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Technical Report on eDNA analysis of water samples for multiple species analysis - 2025 Samples collected from the Mackenzie, Skeena, and Fraser watersheds

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Summary

Previously validated Kokanee (Sockeye) Salmon (*Oncorhynchus nerka*), Rainbow Trout (*Oncorhynchus mykiss*), Burbot (*Lota lota*), Bull Trout/Dolly Varden (*Salvelinus confluentus* and *Salvelinus malma*), Arctic Grayling (*Thymallus arcticus*), Chinook Salmon (*Oncorhynchus tshawytscha*), and Coho Salmon (*Oncorhynchus kisutch*) quantitative PCR assays were utilized to test for the presence of environmental DNA (eDNA) in the provided water filter samples. There were 93 water filters submitted from the Mackenzie, Skeena, and Fraser sytems. These were divided into 12 different groups for testing (combinations of two to four species per group). A droplet digital PCR (ddPCR) platform was used to test paired (duplexed) assays for the presence of species-specific eDNA. A temperature gradient test was previously undertaken to determine the optimal conditions for the duplexed testing. Reference tissue samples and synthetic Genomic Block DNA sequence yielded positive signals as expected. The following report contains a summary of the data obtained from the analysis of water filter samples for the presence of eDNA. Each filter isolate was first tested in duplicate (the first and second elutions of the eDNA extraction), and the corresponding quantities (copy number of target DNA per reaction well estimated by positive droplet number) were used to determine presence or absence. A minimum of 4 positive droplets were required for a positive detection result. An internal control (bacterial phage lambda DNA) and ePlant assay were used to assay for the presence of PCR inhibitors. Overall, Arctic Grayling was not positively detected, while Rainbow Trout was in many samples.Chinook salmon was detected in 11 samples, all of which were from a Fraser site. Coho Salmon eDNA was only found in 3 of the Skeena water filters. Sockeye Salmon was only positively detected in sample F60813, and Burbot in sample F40915. Bull Trout/Dolly varden was found in 16 sites.

Methods

Published TaqMan assays for Kokanee/Sockeye Salmon, Rainbow Trout, Bull Trout, Burbot, Arctic Grayling, Chinook Salmon, and Coho Salmon were paired to test for eDNA - one with a FAM reporter dye and the other with a VIC reporter dye were used for this work (Hellberg et al., 2010; Laramie et al., 2015; Pilliod et al., 2016; Rodgers et al., 2017; Wilcox et al., 2013). The Bull Trout assay does not distinguish between Bull Trout and Dolly Varden. To confirm assay performance we previously tested the assays in the presence of synthetic genomic DNA (gBlock – synthesized double stranded DNA that has a perfect match to each assays primers and probes) and against a panel of DNA isolated from tissue samples collected from northern BC fish samples (this included Coho, Chinook, Sockeye, Rainbow Trout, Bull Trout and Dolly Varden) to confirm no cross-reactivity. The published Burbot assay (Rodgers et al., 2017) has not yet been tested against our panel of local tissue samples but was tested for specificity in the

original publication and has been validated with our synthetic standard gBlock at the time of analysis. The same reporter dyes are used for an inhibitor/eDNA quality test - a duplex reaction detecting “lambda” (added bacterial phage lambda DNA) and “ePLANT” (plant chloroplast DNA found in most/all water sample). Briefly, following the Bio-Rad laboratories protocol, samples (5 µL of extracted eDNA) were run in 20 µL duplex reactions using Mastermix for Probes (no UTP). Droplets (~15,000 – 20,000 micro reactions) were generated using the AutoDG (Bio-Rad Laboratories) and subjected to PCR. Droplets were assessed for detection (amplification) of target eDNA using a QX200 Droplet Reader (Bio-Rad Laboratories).

Environmental DNA (eDNA) was isolated from the provided water filters using the DNeasy Blood and Tissue Kit (Qiagen) following a modified protocol for water filters. For each filter, an “i” and “ii” eDNA elution was collected. After the initial sample “i” eDNA was eluted from its purification column, a second volume of elution buffer was run through it to collect any DNA that remained bound to it. For each filter, the first “i” and second “ii” elutions were analyzed for the presence of target eDNA. Each test initially analyzed 1/10 of the total elution volume. For retested samples an additional 1/10 of the volume was tested (1/5 of the total). Sites were considered positive if 4 or more droplets were positive at the appropriate amplitude – this corresponds to 5-6 copies, and any samples with 2 -3 positive droplets were retested. If additional positive droplets summing to 4 or more were detected, then the sample was considered “POSITIVE” for that species. If not, the sample was considered negative. In the event that only 2 to 3 droplets were detected, the sample was considered “NEGATIVE” (species not detected).

To control for false negative results, exogenous lambda DNA was added to a master mix to test for the presence of inhibitors in samples with no detections. An ePlant assay was also used to test for eDNA degradation. In cases of significant deviations from expected quantities (possible inhibition/degradation), an additional DNA purification (ZYMO Research) is performed prior to retesting.

Results

Results of testing (and retesting when applicable) are shown in the attached summary tables for each “Lab Testing Group”. Each testing group contains a unique combination of species assays. Lab Testing Group 7 was combined with another group. Lab extraction controls were negative for all eDNA assays (data spreadsheet). Tests for PCR inhibition and degradation with internal bacterial lambda control and chloroplast eDNA (ePLANT) indicated several negative samples required retesting. These samples had both elutions combined and cleaned before retesting (included in retest plate, data spreadhseet).

Three samples in Group 13 from the Mackenzie system had positive detection of Rainbow Trout eDNA in the filter control blank filters. Of these, M41361 and M41365 showed positive detections of less than 12 copies. Results from eDNA samples taken at this time should be considered with some caution and associated samples with a similar low detection level should not be interpreted as positive form Rainbow Trout. Most samples positive for Rainbow Trout in this group had detections well above this level of background contamination. The filter control sample M41351, however, has a Rainbow Trout detection level comparable to other positive sites (636 copies). This filter was retested in case of a plate preparation error, and this level of detection was confirmed. We note a possible transciption error with sample M41357. This sample has a low Rainbow detection level of 5.38 copies (equal 4 drops), similar to that observed in other positive control filer blanks, M41361 and M41365.

Table 1 – Summary of presence/not detected results for Kokanee/Sockeye Salmon (SOCK), Rainbow Trout (RAIN), Bull Trout/Dolly Varden (BULT), and Burbot (BURB), Arctic Grayling (GRAY), Chinook Salmon (CHIN), and Coho Salmon (COHO). Results are divided into Testing Groups. Copy numbers are estimated from the number of positive droplets, summed between replicate samples. Filters with sufficient droplets needed for a positive call are highlighted in green. Detections below the cutoff that required retesting and retests that still did not exceed the cutoff number are highlighted in yellow. Field blanks with positive detections are red.

GROUP 1										
		DNA copies detected				DNA copies detected - RERUNS				
Site ID	Call	BURB	BULT	GRAY	RAIN	BURB	BULT	GRAY	RAIN	Notes
M10139	RAIN	0.00	0.00	0.00	200.34					
M10140	RAIN	0.00	0.00	0.00	103.93					

GROUP 2										
		DNA copies detected				DNA copies detected - RERUNS				
Site ID	Call	COHO	SOCK	BULT	CHIN	COHO	SOCK	BULT	CHIN	Notes
S60274	NEGATIVE	0.00	0.00	0.00	0.00					
S60275	NEGATIVE	0.00	0.00	0.00	0.00					
S60276	NEGATIVE	2.62	0.00	0.00	0.00	0.00				COHO 2 DROPLETS, RETEST 0

GROUP 3										
		DNA copies detected				DNA copies detected - RERUNS				
Site ID	Call	BULT	RAIN	COHO	SOCK	BULT	RAIN	COHO	SOCK	Notes
S31493	RAIN	0.00	207.95	0.00	0.00					
S60377	NEGATIVE	0.00	0.00	0.00	0.00					

GROUP 4						
		DNA copies detected / RERUNS				
Site ID	Call	CHIN	BULT	CHIN	BULT	Notes
F20401		0.00	0.00			
F20402	CHIN BULT	101.71	5.13			

GROUP 5										
		DNA copies detected				DNA copies detected - RERUNS				
Site ID	Call	BULT	RAIN	CHIN	SOCK	BULT	RAIN	CHIN	SOCK	Notes
F60503	RAIN	0.00	9.86	0.00	0.00					
F60504	RAIN	0.00	3.71	0.00	0.00		1.16			RAIN 3 DROPLETS, RETEST 1
F60505	RAIN	0.00	47.67	0.00	0.00					
F60506	RAIN CHIN	0.00	2558.89	138.22	0.00					
F60507	RAIN CHIN	0.00	3734.66	49.74	0.00					

GROUP 6										
		DNA copies detected				DNA copies detected - RERUNS				
Site ID	Call	CHIN	RAIN	COHO	SOCK	CHIN	RAIN	COHO	SOCK	Notes
S60678	RAIN COHO	0.00	281.32	18.86	0.00					

GROUP 8										
		DNA copies detected				DNA copies detected - RERUNS				
Site ID	Call	CHIN	BULT	COHO	SOCK	CHIN	BULT	COHO	SOCK	Notes
F60808	NEGATIVE	0.00	0.00	0.00	0.00					
F60809	NEGATIVE	0.00	0.00	0.00	0.00					
F60810	NEGATIVE	0.00	0.00	0.00	0.00					
F60811	CHIN	7.83	0.00	0.00	0.00					
F60812	BULT	0.00	25.54	0.00	0.00					
F60813	CHIN BULT SOCK	235.05	99.39	0.00	559.65					
F60814	NEGATIVE	0.00	0.00	0.00	0.00					BLANK
S50879	BULT COHO	0.00	300.86	2.73	0.00			2.52		COHO 2 DROPLETS, RETEST 2
S60880	BULT COHO	0.00	188.87	12.01	0.00					
S60881	NEGATIVE	0.00	2.56	0.00	0.00		0.00			BULT 2 DROPLETS; RETEST 0

GROUP 9										
		DNA copies detected				DNA copies detected - RERUNS				
Site ID	Call	CHIN	BULT	BURB	SOCK	CHIN	BULT	BURB	SOCK	Notes
F40915	CHIN BURB	393.06	0.00	14.51	0.00					

GROUP 10										
		DNA copies detected				DNA copies detected - RERUNS				
Site ID	Call	CHIN	BULT	BURB	RAIN	CHIN	BULT	BURB	RAIN	Notes
F41016	RAIN	0.00	0.00	0.00	50.81					
F41017	RAIN	0.00	0.00	0.00	10.38					
F41018	NEGATIVE	0.00	0.00	0.00	0.00					BLANK
F41019	CHIN RAIN	17.89	0.00	0.00	9.52					
F41020	CHIN RAIN	291.24	0.00	0.00	230.12					

GROUP 11										
		DNA copies detected				DNA copies detected - RERUNS				
Site ID	Call	CHIN	BULT	COHO	RAIN	CHIN	BULT	COHO	RAIN	Notes
F61121	RAIN	0.00	0.00	0.00	163.31					
F61122	RAIN	0.00	0.00	0.00	50.39					
F61123	CHIN RAIN	3.73	2.44	0.00	111.02	4.18	1.26			BULT 2 DROPLETS, RETEST 1, BELOW CUTOFF
F61124	CHIN RAIN	3.71	0.00	0.00	113.63	2.81				CHIN 3, RETEST 2
F61125	RAIN	0.00	0.00	0.00	114.91					
F61126	CHIN RAIN	40.22	0.00	0.00	628.71					
F61127	RAIN	0.00	0.00	0.00	1227.17					
F61128	RAIN	0.00	0.00	0.00	97.38					
F61129	NEGATIVE	0.00	0.00	0.00	0.00					
F61130	NEGATIVE	0.00	0.00	0.00	0.00					BLANK
F61131	BULT RAIN	0.00	236.29	0.00	74.69					
F61132	BULT RAIN	0.00	270.26	0.00	47.50					
F61133	NEGATIVE	0.00	0.00	0.00	0.00					BLANK
F61134	NEGATIVE	0.00	0.00	0.00	0.00					BLANK
F61135	RAIN	0.00	0.00	0.00	134.93					
S61182	RAIN	0.00	0.00	0.00	0.00	0.00	0.00	0.00	82.37	eP low; lambda low, cleaned: negative, diluted: RAIN
S61183	NEGATIVE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	eP low, retests negative, BLANK
S61184	RAIN	0.00	0.00	0.00	712.07					
S61185	RAIN	0.00	0.00	0.00	933.94					
S61186	RAIN	0.00	0.00	0.00	75.17					
S61187	RAIN	0.00	0.00	0.00	255.24					
S61188	BULT	0.00	299.43	0.00	0.00					
S61189	BULT	0.00	246.31	0.00	0.00					
S61190	BULT	0.00	40.09	0.00	0.00					
S61191	BULT RAIN	0.00	166.71	0.00	141.01					
S61192	BULT RAIN	0.00	107.03	0.00	77.83					

GROUP 12						
		DNA copies detected		RERUNS		
Site ID	Call	BULT	RAIN	BULT	RAIN	Notes
F21236	RAIN	0.00	79.71			
F21237	NEGATIVE	0.00	0.00			
F21238	NEGATIVE	0.00	0.00	3.76	0.00	eP low, clean BULT 2 DROPLETS, RETEST 1, BELOW CUTOFF
M21241	NEGATIVE	0.00	2.63		0.00	2 DROPLETS, RETEST 0, BLANK
M21242	RAIN	0.00	2.63		6.79	2 DROPLETS, RETEST 5
M21243	NEGATIVE	0.00	0.00			BLANK
M21244	NEGATIVE	0.00	0.00			
M21245	NEGATIVE	0.00	0.00			
M21246	NEGATIVE	0.00	0.00			

GROUP 13										
		DNA copies detected				DNA copies detected - RERUNS				
Site ID	Call	BULT	GRAY	RAIN	SOCK	BULT	GRAY	RAIN	SOCK	Notes
M41347	RAIN	0.00	0.00	2591.71	0.00					
M41348	RAIN	0.00	0.00	316.58	0.00					
M41349	RAIN	2.58	0.00	528.61	0.00	1.36				BULT 2 DROPLETS, RETEST 1, BELOW CUTOFF
M41350	NEGATIVE	0.00	0.00	0.00	0.00					BLANK
M41351	RAIN	0.00	0.00	636.29	0.00					BLANK
M41352	NEGATIVE	0.00	0.00	0.00	0.00					BLANK
M41353	BULT RAIN	6.23	0.00	614.55	0.00					
M41354	BULT RAIN	6.99	0.00	548.42	0.00					
M41355	NEGATIVE	0.00	0.00	0.00	0.00					BLANK
M41356	RAIN	0.00	0.00	409.47	0.00					
M41357	RAIN	0.00	0.00	9.91	0.00					
M41358	RAIN	0.00	0.00	490.76	0.00					
M41359	RAIN	0.00	0.00	1174.38	0.00					
M41360	RAIN	0.00	0.00	1378.56	0.00					
M41361	RAIN	0.00	0.00	0.00	0.00	0.00	0.00	11.62	0.00	eP low, retests negative, clean: RAIN, BLANK
M41362	RAIN	0.00	0.00	177.23	0.00					
M41363	RAIN	0.00	0.00	1633.66	0.00					
M41364	RAIN	0.00	0.00	540.87	0.00					
M41365	RAIN	0.00	0.00	5.38	0.00					Ep low, BLANK
M41366	BULT RAIN	5.37	0.00	267.92	0.00					
M41367	RAIN	0.00	0.00	407.15	0.00					
M41368	RAIN BULT	2.69	0.00	1232.55	0.00	3.99				BULT 2 DROPLETS, RETEST 3
M41369	RAIN	0.00	0.00	1063.72	0.00					
M41370	BULT RAIN	3.67	2.54	8.57	0.00	1.32	0.00			BULT 3 DROPLETS, RETEST 1; GRAY 2 DROPETS
M41371	RAIN	0.00	0.00	23.75	0.00					
M41372	NEGATIVE	0.00	0.00	0.00	0.00					
M41373	NEGATIVE	0.00	0.00	0.00	0.00					

Please let us know if you require further clarification or details of the results and interpretations presented here.

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