Report on the 2023 eDNA Survey of Study Creeks in Nechako watershed from Prince George to the Upper Fraser.

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Executive Summary

As part of an ongoing environmental DNA (eDNA) survey of creeks and rivers in the Nechako watershed we filtered water samples from 39 creeks/rivers. The geographic scope of the sampling ranged from creeks/rivers flowing into the Nechako River from the Chilako River just west of Prince George to the Nechako Canyon adjacent to the Cheslatta Falls, and further west including Ormand Creek and the Endako River emptying into Fraser Lake. This survey included 64 sites in our main study, each of which were sampled at up to three timepoints, i.e., June, July and August. Environmental DNA was extracted from each site and assessed for the presence of four salmonid species, chinook, sockeye and coho salmon and rainbow trout. Our data show the widespread use of the study area by both rainbow trout and juvenile chinook salmon, as most creeks showed the detection of these species at, at least, one site throughout the sampling periods. Positive sockeye detections are more complicated reflecting the complex relationship among spawning sockeye and kokanee stocks and possible first year juvenile sockeyes. Consistent with the straying sockeye observed in our fall 2022 survey, sockeye salmon were also detected at various sites in our June survey. In the upper Nechako sites in Greer and Swanson Creeks, sockeye was detected at all three timepoints throughout the summer, which may indicate the presence of juvenile sockeye. In contrast, very strong detections in the lower Fraser in August at the Cluculz Creek site are consistent with the outflow spawning of kokanee in Cluculz Lake. In contrast to all previous surveys, we detected coho at many creeks in our late August samples. This likely reflects the fact that sites were added between Prince George and Fraser Lake allowing the detection of the coho migrating to their spawning locations. However, long-term survey sites around Vanderhoof, in Murray, Moss and Clear creeks also show evidence of coho presence not found in the previous 2000-2022 sampling years. In addition, coho were detected for the first time in the upper Nechako, in the late August sampling of Cutoff Creek.

In summary, clear evidence of habitat use by all four species was found throughout the study area. These data provide new information on the salmonid distribution in the watershed and baseline information at specific sites associated with restoration projects in the Vanderhoof area at Murray, Knight and Clear creeks.

Methods

In 2023, we took water/eDNA samples from 39 different creeks/water bodies (Appendix 1). In keeping with our ongoing sampling program, we sampled 11 creeks that have been sampled regularly since 2020. We expanded the number of creeks sampled in 2023, as well as increasing the number of locations on selected creeks. We also continued our examination of how salmon are utilizing a beaver dam complex on the bottom 1 km section of Murray Creek.

The selection of additional creeks within the Nechako watershed was facilitated by conversations with project partners. Chelton van Geloven, the then source water hydrologist for the BC Ministry of the Environment, suggested sampling 6 new creeks within the Chilako watershed. We also worked with Mr. van Geloven to sample 17 creeks from a river boat at/near the confluence of these creeks with the mainstem of the Nechako from Prince George (Chilako River) all the way to the Upper Nechako (Cutoff Creek, ~18 km downstream of the Kenney Dam).

We also worked with Jeff Beardsal, contractor for the Carrier Sekani Tribal Council (CSTC), to determine sampling sites within regions of the Endako River where the Endako River chinook salmon are known to spawn. We traveled with Mr. Beardsall and two CSTC fisheries technicians on our first sampling trip along the Endako River and Shovel Creek. Mr. Beardsall accompanied us on our second trip to the Endako where sampling was abandoned because we did not want to disturb the spawning chinook salmon. We also sampled numerous locations on the Endako River both upstream and downstream of Shovel Creek, including Tchesinkut Creek.

Most of the sampling locations are close (generally within 2 km) of the confluence with the Nechako River, the Chilako River, or Fraser Lake (noted with codes xx00/01). In a few creeks, additional samples were collected further upstream to coincide with restoration projects planned by the Nechako Environment and Water Stewardship Society (NEWSS). This included adding new sampling locations along Murray Creek and a new site on Eden Creek (a tributary of Clear Creek). We were also able to sample the mainstem of the Nechako both above and below the confluence with the Cheslatta River in November due to reports of potential spawning of coho salmon in this region. Sampling was conducted at three sampling periods or blocks that coincided with the months of June, July and August (coded in our data as SD301, 302, and 303). Sampling of the beaver dam study (21 Oct 2023) and the sampling of the Upper Nechako mainstem (10 Nov 2023) is coded as SD304. For most sites, two x 1 liter water samples (A and B) were collected from each sampling site, stored at 4°C and filtered within 24 hours of collection in the lab established at North Nechako Secondary School (NVSS). Filtering was conducted by the high school students under the supervision of B, Murray and/or NVSS teachers. Water samples were filtered onto 0.45 um MCE filters, with up to two filters collected per A or B water sample, depending on the saturation point of the filtering. Saturation of filters was determined when flow of water through the filters decreased to less than one drop (~ 2 ml) per 10 seconds. Depending on the water characteristics of the site, between 250 ml – 1000 ml were sampled per filter. The “A” filters were stored in 95% ethanol prior to DNA extraction while the “B” filters were stored in silica desiccant beads. All samples were subsequently stored at -20°C prior to DNA extraction. To test for cross contamination during filtering, 250 ml of distilled water was filtered every second sample (termed filter controls). Filter controls were stored in 95% ethanol.

At sites sampled in October and November, i.e., the SD304 sites, sampling was conducted using an OSMOS eDNA sampling backpack (Halltech Scientific, Guelph Ontario). For the beaver dam study, 1 liter of water for both the A and B samples was filtered through 1.5 mm Glass filters and stored with silica desiccant prior to analysis. For the Nechako mainstem, 4 liters of water were filtered for the A and B samples. On each sampling day, a start-of-day and end-of-day filtering control was conducted using 1 liter of distilled water.

Environmental DNA (eDNA) was extracted in the dedicated eDNA lab at UNBC and analysed with the protocols developed. If applicable, both filters were combined for eDNA extraction, therefore 500-1000 ml of filtered water were assessed per A or B replicate (except for SD304 of the Nechako mainstem where 4 L of water was filtered). Environmental DNA was isolated using the DNeasy Blood and Tissue Kit (Qiagen) following a modified protocol for water filters. For each filter, a first and second eDNA elution was collected. . We have found that the second elution contains ~2/3 of the eDNA compared with the first elution, but has fewer impurities interfering with the eDNA PCR assays (unpublished, B. Murray). For each filter, both elutions were analyzed for the presence of target eDNA. To test for cross contamination during extraction, filters were processed with an extraction control (an untreated filter) for each extraction batch.

Species-specific assays were identified in primary literature (Table 1) and developed for use with droplet digital PCR (ddPCR) in previous studies (Ref PSF reports). Results of the assay validation are shown. All assays were purchased and tested against a synthesised DNA positive control (gBlock, IDT Inc.) and a panel of local salmonid species. This panel included sockeye/kokanee from the Peace, Fraser and Columbia systems (N=19); coho, chinook and rainbow trout from the Fraser (N = 12, 5 and 10 respectively); and 1-3 samples of Arctic grayling, bull trout, brook trout and northern pike minnow. A “Good” result indicated the assay yielded a strong positive signal in all target samples, with no signal found in any of the non-target species samples.

Table 1. Published quantitative PCR assays developed for use with ddPCR.

Species	reference	gene	Primer 1/2/Probe	ddPCR Probe	Result
Chinook	Hellberg et al 2010	*COI*	ACCATTATTAACATAAAACCTCCAG GTAATGCCTGCTGCCAGGA VIC-CGTTTGAGCCGTGCTA-MGBNFQ	FAM	Good
Rainbow	Hellberg et al 2010	*COI*	ACCATTATTAACATAAAACCTCCAG GTAATGCCTGCTGCCAGGA VIC-CGTTTGAGCCGTGCTA-MGBNFQ	VIC	Good
Coho	Pilliod and Laramie 2016	*CYTB*	CCTTGGTGGCGGATATACTTATCTTA GAACTAGGAAGATGGCGAAGTAGATC 6FAM-TGGAACACCCATTCAT-MGBNFQ	FAM	Good
Sockeye	Hellberg et al 2010	*COI*	GGAAACCTTGCCCACGCG AAAAGTGGGGTCTGGTACTGAG VIC-CTCTGTTGACTTAACCATC-MGBNFQ	VIC	Good

Validated primers were combined in dual assays (a reaction combination of two assays with differing reporter dyes). Dual assays allow for the presence of two species to be assessed in a single reaction. In this study two dual assays were used, Chin-COI-FAM/Rain-COI-VIC and Coho-Cytb-FAM/Sock-COI-VIC. The sensitivity (S) of the reactions was tested via analysis of a dilution series of the known DNA templates (PSF report again 2022). For both dual assays used in this study, the sensitivity was confirmed to extend down to single copy numbers of template molecules, indicating that combining reactions did not reduce the expected sensitivity of the individual assays.

To test for species-specific eDNA using the dual assays noted above, samples were run in 20 µl reactions using mastermix for probes (no UTP) (Bio-Rad Laboratories) and 5 µl of the extracted eDNA (1/10 of an elution volume). Droplets (~18,000/sample) were generated using the Automated Droplet Generators (Bio-Rad), subjected to PCR and analyzed on a QX200 Droplet Reader. Samples showing 4 or more positive droplets were considered a positive detection. Samples with three positive droplets were considered a possible detection and reanalysed. If two or more positive droplets were found in the reanalysis, the sample was considered a weak positive detection. If only one droplet was positive the sample was listed as possible.

Results and Discussion

No evidence of cross contamination (i.e., no positive assay detections) was found in either the internal ddPCR controls (no template controls) or the extraction controls (N=29).. For the SD301 (N=19), SD302 (N=17) and SD304 (N=4) time point samples all filter controls were also blank. For SD303, two filter controls indicated the presence of target species, while two additional filter controls showed possible signals (i.e., 3 positive droplets). In each case the filter controls results were matched to the samples filtered on that day/run and the level of positives observed in the filter controls removed from the sample data. This allowed for positive samples with stronger positive signals than the filter controls to be retained for the survey.

A summary of positive results for each target species, along with a quantitative estimation of signal strength, is shown in Table 2. Not all sites were sampled at all timepoints, due to a number of constraints, including access by river boat, water flow, and other sampling considerations. The overwhelming majority of sites (117/121) showed at least one target fish detected. Of the negative sites, two (MU07 and MU09), are associated with restoration sites on Murray Creek and salmonids were not expected to be present (see below). The Engen Creek site, EG01, is a small creek that has intermittent connection to the Nechako, while Endako 13 is part of a system that contains both rainbow and chinook at other sites. The detection of at least one species of interest at all other sites illustrates the sensitivity of the eDNA sampling method.

Rainbow trout: As rainbow trout are an abundant salmonid found throughout the study area, with similar environmental requirements to the other salmonids, they are used here as a “likely positive” control for the survey. Of the 28 creeks sampled flowing directly into the Nechako, only Engen, Knight, Smith, Tahultzu and Targe did not show evidence for the presence of rainbow trout. At all other creeks the detection of rainbow trout ranged from weak (4-10 copies detected) to very strong (>1000 copies detected). The majority of detections with “very strong” signals were for rainbow trout.

Table 2. Summary of eDNA survey by sampling period and species detected. The relative signal strength of the eDNA detected (in terms of copy number detected in 1/10 of the elution volume) is shown in colour. Sites associated with ongoing sampling 2000-24 are noted in bold and italics, while sites associated with restoration projects are indicated with an asterisk.

Site	Site code	June SD301 Chin	Rain	Coho	Sock	July SD302 Chin	Rain	Coho	Sock	Aug SD303 Chin	Rain	Coho	Sock
Chilako													
Chilako Main	CK01	20-50	4-10	-	-					-	4-10		-
Beaverly	BE02					-		-	-				
Gregg	GG05					-		-	-				
Dalh	DH01					20-50		-	-				
Chehischic	CH08					-	4-10	-	-				
Unknown tribe	CK-un1					20-50		-	-				
site 2	CK-un2					-		-	-				
Butcherfields	BU01					10-20		-	-				
Nechako PG - Vanderhoof													
Sweden	SN01	50-100		-	4-10					50-100		20-50	-
Tatentelichick	TK01	10-20		-	-					4-10		20-50	4-10
Breeze	BZ01			-								4-10	-
Zelkwas	ZK01	50-100		-	-					10-20		20-50	-
Hutchenson	HN01			-	4-10							-	10-20
Cluculz	CZ01			-	10-20							-	
Hullat	HL01											4-10	-
Sinkut	SU01	50-100	20-50	-	-					20-50	4-10	4-10	-
Vanderhoof													
Knight	***KN01****	20-50	-	-	-								
Murray	***MU01***	20-50	20-50	-	4-10	4-10	10-20	-	-	-	20-50	4-10	-
	MU02	-		-	-	-		-	-	-			-
	MU03	-	20-50	-	-	-	50-100	-	-	-	20-50	4-10	-
West arm	***MU04***	-	50-100	-	-	-		-	-	-	20-50	-	-
	MU4.1*					-	20-50	-	-	-	4-10	-	-
	MU05	-		-	-	-		-	-	10-20		-	-
	MU06	-		-	-	-		-	-	-		-	-
East arm	MU07*					-	-	-	-	-	-	-	-
	MU09*					-	-	-	-	-	-	-	-
Clear	***CL01***			-	-			-	-		20-50	-	-
	CL02	-	50-100	-	-	-	20-50	-	-	-	20-50	-	-
Eden (CL trib)	EN01*	-	50-100	-	-	-		-	-	-		20-50	-
	CL03*	-	50-100	-	-	-	50-100	-	-	-	20-50	4-10	-
	CL04*	-	50-100	-	-	-		-	-	-	20-50	4-10	-
Moss	***MS01***			4-10	-			-	-			4-10	-
Vanderhoof - Fort Fraser													
Engen	EG01	-	-	-	-								
Halsey	HY01											4-10	-
Kluk	KK01			-	20-50							-	-
Nine Mile	9M00			-	4-10							4-10	-
	9M01	-		-	-	-		-	-	4-10			-
Tatsutani	TC00											-	-
	TC01	50-100		-	-	20-50		-	-	50-100		-	-
Dog	***DC01***			-	4-10			-	-	50-100		-	-
Fraser Lake													
Ormond	***OR1***			-	-			-	-			-	-
Endako													
Endako	ED13					-	-	-	-				
	ED18					-		-	-				
	ED22					4-10	4-10	-	-				
	ED26					4-10		-	-				
Tchesinkut (ED trib)	TH01					10-20		-	-				
	ED52	4-10	50-100	-	-								
	ED54	20-50		-	-								
	ED55	-	4-10	-	-								
Shovel (ED trib)	SH00	20-50		-	-								
	SH01	20-50		-	-								
	ED66						4-10	-	-				
	ED66.1					-	4-10	-	-				
Upper Nechako													
Smith	SM01	10-20	-	-	-								
Tahultzu	TU01		-	-	10-20					50-100	-	-	-
Targe	TE01		-	-	-		10-20	-	4-10				
Greer	***GR01***			-	4-10		20-50	-	4-10			-	4-10
	GR03			-	-			-	-			-	-
Swanson	***SW01***		20-50	-	4-10			-	10-20			-	20-50
	SW02			-	-			-	-			-	-
Cutoff	CT00									4-10	-	4-10	-
	CT01									4-10	4-10	4-10	-
Twin	***TW01***			-				-	-			-	-
	TW02			-	-			-	10-20				

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

Chinook: Consistent with our previous studies in 2000-2022, chinook salmon are detected at most creeks, except Engen, that directly flow into the Nechako River or Fraser Lake (27/28) and in 3 of the 6 tributaries of the Chilako river. The detection throughout June – August and the association of juvenile chinook fry trapped at many locations, illustrates the importance of this habitat for the rearing of juvenile chinook. Most of the sampling locations are close to confluences or within 2 km (samples labelled xx00 or xx01). In selected creeks, additional samples were collected further upstream. In the agricultural zone around Vanderhoof, chinook are only found at the lower sites of Murray and Clear creeks. In contrast, creeks in the upper Nechako, Swanson (SW02 ~ 2.5 km upstream) and Greer (GR03 ~ 15 km), also have chinook present at upper sites. Sites around the known spawning locations on the Endako River (~52-54 km upstream of Fraser Lake) were positive for chinook salmon in the June sampling period. These positive results, that were prior to the spawning run, is evidence for the continued use of the area by juvenile chinook.

Sockeye/Kokanee: Although sockeye are known to migrate through the system, they have not been detected in our previous analysis of associated creeks (2000 – 2022), except for the August/September sampling in 2022 where we found evidence of straying throughout the more limited survey of the study area. In 2022, sockeye were detected in the late summer samples of Murray, Clear, Dog, Twin and Greer creeks (Murray and Booth 2023; Table 4). Consistent with this, a Sockeye signal was detected in Murray (MU01), Dog, Twin and Greer creeks in the June samples of 2023. It is not clear if this is a residual signal from the straying adults of the previous year or representative of juvenile sockeye. In Vanderhoof sites (i.e., Murray) sockeye are not detected at later time points, however, in the upper Nechako sites in Greer and Swanson creeks, sockeye are detected throughout the season, suggesting the presence of juvenile fish throughout the summer. Sites in the lower Nechako between Prince George and Vanderhoof also show sockeye signal in the June and August samplings. Here caution should be taken as Cluculz Lake is home to a population of kokanee. Indeed, the very strong signal noted in the August sample may represent the spawning of kokanee in this outflow stream. Very strong signals were previously associated with known spawning runs in both the Stuart and Takla systems (Murray and Booth 2022).

Coho: Coho were not detected in our previous eDNA surveys (2000-2022). In 2023, a coho signal was detected in Moss creek in June and at multiple sites in the late August sampling. Many of these sites are located between Prince George and Fraser Lake and likely represent coho migrating to their spawning locations. Note that many of these sites were not surveyed in previous studies. More surprising, coho were detected at some upper sites in both Murray and Clear creeks. These creeks were surveyed in 2000-2022, and coho was not previously detected. These unique detections are similar to the detections of sockeye salmon noted in the same area in 2022. Coho were also detected at sites upstream of Fraser Lake, in the upper Nechako, at two Cutoff Creek sites.

Restoration sites: A number of sites were chosen to coincide with ongoing or planned restoration (Table 2, noted with asterisks). The Clear/Eden creek sites, CL04 and CL03/EN01, are located upstream and downstream, respectively, of previous restoration projects. All sites show the presence of rainbow trout throughout the study period. Although chinook are not found in this location, which is upstream of a large beaver dam complex, coho, potentially straying adults,

were detected at all three sites in the August sampling period. The Knight Creek site, KN01, is downstream of ongoing stream restoration efforts and upstream of possible culvert improvements. In 2023, the June sampling showed the presence of chinook salmon, while in previous years both chinook and rainbow have been detected (Murray and Booth 2022). Unfortunately, the stream no longer had a surface flow in July and August time periods and could not be sampled. Murray Creek has multiple sites with planned restoration activities, which were sampled in only the July and August time points. Site MU4.1 is upstream of planned culvert improvement. Rainbow trout were detected in both July and August. Sites MU07 and MU09 are located on the east arm of Murray creek where multiple restoration activities are planned. Neither site yielded evidence of salmonid presence in the 2023 sampling. MU08 (not shown) is located between the sites but could not be sampled due to lack of surface water in July and August. The presence or absence of salmonids in these Murray Creek sites should be considered baseline data for evaluation of the planned restoration projects.

November sampling (SD 304): As a number of stakeholders were interested in exploring the use of the Nechako mainstem by late migratory species, like coho, we undertook a sampling of the upper Nechako from the Nechako Canyon, adjacent to the Cheslatta Falls, to Greer creek recreation site (Table 3). Similar to the main survey (Table 2), Greer creek (GR01) has positive detections for chinook and rainbow trout, however, coho are not detected at this time. A similar pattern is observed in the mainstem sites, NK49-74, with a weak signal detected for both chinook and rainbow. As these samples are taken from the mainstem of the river, the point source is uncertain, as fish could reside in either the mainstem or upstream creeks. Sockeye was also detected at the Twin Creek site, which represents a sample of water upstream of the inflow from Twin Creek. The Nechako Canyon sites are technically part of the Nechako River, but in reality, are now part of a creek flowing through the old river canyon. Since the creation of the Kenney Dam, the vast majority of the Nechako water flows through the Cheslatta spillway and over Cheslatta falls downstream of the Canyon sites. The widescale distribution of chinook in the study area is highlighted by the positive detection of chinook (likely juvenile) within the Nechako Canyon which represents the most upstream creek in the system. Contrary to the expectations of stakeholders, no coho were detected in this survey.

Table 3. Results of eDNA survey of the mainstem of the Upper Nechako, 10 November 2023.

Site	site code	Chin	Rain	Coho	Sock
Greer Creek	GR01		20-50	-	-
Nechako Mainstem					
Greer CK Rec site	NK49*	4-10	4-10	-	-
Cutoff Ck Rec site	NK70	4-10	4-10	-	-
Twin Creek	NK74	4-10	20-50	-	4-10
Nechako Canyon	NK83	4-10	20-50	-	-
	NK84	-		-	-

	Positive drop.	
-	0	Not detected
	3	Possible
	4-10	Weak
	10-20	
	20-50	Good
	50-100	
	sup. 100	Strong

* Km from Fort Fraser Bridge

Analysis of the beaver dam complex on lower Murray Creek: Since 2021 we have been investigating the salmonid usage of a growing beaver dam complex on the lower section of Murray Creek (Figure 1). To illustrate the trends in the data, the results from 2023 have been

added to those of the previous seasons (Table 4). All sampling has been conducted in October and involves walking the stream and sampling the pools created by each beaver dam. As a beaver dam complex is dynamic over the years, dams are added from 2021 to 2022 and lost/breached in 2023. These are noted in gray and therefore not sampled. Rainbow trout eDNA is detected throughout the complex in varying signal strength in each of the ponds. In contrast, the strongest chinook detection is in the pre-complex site and then generally reduces in intensity moving upstream. In 2023, chinook are no longer detected at the uppermost beaver ponds, which corresponds to the largest dams in terms of height (not shown). Sockeye are not detected in 2021 or 2023, but consistent with the straying observed in the larger geographic area in 2022, they are detected in multiple ponds in 2022. In summary, over the course of a three-year study, salmonids are found in most ponds, and we find little evidence, except for the largest dams, for beaver dams acting as a barrier to fish passage.

Figure 1. Relative locations of beaver dams in lower Murray Creek, October 2022. Beaver dams are noted in red, except for the first location, BD01, which is downstream of the first dam and about 200 m from the Nechako River.

Table 4. Results of a three-year study of the usage of beaver dam pools in lower Murray Creek by salmonids in October. Coho were not detected in any year at any site (results not shown).

		2021			2022			2023		
Site	site code	Chin	Rain	Sock	Chin	Rain	Sock	Chin	Rain	Sock
Pre complex	BD01		20-50	-		20-50			20-50	-
Beaver Dam 1	BD02		4-10	-		20-50				
Beaver Dam 2	BD03		20-50	-		20-50	-		20-50	-
Beaver Dam 3	BD04			-			4-10	20-50	10-20	-
Beaver Dam 4	BD05	20-50	20-50	-		20-50		10-20	10-20	-
Beaver Dam 5	BD06	4-10	20-50	-		20-50		10-20	4-10	-
Beaver Dam 6	BD07					20-50		4-10	4-10	-
Beaver Dam 7	BD08	4-10	20-50	-	20-50	20-50		20-50	4-10	-
Beaver Dam 8	BD09				20-50	10-20	4-10	20-50	20-50	-
Beaver Dam 9	BD10				10-20	10-20	4-10			
Beaver Dam 10	BD11*	4-10	20-50	-	10-20	10-20	20-50	-	10-20	-
Beaver Dam 11	BD12				4-10	20-50		-	20-50	-

* = MU01 (Table 2)

	Positive drop.	
-	0	Not detected
	3	Possible
	4-10	Weak
	10-20	
	20-50	Good
	50-100	
	sup. 100	Strong
	not sampled	

Recommendations: The strength of the multiple timepoint sampling to understand the habitat usage of salmonids in the study area is clearly illustrated in this survey. Attempts should be made in 2024, to expand this approach to as many timepoints as possible. Targeting of additional timepoints in the Chilako system should be a priority in 2024. As water flow limits river boat sampling, only June and late August sampling is likely possible.

In 2023, most samples were collected in 1-liter bottles and returned to NVSS for filtering. Although this approach had proven successful for a number of years, the additional transfer steps can lead to filter control issues noted in the August sampling period and the possibility of sample mix up. The SD304 timepoint samples were conducted with a backpack sampler allowing for instream sampling. The results of the SD304 samples and the successful use of this strategy in another project in the Peace Williston area recommend the use of this approach in the 2024 season. The reduction of one transfer step and removal of filtering at NVSS, simplifies the eDNA sampling procedure and reduces the chances of transcription errors.

References:

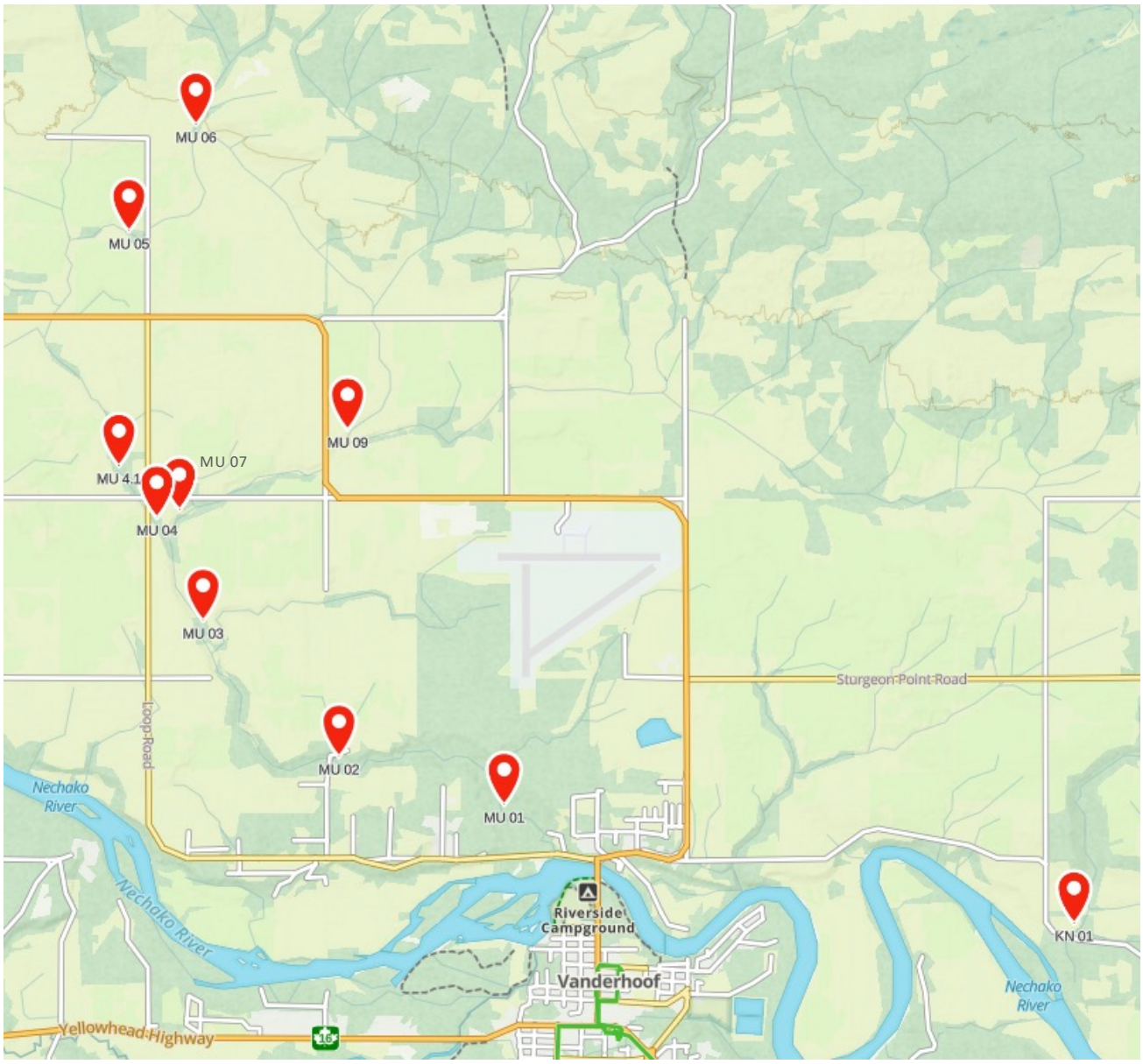
Hellberg, R.S.R., Morrissey, M.T., and Hanner, R.H. (2010) A multiplex PCR method for identification of commercially important salmon and trout species (*Oncorhynchus* and *Salmo*) in North America. Journal of Food Sciences, 75:C595-C606. DOI 10.1111/j.1750-3841.2010.01752.x.

Murray, B.W. and Booth, B. (2022) Using Environmental DNA (eDNA) to Assess the Distribution of Juvenile Salmonids in the Nechako Basin. Pacific Salmon Foundation, Community Salmon Program Final reports, CSP_20S_127, CSP_21S_116 and CSP_22S_121.

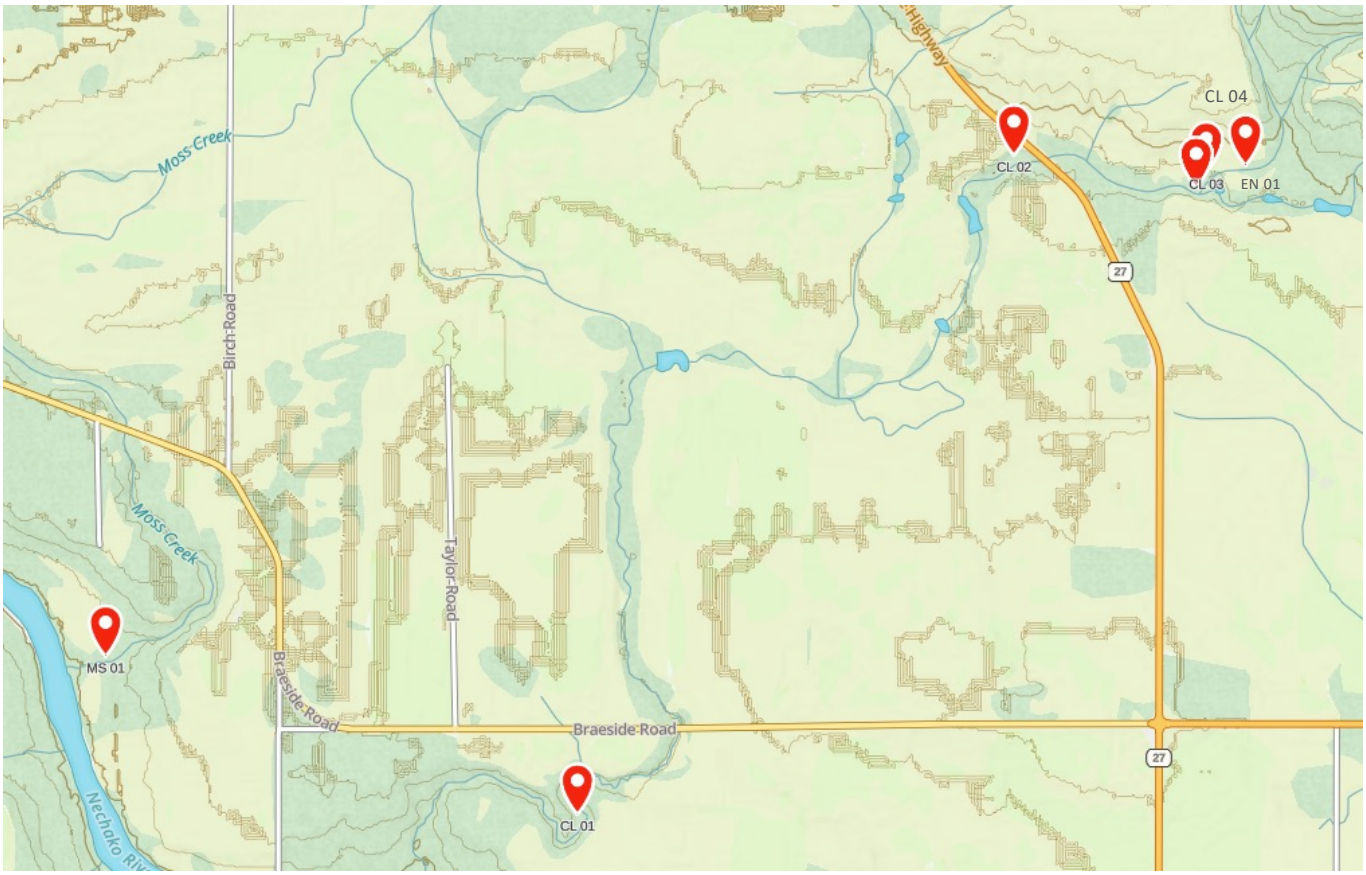
Pilliod, D.S., and Laramie, M.B. (2016) Salmon redd identification using environmental DNA (eDNA): U.S. Geological Survey Open-File Report 2016–1091, DOI 10.3133/ofr20161091.

Appendix 1. Location of sampling sites

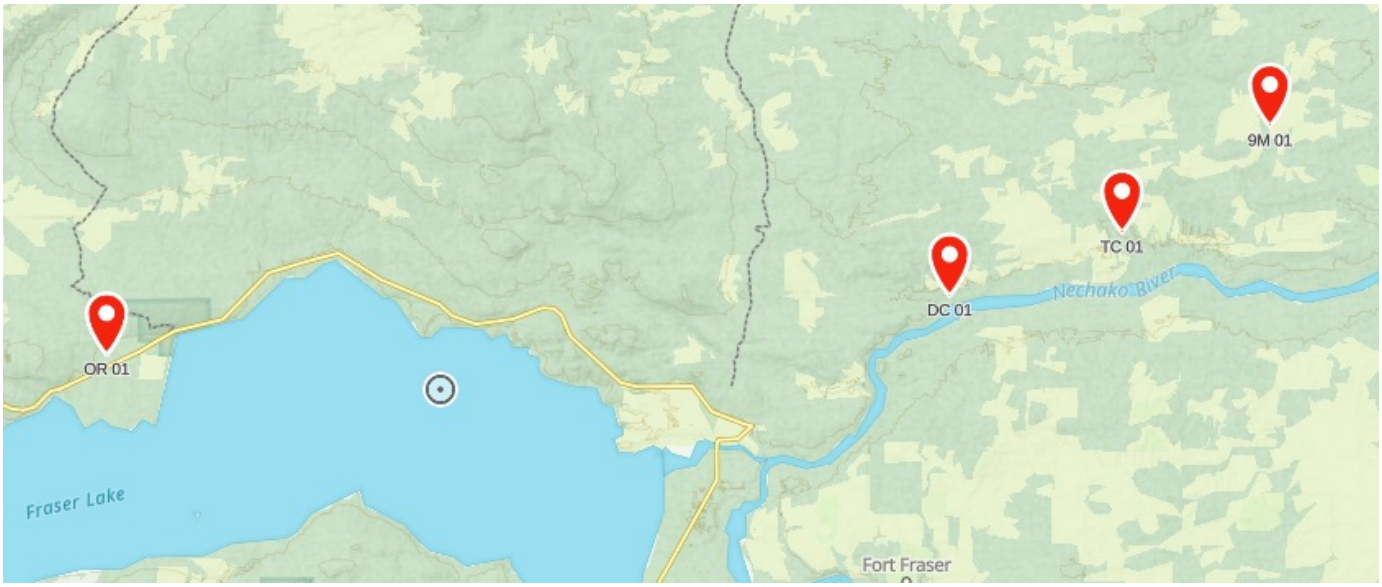
Murray Creek and Knight Creek sites



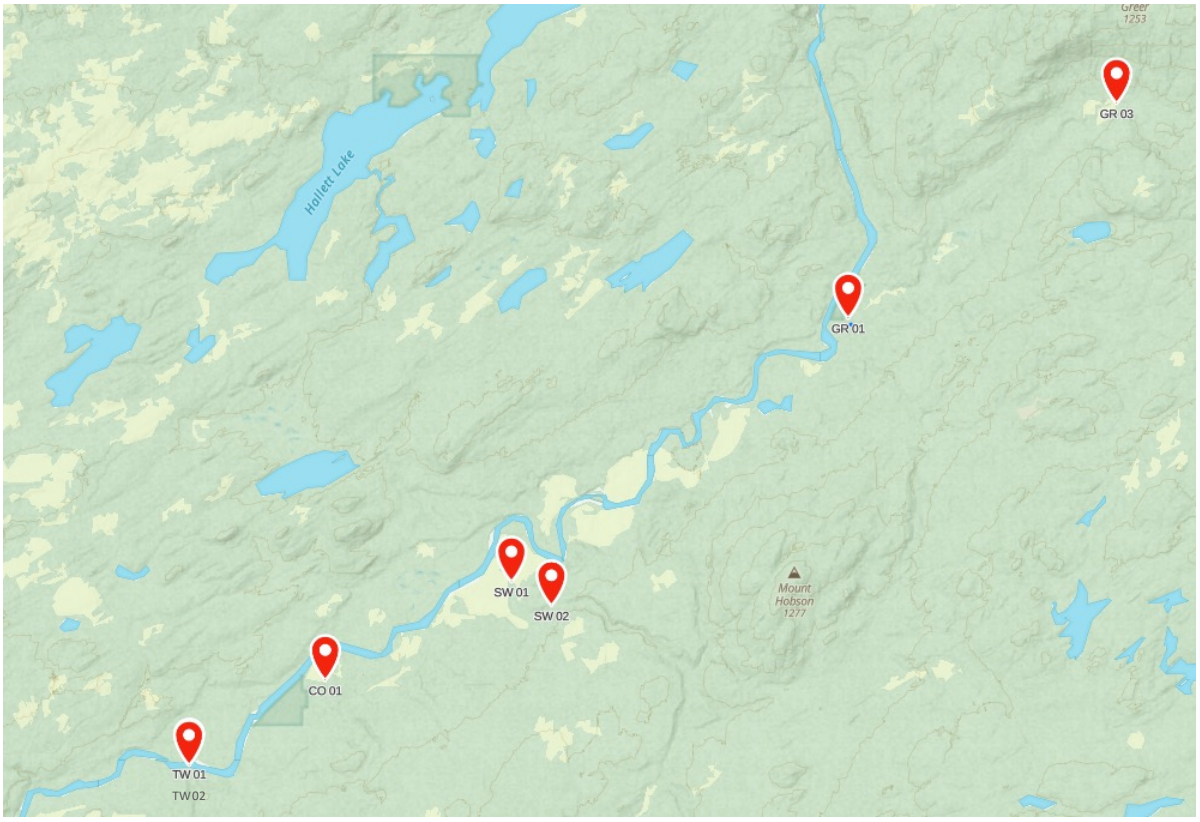
Clear and Moss Creek sites



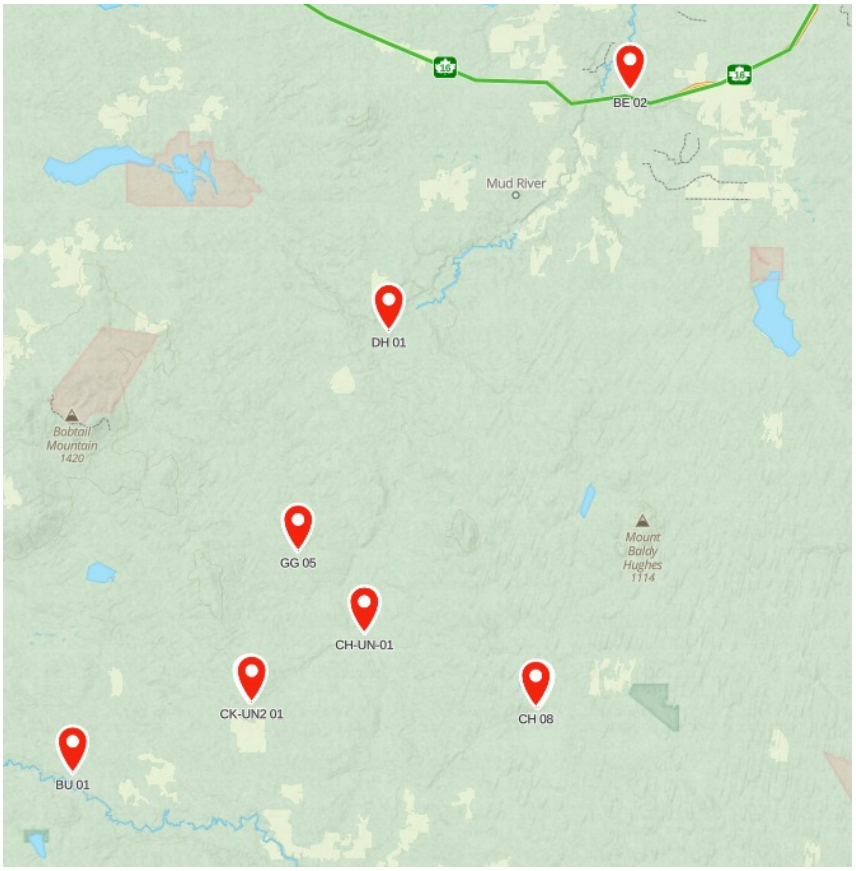
Fort Fraser sites



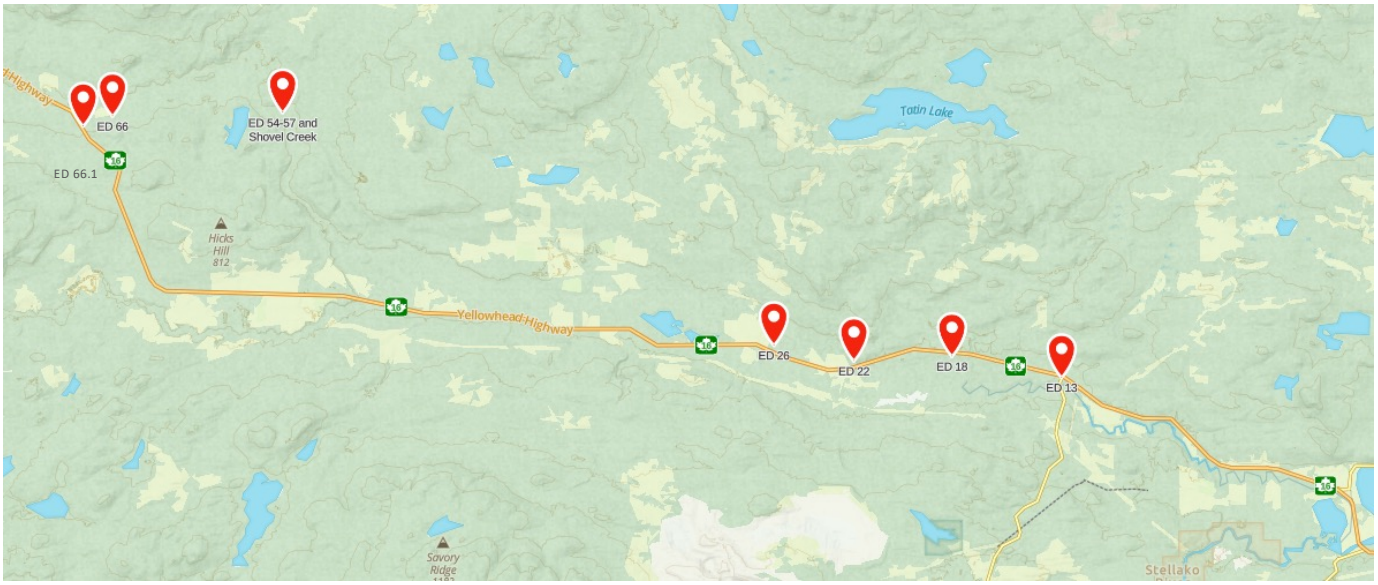
Upper Nechako sites



Chilako watershed sites

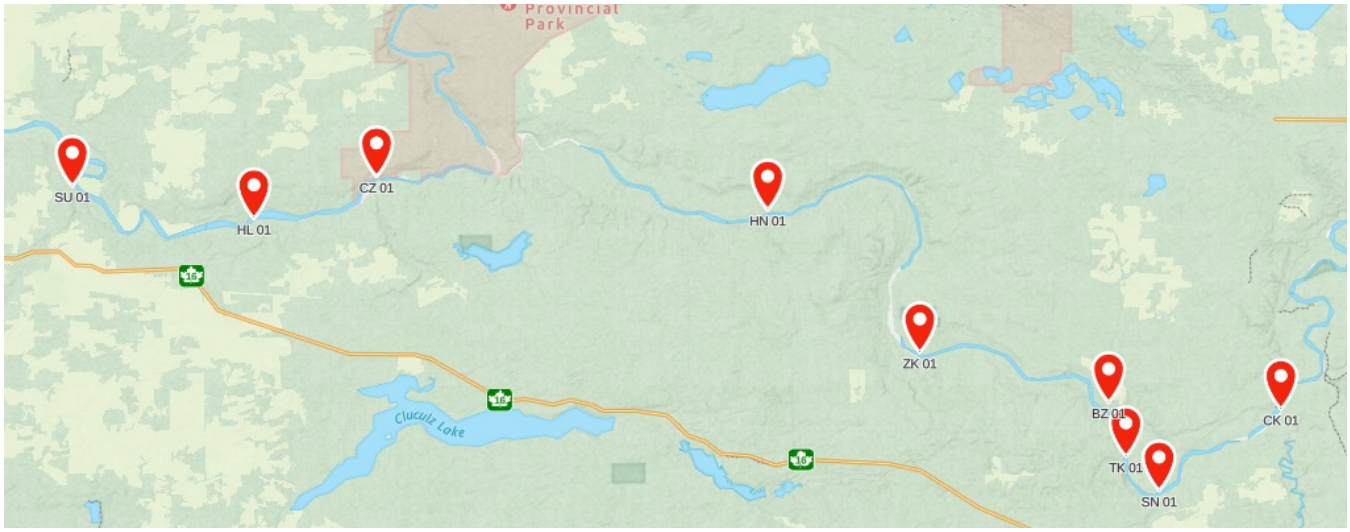


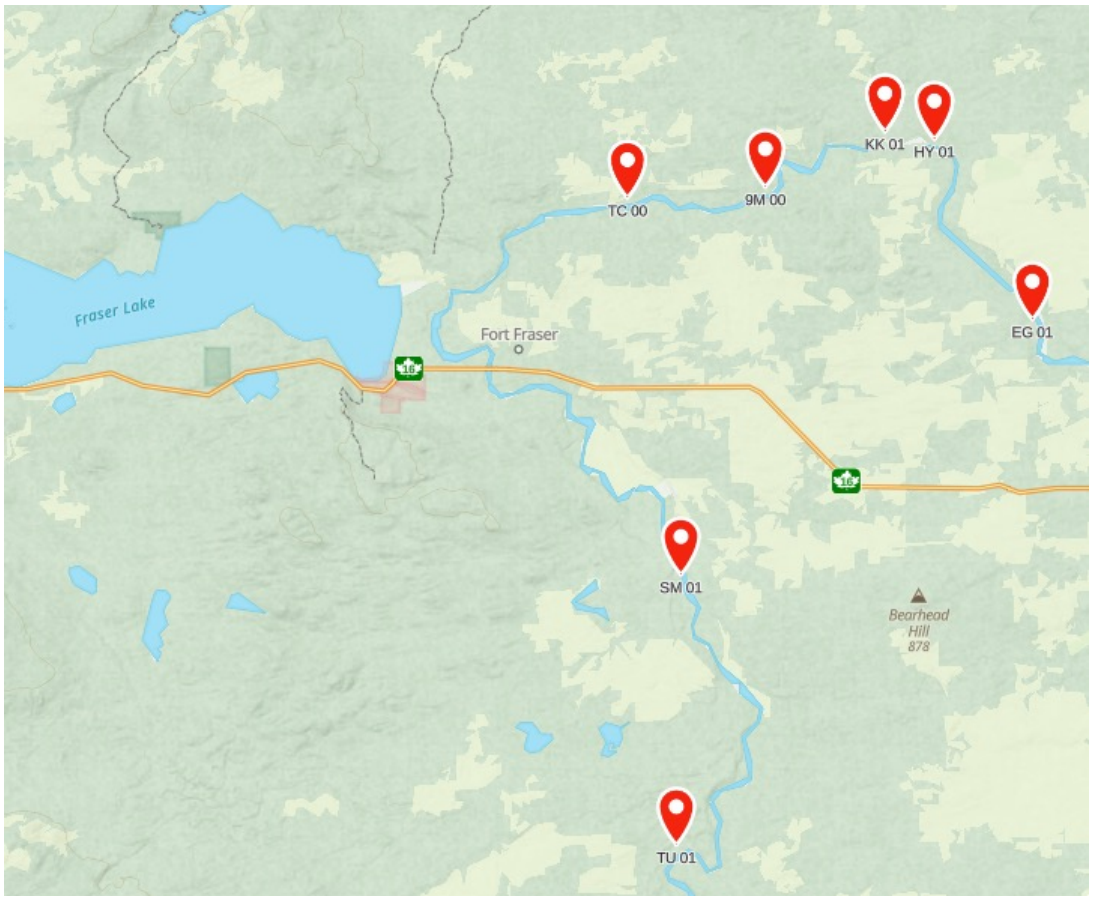
Endako River sites



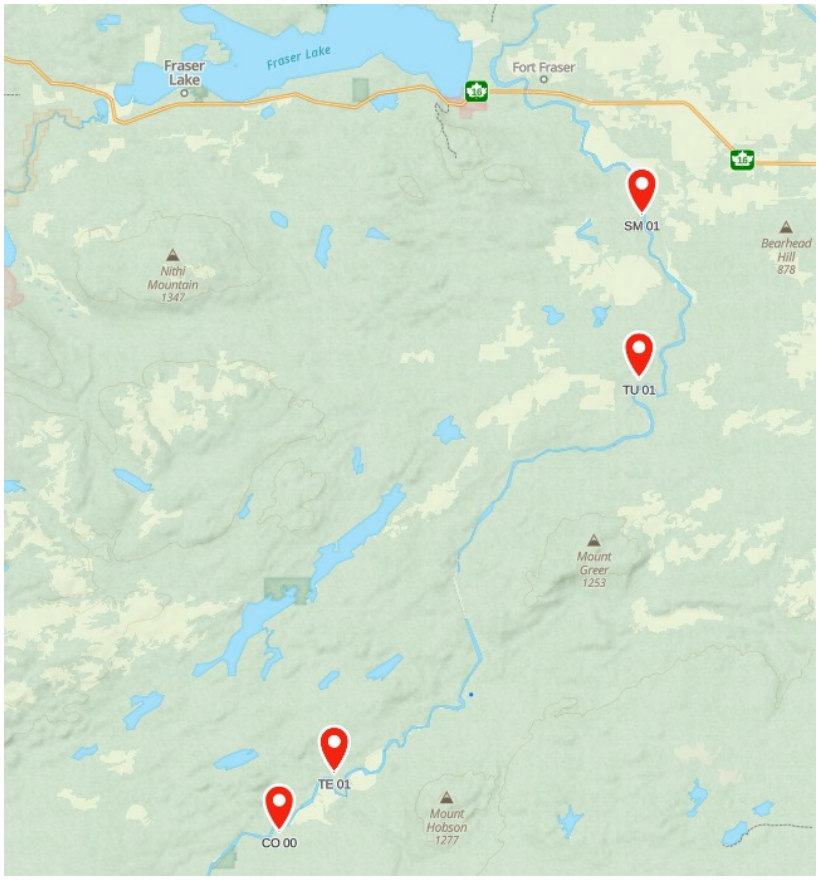
Endako River/ Shovel Creek sites

Sites sampled from river boat





Sites sampled from river boat continued



November Sampling of the Nechako Mainstem