vanderhoof to the Nautley River (NFCP Technical Committee, 2023). Various creeks were sampled in this zone (Figure 3). Starting in 2021, sites on three creeks (DC01, TC01 and 9M01) with road access (i.e., Dog Creek FSR and adjacent roads) were sampled 3 times per year. In 2023, additional sites were included as part of the bi-yearly riverboat survey.

"Dog" Creek: This creek was labeled as Dog Creek in our survey; however, this is an un-named creek that is the first creek downstream of the confluence with the Nautley River. The site, DC01 is with 50 m of the confluence while DC00 is located less than 20 m downstream of DC01. These sites show consistent detection of Chinook throughout all 3 times points over the 5-year survey.

Tatsutnai and Nine Mile Creeks: These are first named creeks downstream and of Nautley with both creeks draining a similar watershed size (~6800 ha) northwest of the Nechako. Similar to 'Dog' Creek, both of these creeks were sampled at an upstream site three times a year, starting in 2021, while confluence sites were sampled 2 times a year, starting in 2023/24. Chinook salmon were detected at Tatsutnai sites (~ 700 m apart) at all time points, showing consistent habitat use. The confluence site on Nine Mile Creek (9M00) showed similar results, while only samples prior to 2024 detected Chinook at the upper site (9M01), 4 km upstream. We suspect a barrier to fish passage has blocked usage of upstream areas by juvenile Chinook at the more recent sampling times.

Figure 3: Sampling sites in the Nechako River – Nautley River to Vanderhoof. The map shown is modified from iMapBC. Reported Chinook observations and Major spawning location reported on iMapBC (April 2026) are shown as well as the relative results of the eDNA survey.



“Dog” Creek: This creek was labeled as Dog Creek in our survey; however, this is an un-named creek that is the first creek downstream of the confluence with the Nautley River. The site, DC01 is with 50 m of the confluence while DC00 is located less than 20 m downstream of DC01. These sites show consistent detection of Chinook throughout all 3 times points over the 5-year survey.

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Kluk, Halsey and Engen Creeks: These creeks were sampled only during the bi-yearly river sampling. Both Kluk and Halsey had consistent detection of Chinook throughout 2023-2025. In contrast, Engen Creek drains into a side channel of the Nechako. In our sampling, this side channel was not always connected to the Nechako. Further, at most times points, except in June 2023 and June 2024, the creek did not connect with the side channel and was either dry or contained isolated pools. At only one time point, June 2024, were Chinook detected at this creek.

Vanderhoof Area: The Vanderhoof area represents our most intensive survey zone (Figure 4). Sampling here started in 2020 and has involved four creeks, Moss, Clear, Murray and Knight. Each creek has a number of locations/creeks, encompassing both past and ongoing restoration sites as well as natural beaver dam complex (Murray Creek).

Figure 4: Sampling sites in the Nechako River – Vanderhoof. The map shown is modified from iMapBC. Reported Chinook observations and Major spawning location reported on iMapBC (April 2026) are shown as well as the relative results of the eDNA survey.



Moss Creek. Moss Creek has the smallest drainage of all creeks studied in this zone. Sample sites here were concentrated in a reach ~ 200 m upstream of the confluence with the Nechako. A perched culvert was replaced by a bridge in proximity to our sample locations in late 2021 just downstream of site MS1.0. As with most sites close to the Nechako Chinook were detected at all years and most time points. The site MS1.5 (about 500 m further upstream) was only sampled in 2024, after the improvement work and Chinook were detected in both time points sampled.

Clear Creek: This creek is the first of our "school district focus" areas and has a variety of sites located in agricultural areas, from the downstream site CL01, which

consistently detects Chinook, to the upstream sites CL03/CL04/EN01. These last three sites are above a major beaver dam complex, in proximity to a significant culvert replacement project that occurred in 2023, and are located in an area were School District 91 has an outdoor teaching facility. Chinook have not been detected at these three upstream sites, except in the late Aug/Sept sampling of 2025. Prior to sampling in 2025 ~ 1000 hatchery fish Chinook were added to this area as part of another study on fish movement (Pearce 2026). Although the existing beaver dam complex is a potential barrier to fish upstream passage, Coho were detected in this zone in the Aug/Sep sampling of 2023 and 2025 hatchery fish were able to move downstream (Pearce 2026.).

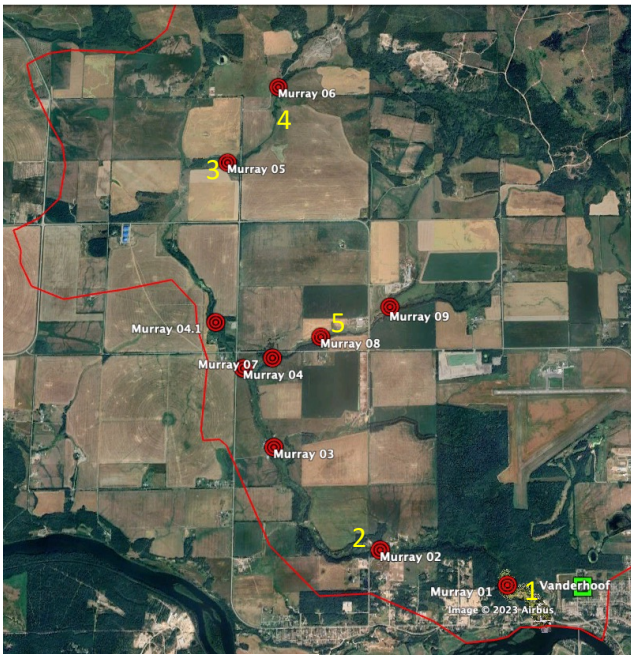
Knight Creek: This creek also flows through the Vanderhoof agricultural area. It was our experience from 2020-2024 that flows at Knight Creek consistently went sub-surface in the later sampling periods resulting in discontinuous flows and the formation of isolated pools thus rendering the lower site (KN01) unsuitable for Chinook as the season progressed. In fact, the NVSS fisheries crew salvaged a large number of Chinook from isolated pools in this area in 2024. As part of the BC SRIF project, BDAs were constructed prior to the 2025 sampling period. Sites KN02, KN03 and KN04, are located just downstream and within the two BDAs constructed in this area. In the spring of 2025, Chinook were found throughout the complex. However, as flows reduced, only sites KN01 and KN02 could be sampled at later time points. Chinook were not detected at KN01/KN02 at the later timepoints. Unlike previous years, we were able to sample flowing water at KN01 indicating that BDAs are able to help maintain surface flows in this section of the creek. Ongoing sampling should be conducted to assess long-term future usage.

Murray Creek: This creek has the largest watershed (~ 12000 ha) of our study creeks in this zone and is the closest creek to the town of Vanderhoof and to NVSS. It is also one of the creeks that is most heavily impacted by agriculture. Given its proximity to Vanderhoof, it has been used as a demonstration site for a range of past and present restoration projects. As a result, this creek has been a focal point of our sampling efforts with nine core sampling sites spread over 12 km of the creek (Figure 5).

Murray Creek best represents the “teaching /educational’ aspects of the project and has become the “learning lab” for High School students on the eDNA project. Rainbow trout are observed and detected at the top-most sites, MU05 and MU06. Here ideal trout habitat is found. MU06 represents the most natural state, being just outside the agricultural belt. Rainbow trout dominated the species caught in minnow traps throughout our study at this location. MU05 is located on a previous restoration site, adjacent to a horse pasture. A combination of rainbow trout and

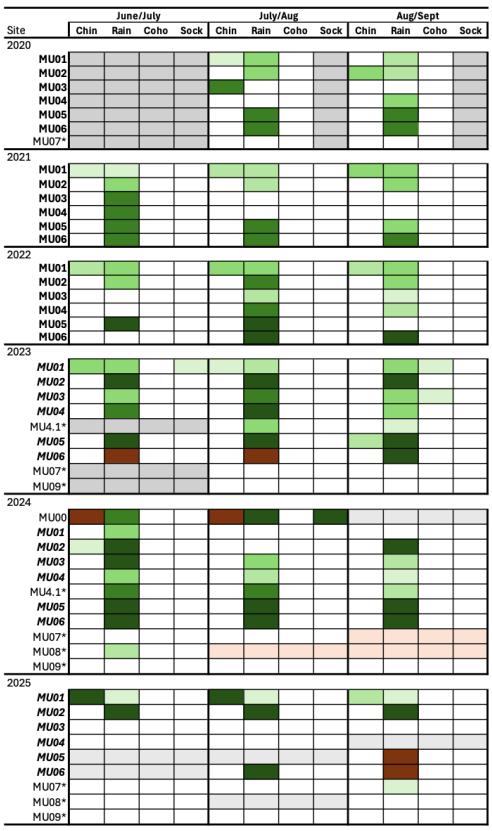
minnow species have been caught at this site. Large (12-24 cm) rainbow trout were also caught in beach seines in constructed over-wintering ponds in this area in 2020. Downstream of MU 05 the creek morphology changes: the gradient is lower, and creek bed material is dominated by fines. In addition, there is a general reduction in over story cover, water temperatures are generally higher, and the areas are more subject to agricultural impacts. This is true for sites MU09, 08, 07, 04 and 03. Minnow trapping results reported in the previous reports (See 2023/2024 reports, Appendix 3) find non-salmonid species (e.g., suckers and various minnow species) dominant. Rainbow trout, although detected in some eDNA samples, have never been observed. At the lower sites MU02 and MU01, Rainbow trout are once again observed, both in minnow trapping and seining efforts and consistently detected in eDNA.

Figure 5: Murray Creek restoration and sampling sites. Sampling sites MU01-09 are shown by red circles. The location of five restoration sites are shown: 1) Bank stabilization/Natural Beaver dam complex; 2) Bank stabilization, riparian planting, overwintering ponds; 3) Culvert replacement, re-establishment of creek bed, riparian planting; 4) Bank stabilization, riparian planting; and 5) Beaver dam analogs for headwater storage (2024/25 NEWSS activity). The eDNA results specific for Murray creeks sites from 2020-2025 are shown to the right of the study map.



Legend

Colour	Strength	# positive
	Weak	4-10
	Solid	10-20
	Solid	20-50
	Strong	50-100
	Strong	sup. 100
	Very Strong	> 1000
	No water	
	Not detected	



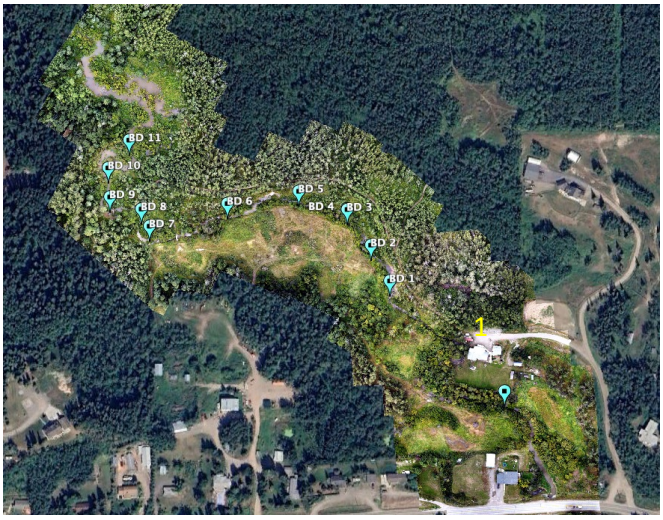
As with other creeks, Chinook are primarily observed at the bottom sites, closest to the Nechako River. Although we have not caught Chinook above MU01 in our minnow trapping efforts, they were noted in earlier studies at/near MU 04 reported on iMapBC (April 2026), and we only occasionally detect them in eDNA sampling.

In summary, the detailed analysis of this creek illustrates the varied and differential use of the creek by a range of fish species, providing both an excellent learning lab for the students, as well as a well-documented baseline for current initialization of the Beaver dam analog at site MU08.

Analysis of the Murray Creek Beaver Dam complex: The general lack of Chinook detection above MU01, situated in the penultimate pond of a natural beaver dam complex, prompted the initiation of yearly Fall eDNA survey of the complex. From 2021 to 2025, up to 12 dams have been noted in the complex (Figure 6). These range from large dams found throughout the study, to smaller dam observed in only one year. Yearly freshets which can disrupt existing dams and the vagaries of beaver activity make the complex dynamic. In general, we observed an increase in dam height over the time period. For example, the most upstream dam of the complex, that also hosts the beaver lodge, grew from 0.7 m in 2021 (Murray and Booth 2021) to just over 2 m in 2025. In contrast some secondary (=smaller) dams were breached and mostly removed between the 2024 and 2025 sampling. Despite the presence of beaver dams, Chinook eDNA was found throughout the complex in 2021 and 2022. In both these years an increasing eDNA signal is observed as the sample is taken further down the complex, showing evidence for habitat use by Chinook throughout the complex. By 2023, Chinook are no longer detected at the top-most/primary pond(s), and detection of Chinook in lower ponds has become more stochastic and relative signal strengths weaker. Overall, these surveys show a dynamic relationship between dam height and Chinook passage through a beaver dam complex and suggest that as dam height increases Chinook passage decreases.

Figure 6: Composite of Natural Beaver dam Complex on Murray Creek and eDNA analysis 2021-2025. The arial map shows the relative locations of the Beaver dams within the complex. The eDNA results specific for beaver dam sites BD01-BD12, analyzed from 2021-2025 are shown below the study map. Coho were not detected and therefore removed for clarity.





	Positive drop.
-	Not detected
4-10	Weak 4-10
	10-20
	Good 20-50
	50-100
	Strong sup. 100
	not sampled - NO beaver dam

* = MU00 (Main Survey)
** = MU01 (Main Survey)

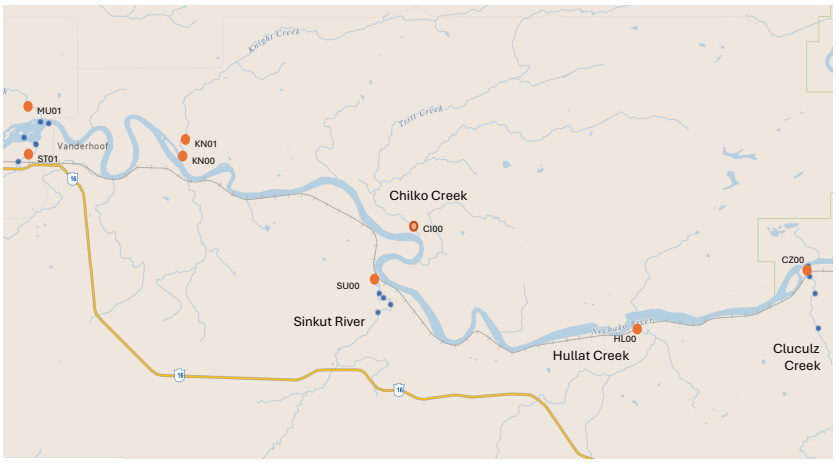
Site	site code	2021 Chin	2021 Rain	2021 Sock	2022 Chin	2022 Rain	2022 Sock	2023 Chin	2023 Rain	2023 Sock	2024 Chin	2024 Rain	2024 Sock	2025 Chin	2025 Rain	2025 Sock
Pre complex	BD01*			-						-			-	-	-	-
Beaver Dam 1	BD02		4-10	-			4-10									-
Beaver Dam 2	BD03			-			-			-	4-10		-			
Beaver Dam 3	BD04			-			4-10			-	-		-			
Beaver Dam 4	BD05			-			-			-	4-10		-	-		-
Beaver Dam 5	BD06	4-10		-					4-10	-	-		-	-		-
Beaver Dam 6	BD07	4-10		-			-	4-10	4-10	-	-		-	4-10	4-10	-
Beaver Dam 6.2	BD7.2															
Beaver Dam 7	BD08	4-10		-			-		4-10	-	-	4-10	-	4-10		-
Beaver Dam 8	BD09						4-10			-	4-10	4-10	-	4-10		-
Beaver Dam 9	BD10										-	4-10	-	4-10		-
Beaver Dam 10	BD11**	4-10		-				-		-	-	4-10	-			
Beaver Dam 11	BD12						-	-		-	-	4-10	-	-		-

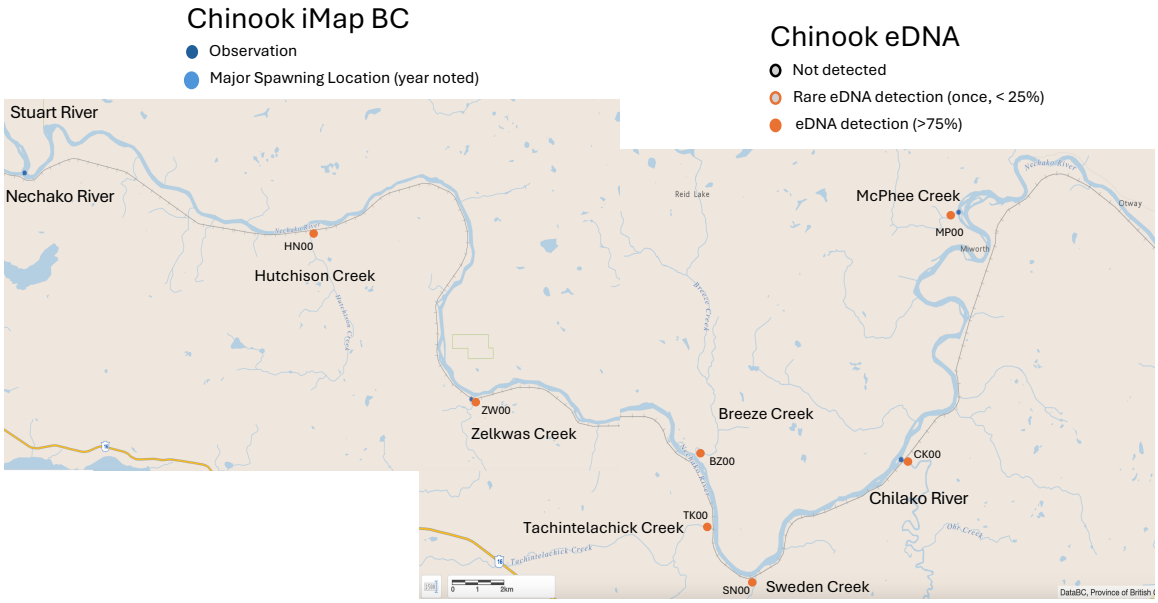
Vanderhoof to Prince George (McPhee Creek): Creeks in this area were sampled by via the bi-yearly river boat samples from 2023 – 2025 (Figure 7). All samples were taken at one location per creek and were located within 100 m of the confluence with the Nechako.

Vanderhoof to Stuart River: Three streams were sampled in this zone. Two have large watersheds (Table 1) and drain medium sized lakes, Sinkut river (Sinkut Lake) and Cluculz Creek (Cluculz Lake). Cluculz Lake, and further upstream Eulatazella Lake, have a known Kokanee populations (Anglers Atlas, Apr 2026). Situated between the two lake systems, Hulatt Creek, drains a smaller area and due to a shallow, rocky approach, was only sampled twice at high flow time points. Consistent habitat use by Chinook at these sites was noted, over 3 years at all time points, except for the sample taken in August 2024 at the Sinkut River (Table 6).

Figure 7: Sampling sites in the Nechako River – Vanderhoof to McPhee Creek.

The map shown is modified from iMapBC. Reported Chinook observations and Major spawning location reported on iMapBC (April 2026) are shown as well as the relative results of the eDNA survey.





Stuart River to McPhee Creek: Six creeks and Chilako River were sampled in this zone. The Chilako was sampled extensively and will be dealt with separately below. The majority of the creeks are found on the south side of the Nechako River and sampling locations were all situated between a CN rail line culvert and the Nechako mainstem. Breeze Creek drains north of the river, while McPhee Creek runs adjacent to the Clesbaoneecheck (Lheidli T'enneh #3) Indian Reserve. As with the creeks above the confluence with the Stuart River, Chinook were consistently detected at most creeks throughout the study period. In Tachintelachick Creek, Chinook were detected at 4 of 6 time points. As noted in previous reports, one area of study in subsequent years could focus on the passage of Chinook above the CN rail line culverts in this zone. This would help define the area of creek being used by juvenile Chinook.

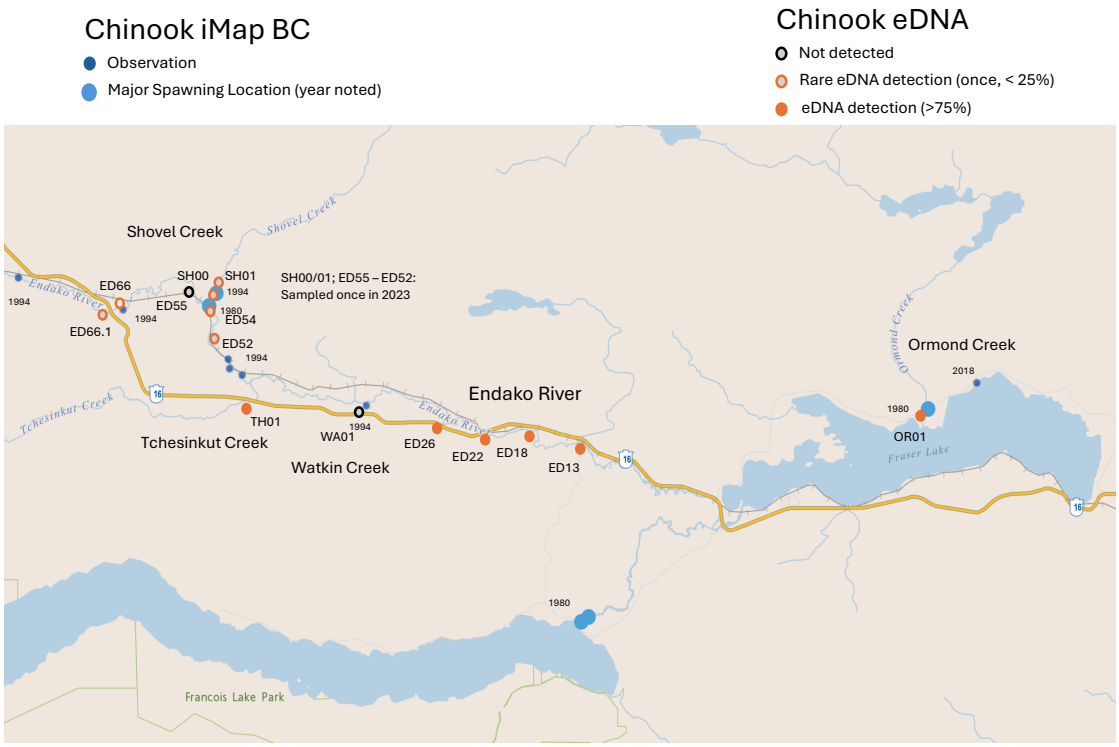
Chilako River: The Chinook in the Chilako River represent another major spawning run in Nechako watershed. Classified as part of the Middle Fraser, Spring 5-2 conservation unit, adults return earlier than the mainstem Nechako spawners (Pacific Salmon Foundation. 2025). We sampled this river in 3 consecutive years, with 2024 being the most extensive (Tables 2-3). In contrast to our sampling of the Nechako, many eDNA samples were taken in the mainstem of the Chilako (Figure 8). Sampling clustered around kilometer 16, 37, 53 and 102 (measured from the river mouth), and adjacent tributary creeks. Of note, Chinook were detected at our upper most sampling location, ~116 km from the confluence of the Chilako with the Nechako River. Widespread use of Chilako tributary creeks by Chinook salmon was also noted in most creeks tested (Beaverley, Dahl, Butcherflats, and two unnamed creeks) except for Gregg and Chehischick Creeks. Chinook were not detected in Gregg Creek in 2023 and 2024 sampling, while Chehischick was only sampled once in 2024. These results again show the widespread use of the Chinook juvenile and adults throughout the system.

Figure 8: Sampling sites in the Chilako River. The map shown is modified from iMapBC. Reported Chinook observations and Major spawning location reported on iMapBC (April 2026) are shown as well as the relative results of the eDNA survey.



Endako River: The Chinook in the Fraser Lake/Endako River system represent the third major spawning run examined in Nechako watershed. Similar to the Chilako run, these fish are classified as part of the Middle Fraser, Spring 5-2 conservation unit (Pacific Salmon Foundation. 2025). A core spawning area for Chinook in the Endako is located in proximity to Shovel Creek ~54 km upstream of the confluence with the Stellako River . Due to the sampling effort to reach these remotes sites, this area was only sampled in 2023 with the fisheries crew from the Carrier Sekani Tribal Council, where presumably juvenile Chinook were detected in the June (pre-spawning) sampling period (Table 3, Figure 9). The remaining sites are located along Highway 16, allowing a wide geographic of site with the same sampling effort. Most sites below the core sampling area, including Tchesinkut Creek, showed consistent detection of Chinook over the 3-year study. Chinook, were not detected in Watkins Creek, located down of the core area, in either 2024 (T2 only) or 2025 (T1 and T2). By the third sampling period this creek did not a have a surface flow. Sites above the core area only detected Chinook in the first and third sampling periods of 2024. This data shows the importance of sites downstream of the core spawning area for rearing.

Figure 9: Sampling sites in the Endako River. The map shown is modified from iMapBC. Reported Chinook observations and Major spawning location reported on iMapBC (April 2026) are shown as well as the relative results of the eDNA survey.

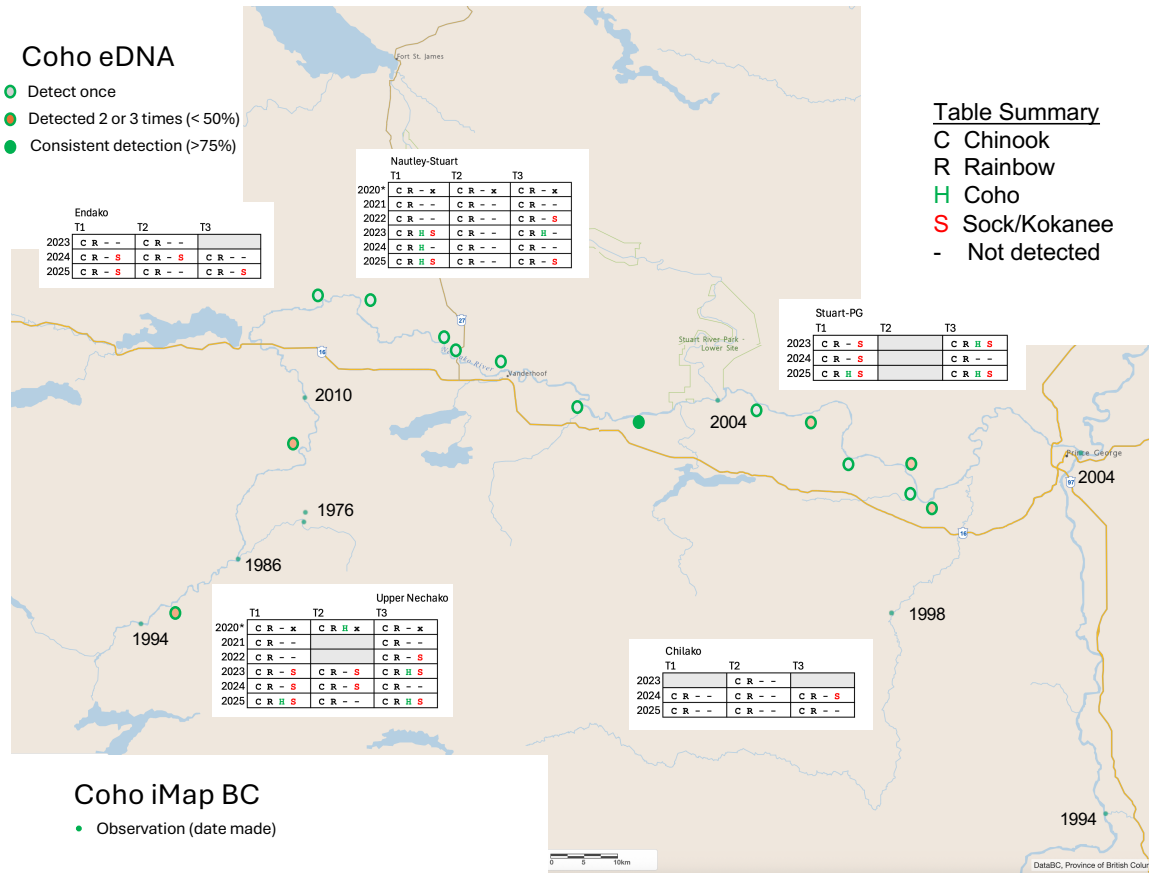


Located on Fraser Lake, Ormond Creek has been sampled since 2021. Except for the initial spring sample in 2021 (T1), Chinook have been detected in this creek in 14 of 15 eDNA sampling times. Indeed, later sampling shows strong or very strong detections in 2023 - 2025 (Table 3 - 5). This shows evidence of the importance of this creek for rearing. Ormand Creek was identified as an important spawning location for Chinook in surveys conducted from 1959 -1978. Given the consistent high eDNA signal detected at this location, we recommend a current re-evaluation of this system to determine its current use a spawning location.

Coho salmon. Interior Fraser Coho are currently recognised as "Threatened" (COSEWIC 2016). The Nechako Coho are part of the Upper/Middle Fraser runs within the Interior Fraser Designated Unit (DU). Although the number of escapements using the Nechako system is not stated, the number of escapements in the Upper Fraser was estimated at a yearly average of 1979 over the course of 2008-2011. Only a proportion of this will represent Nechako Coho runs, and the fish in this system are rarely observed. IMapBC lists eight total observations (Figure 10) from 1976 to 2010 (B.C. Conservation Data Centre. April 2026). Interior Fraser Coho run from Mid-November to Mid-January, with the majority of observations being noted in this time period. The presence of Coho in the Nechako system was confirmed by our eDNA analysis, which detected Coho at 14 creeks (based on 24 positive time points) from 2020-2025, more than doubling the previous observations. Detections varied by year (Table 5), with no detections in 2021, 22 and 24; a single detected in 2020; and larger numbers in 2023 (13 creeks) and 2025 (5 Creeks). As spawning runs occur in the winter, all detections in our survey can be assumed to be from juvenile Coho. No Coho were detected in either the Endako or Chilako systems. While the Chilako system is noted as a possible area for Coho habitat (COSEWIC 2016), our survey does not support this hypothesis.

The majority of detections were noted in the creeks between Prince George and Vanderhoof (Sweden to Moss). Within this area, multiple time point detections were noted for Sweden (3 of 6), Breeze (3 of 6), Hutchison (2 of 6) and Hulatt (2 of 2) creeks. This area represents a key zone for the further study of the Juvenile Coho habitat use with the Nechako. Only in the Upper Nechako sites in Cut off (2 of 10) and Tahultzu (2 of 6) creeks were Coho gain detected at multiple time points. This data shows the widespread use of the Nechako River by juvenile Coho and indicates years of high abundance (2023 and 2025) and area of interest for future study.

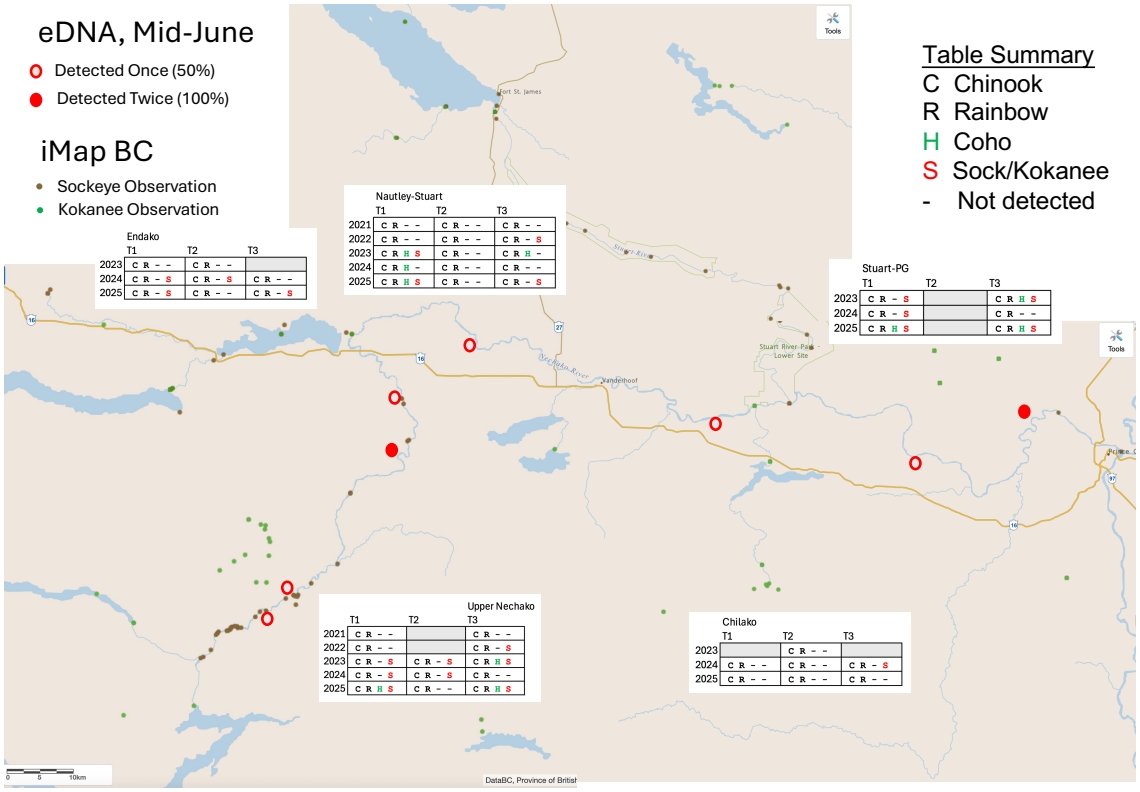
Figure 10: eDNA detection of Coho within the study area. The map shown is modified from iMapBC. Reported Coho observations reported on iMapBC (B.C. Conservation Data Centre. April 2026) are shown as well as the relative results of the eDNA survey. Inserted tables represent regional findings from 2020-2025 surveys, shown by time and fish species: Chinook – Rainbow – Coho – Sockeye.



Sockeye salmon. In contrast to Coho, Sockeye salmon have substantial spawning runs that occur throughout our study area (Pacific Salmon Foundation 2025; DFO 2009), and Sockeye detection is higher than that noted for Coho (Table 5). The detection of juvenile Sockeye in our eDNA study, however, is confounded both by the occurrence of spawning adult runs and Kokanee systems in our study time/area. Early summer and summer runs occur in both the both Stuart and Fraser Lake systems (Pacific Salmon Foundation 2025). Figure 11 shows the iMapBC observations of both Sockeye and Kokanee in the study and limits eDNA detection sites to those collected around mid-June (B.C. Conservation Data Centre. April 2026). In only 2024 and 2025 were samples collect prior to June 18, representing possible juvenile Sockeye. Of note, is the occurrence of multiple eDNA

detections in the Upper Nechako. This area should not be influence by the early lake spawning sockeye ecotypes, or their juveniles. The Upper Nechako Sockeye represent the rarer river spawning ecotype within the study area that is part of the Middle Fraser river-type conservation unit (DFO 2009). Although Kokanee are found in the Targe/Hallett Lake/Creek system, they are not observed in the mainstem, and creeks upstream (Cutoff) and downstream (Smith and Tahultzu) not associated with Kokanee show positive eDNA detections. Similar to Coho, Tahultzu Creek is noted for having positive detections on both 2024 and 2025. This creek flows into a large shallow portion of the mainstem, an area which the data indicates may be of importance for the rearing of both Coho and Sockeye juvenile in the Upper Nechako.

Figure 11: eDNA detection of Sockeye/Kokanee within the study area. The map shown is modified from iMapBC. Reported Sockeye/Kokanee observations reported on iMapBC (B.C. Conservation Data Centre. April 2026) are shown as well as the relative results of the eDNA surveys conducted in 13 June 2024 and 17 June 2025. Inserted tables represent regional findings from 2020-2025 surveys, shown by time and fish species: Chinook – Rainbow – Coho – Sockeye/Kokanee.



Restoration sites and Metabarcoding Analysis

In addition to our ongoing eDNA survey throughout the system, a number of sites were chosen to coincide with ongoing or planned restoration activities being conducted by NEWSS (Tables 3-5, noted with asterisks). In 2025, key sites were chosen for a metabarcoding analysis. Reported in Appendix 2, this data represents critical baseline data for the assessment of aquatic diversity within the Vanderhoof area. This data will allow for the ongoing assessment of restorations projects.

Sites within four creeks within the Vanderhoof were chosen for the metabarcoding study (Table 6). At these sites eDNA from the early (T1) and later (T3) time points were analysed by metabarcoding to assess a wider a range of aquatic diversity. The Clear/Eden Creek sites, CL04 and CL03/EN01, are located upstream and downstream, respectively, of the culvert replacement and subsequent fencing of the area by NEWSS in 2023. The Knight Creek site, KN01, is just downstream of ongoing NEWSS restoration efforts (beaver dam analogs installed in late 2024 and major in-stream improvements and bank revetments (2021). Unfortunately, the stream no longer had a surface flow in July and August time periods of some year and therefore no samples were possible for analysis. Murray Creek has multiple sites with both previous and ongoing NEWSS restoration activities. MU05 is located with an area of creek that had extensive in-stream restoration work, and well as the construction of overwintering ponds that were constructed in 2015 prior to the initiation of this project. MU07 - MU09 are located on the east arm of Murray Creek where a beaver dam analogs were established at MU08. Similar to the Knight Creek sites, MU07 and MU08 were found not to have a surface flow in some years at the later yearly time points.

Table 6: Sites included in Metabarcoding study (Appendix 2). X indicates an eDNA sample was analyzed from that timepoint (62 sites in total). Bolded sites indicate long term study sites.

		2023		2024		2025		
Creek	Site	T1	T3	T1	T3	T1	T3	Total
Knight	***KN01***	**X**		**X**		X	X	4
	KN02					X	X	2
	KN03					X		1
	KN04					X		1
	MU01	X	X	X	**X**	X	X	6
Murray	***MU03***	X	**X**	X	X	X	X	6
	MU06	X	X	X	X	X*	X	6
	MU07			X		X	X	3
	MU08			X		X	X	3
	MU09	X*	X	X	X	X	X	6
Clear	***CL01***	X	X	X	X	X	X	6
	CL03	X	X	**X**	**X**	X	X	6
	CL04	X	X	**X**	**X**		X	6
Moss	***MS01***	X	X	X	X	X	X	6

No samples collected * No T1 sample, sample from T2

In order to assess the sensitivity of the metabarcoding method for detection of salmonids a preliminary comparison of ddPCR and metabarcoding (myfish marker only) results was conducted. Congruence of species detection was noted at 65% (52 of 80) of the positive sites, while 30% (24 of 80) showed ddPCR only and 5% (4 of 80) metabarcoding only. The expected increased sensitivity of ddPCR versus metabarcoding in species detection is illustrated by the improved congruence noted to 77.4% (48 of 62) when ddPCR sites showing weak (4-10 positive droplets) are removed from the comparison, with 16.1% (10 of 62) ddPCR only and 6.5% (4 of 62). Metabarcoding only site, likely also represent lower quantitative signal and overlap increases to 82.8% (48 of 58) when these values are removed.

Minnow Trapping Results - 2025

Minnow trapping was conducted on a sub-sample of the eDNA survey sites within the Vanderhoof area in 2025. Results of this component of our project are presented in Appendix 3.

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References

B.C. Conservation Data Centre. 2026. CDC iMap [web application]. Victoria, British Columbia, Canada. Available: https://maps.gov.bc.ca/ess/hm/cdc/ (Accessed April, 2026).

COSEWIC. 2016. COSEWIC assessment and status report on the Coho Salmon Oncorhynchus kisutch, Interior Fraser population, in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xi + 50 pp.

COSEWIC. 2018. COSEWIC assessment and status report on the Chinook Salmon *Oncorhynchus tshawytscha*, Designatable Units in Southern British Columbia (Part One – Designatable Units with no or low levels of artificial releases in the last 12 years), in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xxxi + 283 pp. Available from: http://www.registrelepsararegistry.gc.ca/default.asp?lang=en&n=24F7211B-1

DFO. 2009. Framework for implementation of the Wild Salmon Policy: Initial lists of Conservation Units for British Columbia. DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2009/055.

DFO. 2021. Recovery Potential Assessment for 11 designatable units of Fraser River chinook salmon, *Oncorhynchus tshawytscha*, part 2: elements 12 to 22. Canadian Science Advisory Secretariat Science. Advisory Report 2021/030

Nechako Fisheries Conservation Technical Committee. 2023. The Nechako Fisheries Conservation Program: Past, Present, Future, https://www.nfcp.org/

NEWSS 2026. Project history 2024: Knight Creek. https://www.newssociety.org/project-history/p2024/knight-creek-3

Pacific Salmon Foundation. 2025. Methods for Assessing Status and Trends in Pacific Salmon Conservation Units and their Freshwater Habitats. The Pacific Salmon Foundation, Vancouver BC, Canada. **Version 13.** Available at https://salmonwatersheds.ca/document/lib_475/ [Accessed 08-05-2026].

Pearce, S. 2026. Evaluation of Fish Passage at Beaver Dam Analogues with a Focus on Non-Natal Tributary Usage by Juvenile Nechako River Chinook Salmon. Interim Report Prepared for the Nechako Environmental Enhancement Fund.

Appendices

Appendix 1: Summary of eDNA results from 2020-2025 in the Nechako watershed by creek and relative time point, 1 pg.

Appendix 2. Biodiversity Assessment of Salmonid Habitats in The Nechako Watershed: Analysis of Four Creeks in the Vanderhoof Area, 2023-2025., RPT183-DR03, 18 pgs.

Appendix 3. Summary of Minnow Trapping, Vanderhoof Area, 2025, 3 pgs.

Appendix 1. Summary of eDNA results from 2020-2025 in the Nechako watershed by creek and relative time point, T1 late-June, T2 late-July, T3 late-August/early-September. Positive detection is noted by C – Chinook, R – Rainbow, H – Coho, and S – Sockeye (S/K – system with Kokanee).

Site	2020			2021			2022			2023			2024			2025		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
Nechako																		
PG - Stuart River																		
Chilako Main**										C R - -	C R - -	- R - -	C R - -	C R - -	C R - S	C R - -	C R - -	C R - -
McPhee													C R - S		C R - -	C R - S		
Sweden										C R - S		C R H -	C R - -		C R - -	C R H -		C R H S
Tachintelachick										C R - -		C R H S	- R - -		C R - -	C R - -		- R - -
Breeze										C R - -		C R H -	C R - -		C R - -	C R H -		C R H S
Zelkwas										C R - -		C R H -	C R - -		C R - -	C R - S		C R - S
Hutchison										C R - S		C R - S	C R - -		C R - -	C R H -		C R H S
Stuart River - Vanderhoof																		
Cluculz										C R - S/K		C R - S/K	C R - -		C R - S/K	C R - -		C R - S/K
Hulatt												C R H -				C R H S		
Sinkut										C R - -		C R H -	C R - -		- - - -	C R - -		C - - S
Vanderhoof																		
Knight***	- - - x	C R - x	C R - x	C - - -	C - - -	C - - -	C R - -			C - - -			C R - -	C R - -		C R - -	- - - -	C R - S
Murray**	C R - x	C R - x	C R - x	C R - -	C R - -	C R - -	C R - -	C R - -	C R - S	C R - S	C R - -	C R H -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -
Stoney				C R - -	C - - -	C - - -												
Clear**	C R - x	C R - x	C R - x	C R - -	- R - -	C R - -	C R - -	C R - -	C R - S	C R - -	C R - -	C R H -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -
Moss**					C R - -	C R - -	C R - -	C R - -	C R - -	C R H -	C R - -	C R H -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -
Vanderhoof - Nautley																		
Engen										- - - -			C - - S		- - - -			
Halsey												C R H -	C R - -		C R - -	C R - -		C R - -
Kluk										C R - S		C R - -	C R - -		C R - -	C R - -		C R - -
Nine Mile*				- R - -	C R - -	C R - -	C R - -	- R - -	C R - -	C R - S	- R - -	C R H -	C R - -	- R - -	C R - -	C R - -	- R - -	C R - -
Tatsutnai*				- R - -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -
Dog				C R - -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - S	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -
Fraser Lake																		
Ormond				- R - -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -
Endako																		
Endako**										C R - -	C R - -		C R - S	C R - S	C R - -	C R - S	C R - -	C R - S
Burns Lake																		
Sauls													- R - -	- R - -	- R - -			
Upper Nechako																		
Smith										C - - -			C R - S		- - - -	C - - -		- - - -
Tahultzu										C - - S		C - - -	C R - S		- - - -	C - H S		C - H S
Greer**	- R - x	C R - x	C R - x	- R - -		C R - -	- R - -		C R - S	C R - S	C R - S	C R - S	C R - -	C R - S	C R - -	C R - -	C R - -	C R - S
Targe										C - - -	C R - S/K		C - - -		- - - -	C R - S/K		- - - S/K
Swanson*	C R - x	C R - x	C R - x	C R - -		C R - -	- R - -		C R - -	C R - S	C R - S	C R - S	C R - -	C R - -	C R - -	C R - -	C R - -	C R - -
Cutoff*	C - - x	C R H x	C R - x						C R - -			C R H -	C R - S		- R - -	C R - -	- - - -	- - - S
Twin*	C R - x	C R - x	C R - x	C R - -		C R - -	C R - -		C R - S	C R - S	C R - S	C R - -	C R - -	C R - -	C R - -	- R - -	C R - -	C R - -

* two sites sampled
** multiple sites (>2)
*** multple sites in 2025

Not sampled due to lack of surface flow
---- no flow - isolated pool(s) sampled

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RPT183-DR03

Biodiversity Assessment of Salmonid Habitats in The Nechako Watershed: Analysis of Four Creeks in the Vanderhoof Area, 2023-2025

May 12, 2026

Prepared for:

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1 Executive Summary

Environmental DNA (eDNA) was used to assess fish and insect biodiversity in the Stuart and Nechako watersheds (BC). In 2025, freshwater eDNA samples were collected for environmental genomics analysis from the survey area. DNA extracts were submitted to the Centre for Environmental Genomics Applications for laboratory analysis.

Following eDNAtec's standard EnviroSeq™ DNA metabarcoding workflow, two DNA markers for fish and insect biodiversity were amplified and sequenced from all samples. DNA was successfully sequenced from 123 of 124 eDNA samples submitted. Across all samples 13 phyla, 368 families, 937 genera, and 817 species were identified. Many families were frequently detected (≥80% of samples), including Chironomidae and Baetidae, Cyclopidae, and Limnephelidae from the phylum Arthropoda, and Naidida and Lumbricidae from the phylum Annelida. We identified four fish families and 9 species of fish, with *Couesius plumbeus* being the most frequently detected species across samples (45%).

The eDNA-based biodiversity assessment presented here and in the supplementary data files provide comprehensive biodiversity data that can be used to monitor and inform management in the survey area. Data have been compiled in the supplementary files to facilitate downstream ecological analyses.

2 Background

Environmental genomics is a powerful, non-invasive approach to biodiversity characterization that relies on the recovery and analysis of genetic material from the physical environment in which organisms live (e.g., water, soil, sediment, air, etc.). DNA is released into the environment from organisms through various mechanisms, including cell shedding and the excretion of various bodily fluids and feces[1–3]. This environmental DNA, or eDNA, can be captured in an environmental sample and analyzed to generate biodiversity data.

Using a DNA metabarcoding approach, DNA is isolated from samples and biodiversity informative regions of DNA are amplified through polymerase chain reaction (PCR). These regions are then sequenced using high-throughput genomic sequencing platforms. The resulting DNA sequences are filtered through quality control and assurance steps and then compared to reference databases where the genomic information for known specimens has been deposited (e.g., GenBank). When a match is found, a taxonomic identification can be assigned to a DNA sequence from an environmental sample[1–3].

There are several advantages to an environmental genomics approach to biodiversity characterization. It is non-invasive and does not require direct observation or the capture of whole biological specimens. Organisms at all trophic levels from algae to large mammals can be detected from the same environmental sample. The approach is highly sensitive, which means rare or elusive species, including endangered and invasive species, can be detected through their DNA in addition to the more common species. It is cost- and time-effective during field sampling and analysis. Furthermore, high-throughput DNA sequencing technology allows for simultaneous analysis of large sets of samples.

While the sensitivity of environmental genomics is one of the many appeals of this technology, it is also one of its potential limitations as contaminants of biological nature can also be detected within an environmental sample. To manage this, many precautions are taken during the acquisition, preservation, and processing of samples to limit the introduction of contaminants and mitigate for any contamination that may have occurred (e.g., collection of negative controls). In addition, stringent quality control measures during data analysis are used to limit false positives.

2.1 Project Description

For this project, environmental genomics was used to characterize metazoan biodiversity, with a focus on fish and insects in the Stuart and Nechako watersheds (British Columbia). Freshwater samples were collected in the field, and DNA was extracted by the laboratory of Dr. Brent Murray. In November 2025 sample DNA extracts were sent to the Centre for Environmental Genomics Applications (CEGA) for analysis. Field blanks and extraction controls were submitted to CEGA as DNA extracts for analysis in March 2026.

This report summarizes the metabarcoding analysis conducted on this sample set and provides biodiversity data to support further ecological analysis and interpretation.

3 Methods

3.1 Sample Submission

A total of 124 freshwater samples and 14 field negative controls were collected in the Stuart and Nechako watersheds (BC) by members of the Murray lab at the University of Northern British Columbia. Samples and negative controls were stored at 4˚C until extraction by the Murray lab and subsequently submitted in two batches to the Centre for Environmental Genomics Applications (CEGA) for analysis. Upon receipt at CEGA, DNA extracts were stored at -20˚C until processing.

3.2 Sample Processing

DNA from samples was extracted by the Murray lab using Qiagen's Blood and Tissue kit with modifications to the bead beating and lysis steps. On receipt at CEGA, all samples were processed following eDNAtec's standard EnviroSeq™ metabarcoding procedure for DNA extracts. DNA was quantified from all samples and negative controls (**Figure 1**, Appendix A: Supplementary Files). Two DNA markers from two genomic regions (12S and COI) were amplified and sequenced to characterize fish and insect biodiversity. Negative controls were added during extraction and PCR and were carried through to sequencing to screen for contamination. Sequencing was completed using the Illumina NovaSeq™6000 instrument with a target depth of one million sequences per sample per marker.

3.3 Data Processing

Sequence data were parsed through CEGA's purpose-built DNA metabarcoding bioinformatic pipeline. Sequences were filtered for quality and length and then denoised to create exact sequence variants (ESVs), each of which represent a unique sequence from the sample. ESVs were assigned taxonomy by comparing the ESVs to reference sequences in the publicly available NCBI nucleotide database (downloaded January 2026). The taxonomy presented here matches the naming conventions used in NCBI's taxonomy database and was assigned based on a sequence similarity score (the product of the % sequence similarity and the % query coverage). The minimum scores for taxonomic assignment at each level were as follows: phylum at 85%, class and order at 90%, family at 95%, genus at 98%, and species at 99%. Taxonomic identifications were verified against the Global Biodiversity Information Facility and the Encyclopedia of Life to ensure that spurious matches resulting from poor reference database coverage were not included in the list. Sequences attributed to animals (Kingdom Metazoa) and other eukaryotes (excluding Fungi) were retained for subsequent analysis.

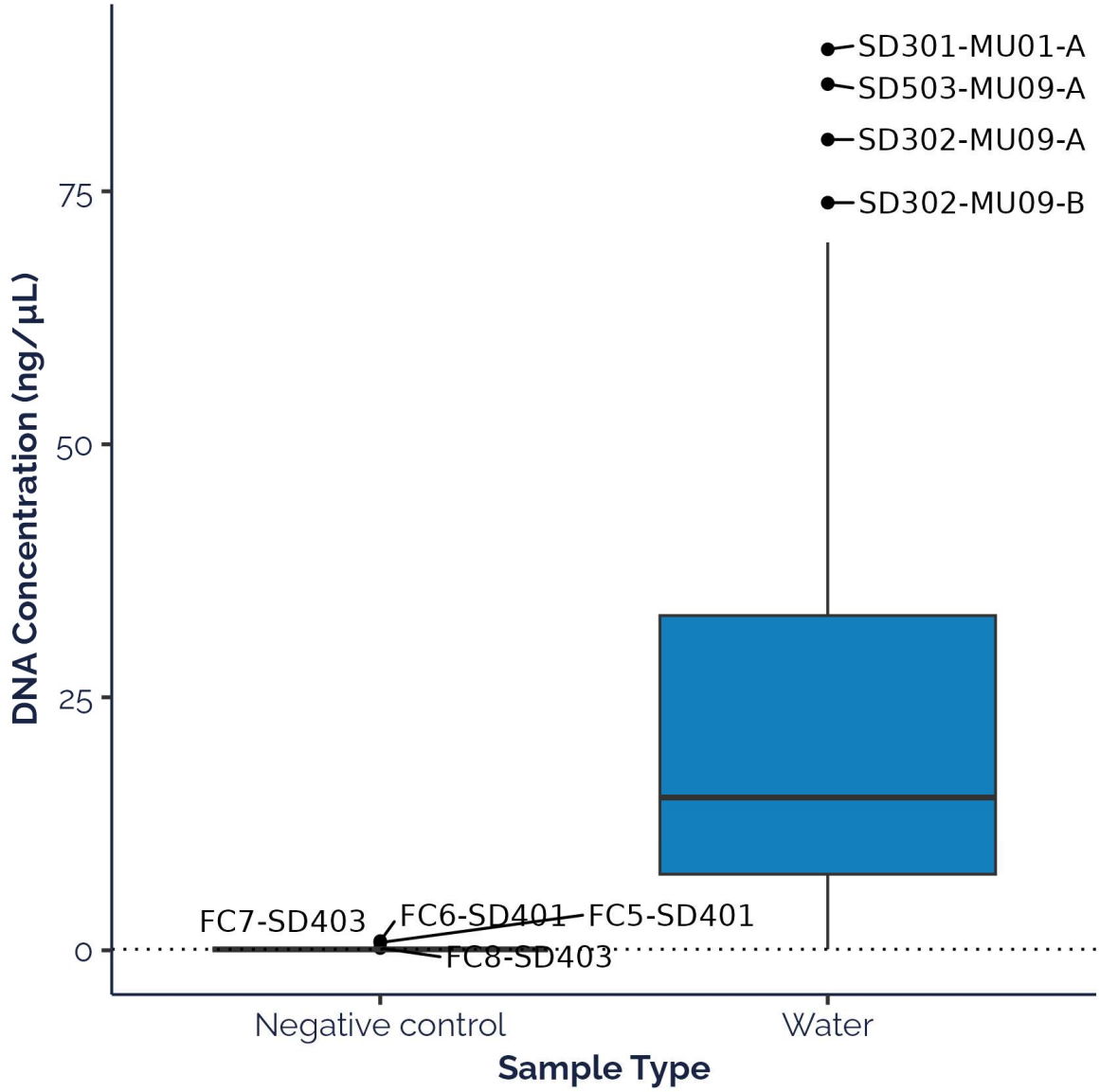


Figure 1 DNA concentrations in all samples after extraction.
Boxplot of DNA concentrations in water samples (n = 123) and negative control samples (n = 19) after DNA extraction. The lines inside the boxes represent group medians; the top and bottom of the boxes represent the 75% and 25% quartiles, respectively; and the whiskers represent 1.5 times the inter-quartile range (IQR). Outliers are labelled with their sample name. This assay has a limit of quantification of 0.1 ng/µL (dotted line). For visualization, any samples below the quantification limit (<0.01 ng/µL) are plotted at the quantification limit.

4 Negative Control Assessment

Sequencing results from the negative controls were screened for contamination and the sequence read counts of any ESVs detected in the negative controls were subtracted from associated samples. No taxa were detected at high read counts (>100 reads) in any lab samples, but several field blanks had high metazoan read counts. Following the contamination mitigation step described above, Lake Chub (*Couesius plumbeus*) was the most effected taxon, which had two ESVs removed from 11 of 46 samples, and one ESV removed from 6 of 57 samples it was initially detected in. Based on available occurrence data for the sampling area, *Oncorhynchus keta* was considered spurious and reported at the genus level. Additionally, the occurrence of several macroinvertebrate taxa was reduced in some sites. In the future samplings, additional care should be taken to reduce the potential for contamination to field blanks and samples.

5 Results

DNA was successfully sequenced from 123 of 124 samples analyzed for this report. Sample SD503-KN01-B was removed from subsequent analyses because it did not yield high quality on-target reads for either marker. A median of 632,527 (min: 5, max: 1581044) sequences were retained per sample per marker after filtering. A total of 13 animal phyla, 368 families, 937 genera, and 817 species were identified based on available reference data. The phylum Arthropoda had the highest number of families identified (298), followed by Chordata (34). The family Chironomidae (Arthropoda) was the most frequently detected, being identified in 99% of samples (**Figure 2**).

A total of four families and 9 fish species were identified. *Couesius plumbeus* (Lake Chub) was the fish species with the highest frequency of occurrence, having been detected in 47% of samples (**Figure 3**). Of salmonid species, *Oncorhynchus mykiss and Oncorhynchus tshawytscha* were each identified in 28% and 27% of sites respectively.

A total of 214 insect families, and 22 species were identified. The chironomid *Micropsectra polita* was the most frequently detected species, occurring in 80% of sites (**Figure 4**).

Complete taxonomy tables listing all metazoan taxa detected across all samples are included in supplementary files (see Appendix A: Supplementary Files for a list of supplementary files and Appendix B for notes on interpreting the data included in the files).

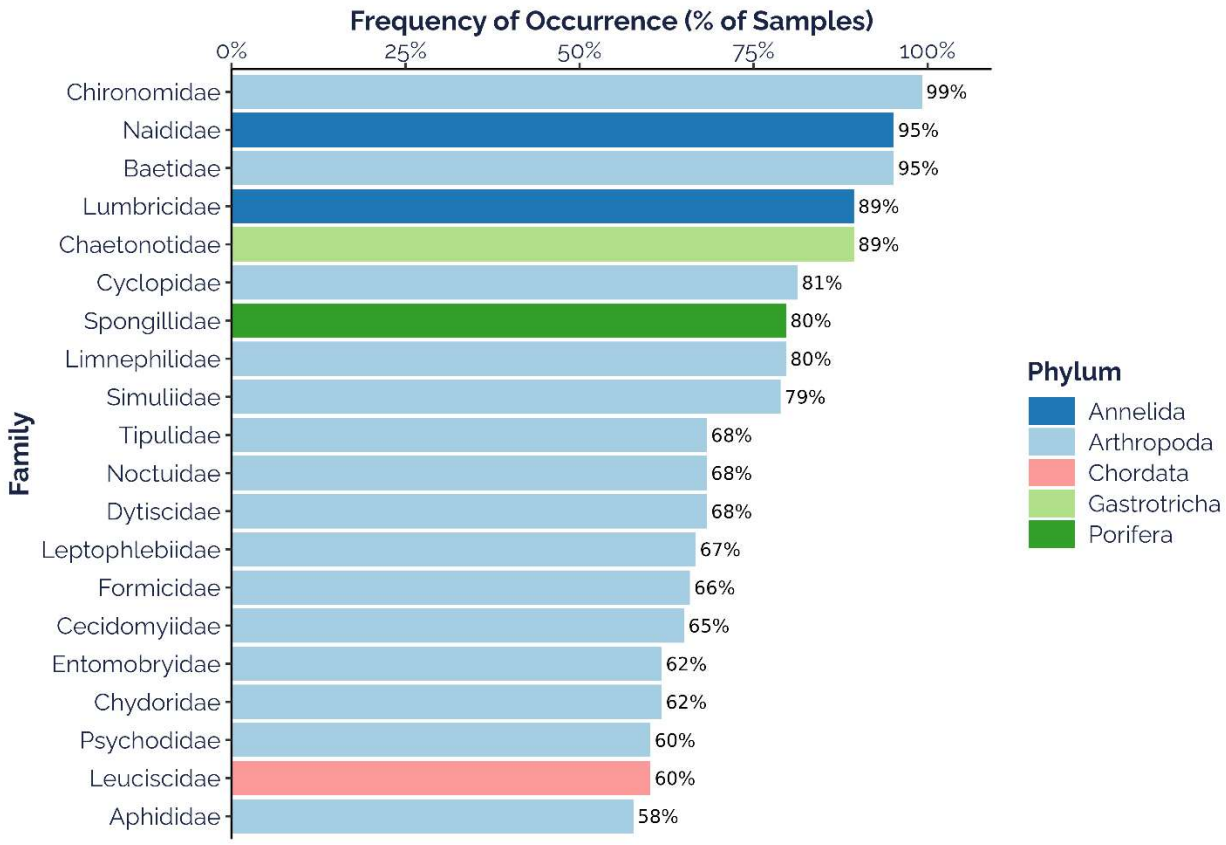


Figure 2 Frequency of occurrence of animal families.

Frequency of occurrence for the top twenty animal families detected (% of samples) across all eDNA samples (n = 123). Families are colored by phylum. Frequency of occurrence rounded to the nearest whole number is listed on the bar for each family.

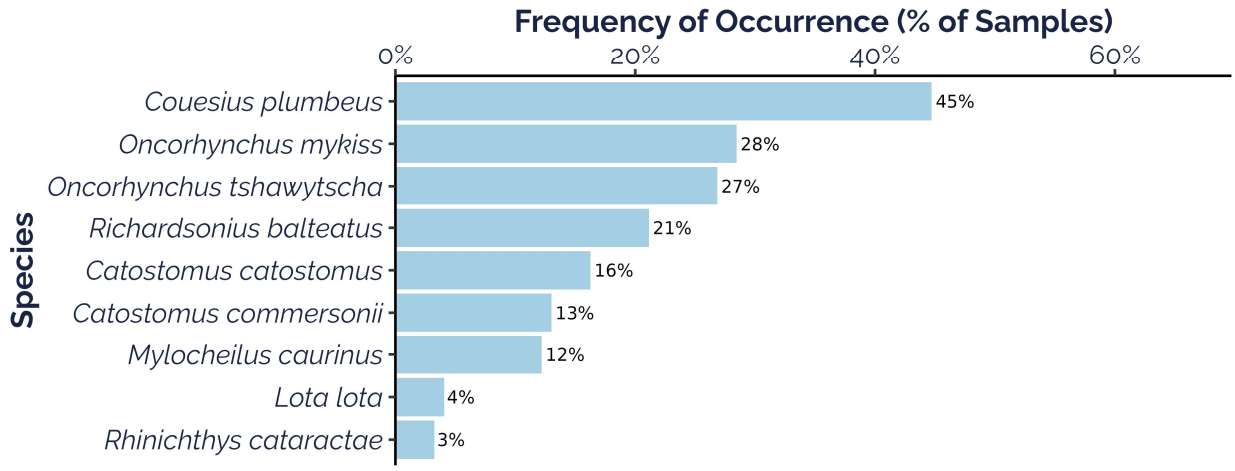


Figure 3 Frequency of occurrence of fish species.
Frequency of occurrence for fish species detected (% of samples) across all eDNA samples (n = 123). Frequency of occurrence rounded to the nearest whole number is listed on the bar for each family.

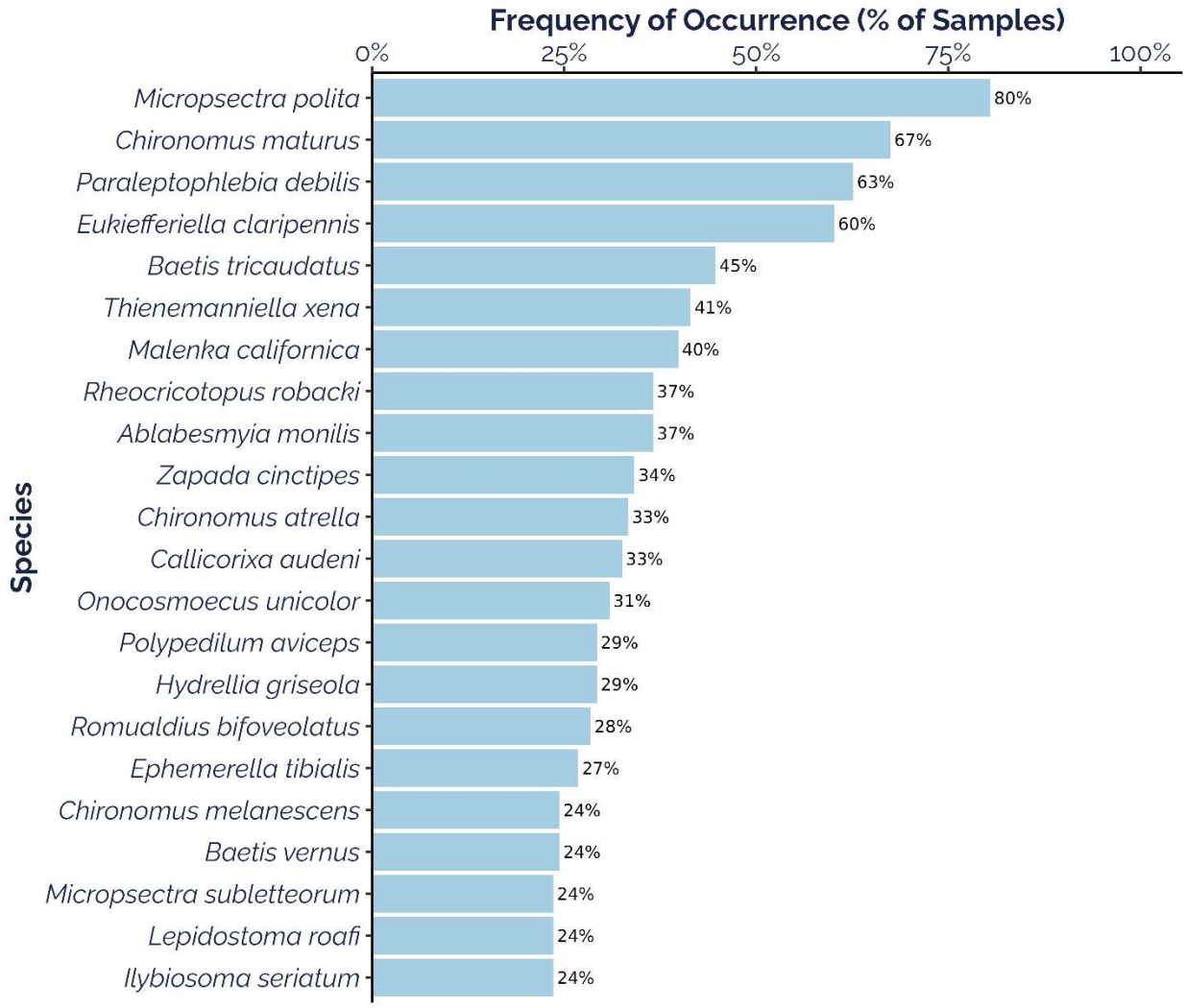


Figure 4 Frequency of occurrence of insect species.
Frequency of occurrence for insect species detected (% of samples) across all eDNA samples (n = 123). Frequency of occurrence rounded to the nearest whole number is listed on the bar for each family.

6 References

1. Baird, D. J. & Hajibabaei, M. Biomonitoring 2.0: a new paradigm in ecosystem assessment made possible by next-generation DNA sequencing. *Mol. Ecol.* **21**, 2039–2044 (2012).
2. Taberlet, P., Coissac, E., Hajibabaei, M. & Rieseberg, L. H. Environmental DNA. *Mol. Ecol.* **21**, 1789–1793 (2012).
3. Thomsen, P. F. *et al.* Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PLoS ONE* **7**, e41732 (2012).
4. Fofonoff, P., Ruiz, G., Steves, B., Simkanin, C. & Carlton, J. National Exotic Marine and Estuarine Species Information System. (2018).
5. Fisheries and Oceans Canada. Canada's State of the Oceans Report, 2012. https://www.dfo-mpo.gc.ca/oceans/publications/soto-rceo/2012/index-eng.html#a6 (2018).
6. Committee on the Status of Endangered Wildlife in Canada. COSEWIC Status Reports. https://cosewic.ca/index.php/en/status-reports.html.
7. IUCN. The IUCN Red List of Threatened Species. Version 2020-2. https://www.iucnredlist.org (2020).
8. Freeland, J. The importance of molecular markers and primer design when characterizing biodiversity from environmental DNA (eDNA). *Genome* **60**, 358–374 (2017).

7 Appendix A: Supplementary Files

Table A1 Supplementary files.
List of all supplementary files to be shared with the report.

File Type	File Name
Table	RPT184-DR03 Supplementary Table 1 R2 DNA Concentrations by Sample
Table	RPT184-DR03 Supplementary Table 3 R3 Taxonomic Occurrence of Animals Detected by COIm3 by Sample
Table	RPT184-DR03 Supplementary Table 4 R3 Taxonomic Occurrence of Animals Detected by 12Sm1 by Sample

8 Appendix B: Interpreting eDNA Results

8.1 Taxonomic Annotation

Taxonomic annotation of environmental DNA is heavily reliant on the quality and completeness of reference databases, which varies between genetic markers[8]. The ESVs with unassigned taxonomies represent organisms whose DNA may not have been sequenced yet by the general scientific community and would therefore not be represented in the publicly available reference databases. Unidentified ESVs are useful in a general analysis of biodiversity and can be informative for comparative biodiversity analyses. As databases continue to be populated and bioinformatics methods are advanced, these unidentified ESVs may be able to be assigned taxonomy in future. Additionally, errors in public reference databases exist and can potentially introduce false positives. Despite our best efforts to minimize the impact of erroneous reference sequences, there is a possibility for false assignments from these errors.

While this report includes a comprehensive list of species detected using environmental genomics, it is possible that some species present in the environment were not detected. False negatives may be due to species being absent from the reference database (as discussed above), or due to a lack of genetic resolution (i.e., high genetic similarity) between sister taxa which can lead to ambiguous or false database assignments. Alternatively, inefficiencies during the PCR stage of sample processing due to the variability in genetic markers between taxonomic groups can bias the recovery of DNA sequences in environmental genomic analyses[8]. A multi-marker approach is used to minimize the potential impact of these limitations by increasing the likelihood of DNA recovery, sequence resolution, and taxonomic annotation for the taxa of interest relative to any one DNA marker.

8.2 Taxonomy in Supplementary Data Files

In supplementary data files presenting taxonomy, rows with taxa that do not have higher resolution taxonomy assigned (i.e., no species or genus level identification) represent sequences that could not be assigned taxonomy at these levels. These rows represent unique observations from rows that were assigned lower-level taxonomy. For example, in Table B1, the observations in row 3 do not include the observations in rows 1 and 2. Rows 1 and 2 represent sequences that were identified to each species respectively and Row 3 only includes sequences that were identified as *Acartia* but could not be assigned a species name.

Table B1 Example taxonomic data.
Example taxonomic data to illustrate how taxonomy results are presented in the supplementary data files.

	phylum	class	order	family	genus	species
1	Arthropoda	Hexanauplia	Calanoida	Acartiidae	*Acartia*	*Acartia tonsa*
2	Arthropoda	Hexanauplia	Calanoida	Acartiidae	*Acartia*	*Acartia hudsonica*
3	Arthropoda	Hexanauplia	Calanoida	Acartiidae	*Acartia*	

eDNAtec is a global leader in environmental genomics offering standardized environmental DNA solutions you can trust to advance environmental sustainability. eDNAtec has an ISO 9001:2015 certified Quality Management System covering all business operations and all stages of project development, sample collection, processing, and analysis. In alignment with this certification, eDNAtec follows rigorous and standardized procedures at all stages of our workflows. We are committed to delivering quality products and services and continually improving our offerings.

EnviroSeq is a fully ISO certified, standardized, reproducible, and robust workflow for environmental DNA analysis that covers program design, calibration, sampling, DNA isolation, DNA amplification, DNA sequencing, data analysis and interpretation.

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Appendix 3. Summary of Minnow Trapping, Vanderhoof Area, 2025

License Number: XE 32 2025

License Holder: School District 91
53 Connaught Street
Vanderhoof, BC
V0J 3A0

Contact Party: Mark Gauthier, 250-567-2284

Prepared By: Jaclyn Boyes and Barry Booth

Sampling Summary:

In 2025, two creeks were sampled at 6 different sampling locations in 2025 (Table 2.1,2.2). Sampling in Knight Creek and Murray was conducted by Jesi Lauzon and a small crew on four days between June 5 and July 7, 2025 (Table 2.3, Figures 2.1-3).

Table 2.1: Sites sampled in 2025

Water Body	Watershed Code	Number of Sites
Knight Creek	180-249800	4
Murray Creek	180-267500	2

Table 2.2: Locations of sites (UTM's) where sampling occurred

Water Body	Reach	Site	UTM		
Knight Creek	2	1	10	438107	5986314
	2	2	10	438118	5986329
	2	3	10	438124	5986333
	2	4	10	438125	5986315
Murray Creek	3	1	10	431611	5987921
	9	2	10	431557	5990611

Table 2.3: Trapping date and number of number of trap sets at each site.

Creek	Site	Date	# of traps
Knight Creek	1	2025-07-07	1
	2	2025-06-06	1
	3	2025-06-06	1
	4	2025-06-06	1
Knight Creek	1	2025-07-02	1
Knight Creek	1	2025-07-07	1
	2	2025-07-07	1
	3	2025-07-07	1
	4	2025-07-07	2
Murray Creek	1	2025-06-26	1
	2	2025-06-26	1
Murray Creek	1	2025-07-08	1
	2	2025-07-08	1



Figure 2.1: Knight Creek site locations in Reach 2.



Figure 2.2: Murray Creek site location in Reach 3



Figure 2.3: Murray Creek site location in Reach 9

Results from Trapping

Overall, we caught a total of 88 individuals from three different species. No anadromous fish species were caught (Table 2.4).

Table 2.4: Total number of each species caught in minnow traps in each creek during 2025 trapping season.

Creek	Sucker	Lake Chub	Brassy Minnow
Knight Creek	6	-	-
Murray Creek		38	44