FSCI Biological Consultants

November 30, 2021 Our File No.: FSCI-21-0023

Michele Lewis
Infrastructure Services, Town of Gibsons
PO 340, 474 South Fletcher Road
Gibsons, BC, V0N 1V0

Re: Habitat/Condition Assessment of Charman Creek, Gibsons, BC, an Urbanized Stream

Dear Michele:

In 2021, ***FSCI*** Biological Consultants completed an assessment of the current condition of aquatic and riparian habitats in Charman Creek. The intent of the assessment/survey was to document specific issues that create a bottleneck to successful and continued salmonid production and stable and productive aquatic and riparian habitats for area wildlife. In addition, the Town of Gibsons (ToG) has requested an opinion of the steps that may be required to remediate and restore Charman Creek to a viable salmonid producing stream.

In order to assist the town of Gibsons and [provide updated information on Charman creek we completed the following activities in 2021:

- Review of the length of Charman Creek from Gibsons Harbour to White Tower Park. The review provided field validation of the mainstream reach breaks, assessment of current/existing viable habitat and basic stream morphological features.

- Mapping channel and bank features/obstructions that present challenges to natural stream processes and recovery.

- Documented observation of rearing fish distribution, including samples collected and analyzed for salmonid eDNA . The use of eDNA is to "confirm" the extent of salmonid distribution by comparing to data collected and stream classification recorded in 2003.

- Collection of benthic macroinvertebrate samples and the sorting of the samples. Macroinvertebrate data collected in 2021 will be compared to data collected in 2018[1]. Benthic invertebrate data will used to calculate the EPT index.

[1] Whitehead Environmental Consultants. 2018. 2018-Water Quality and Biology Baseline Monitoring Report. Prepared for Infrastructure Services, Town of Gibsons, Gibsons, BC.

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- The installation of continuous data loggers, recording water temperatures during the critical summer period. Loggers are installed downstream of White Tower Park ponds and above the Gibsons Marina at Dougall Road. Data will be summarized using the 7-day maximum daily temperature.

The results of the above field work have been summarized and are presented for further discussion regarding the remediation and protection of Charman Creek.

Charman Creek Watershed

Charman Creek is an urban stream located within the boundaries of the Town of Gibsons, BC (**Figure 1**). The approximate watershed or collection area for the creek is 156-ha and includes components of the towns stormwater management infrastructure[2]. Stream length also varies for a similar reason but the estimated length of the daylighted portion of primary stream channel is approximately 2100-m.

The creek supports salmonids including, coastal cutthroat trout (*Oncorhynchus clarkii clarkii*) and coho salmon (*Oncoryhnchus kisutch*). In recent years both these species have been reported present. In addition, the SCRD habitat atlas suggests cutthroat trout reside in the upper reaches, but the data that supports trout presence is not definitive.

Methods

Habitat Assessment

In July and August 2021, a desktop review and field assessment was completed on Charman Creek. The entire length of Charman Creek was walked from the estuary at Gibsons Marina to White Tower Park in upper Gibsons.

The desktop review was completed using digital mapping data and topography information provided by the Sunshine Coast Regional District. This information was used to delineate reaches within the mainstem. Reaches were defined as lengths of stream consisting of similar channel morphology, habitat, flow and slope. Reach breaks were then added to a map and length determined.

A habitat assessment, used to determine macrohabitat features and quality of salmonid and aquatic habitats generally, was modified following the first day in the field. The lack of summer base flows throughout entire length of Charman Creek prevented any meaningful delineation of channel habitat features. The assessment process was then modified to document the following channel features by reach:

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[2]URBAN Systems. 2019. Town of Gibsons Stormwater Management Plan. Prepared for Infrastructure Services, Town of Gibsons, Gibsons, BC.

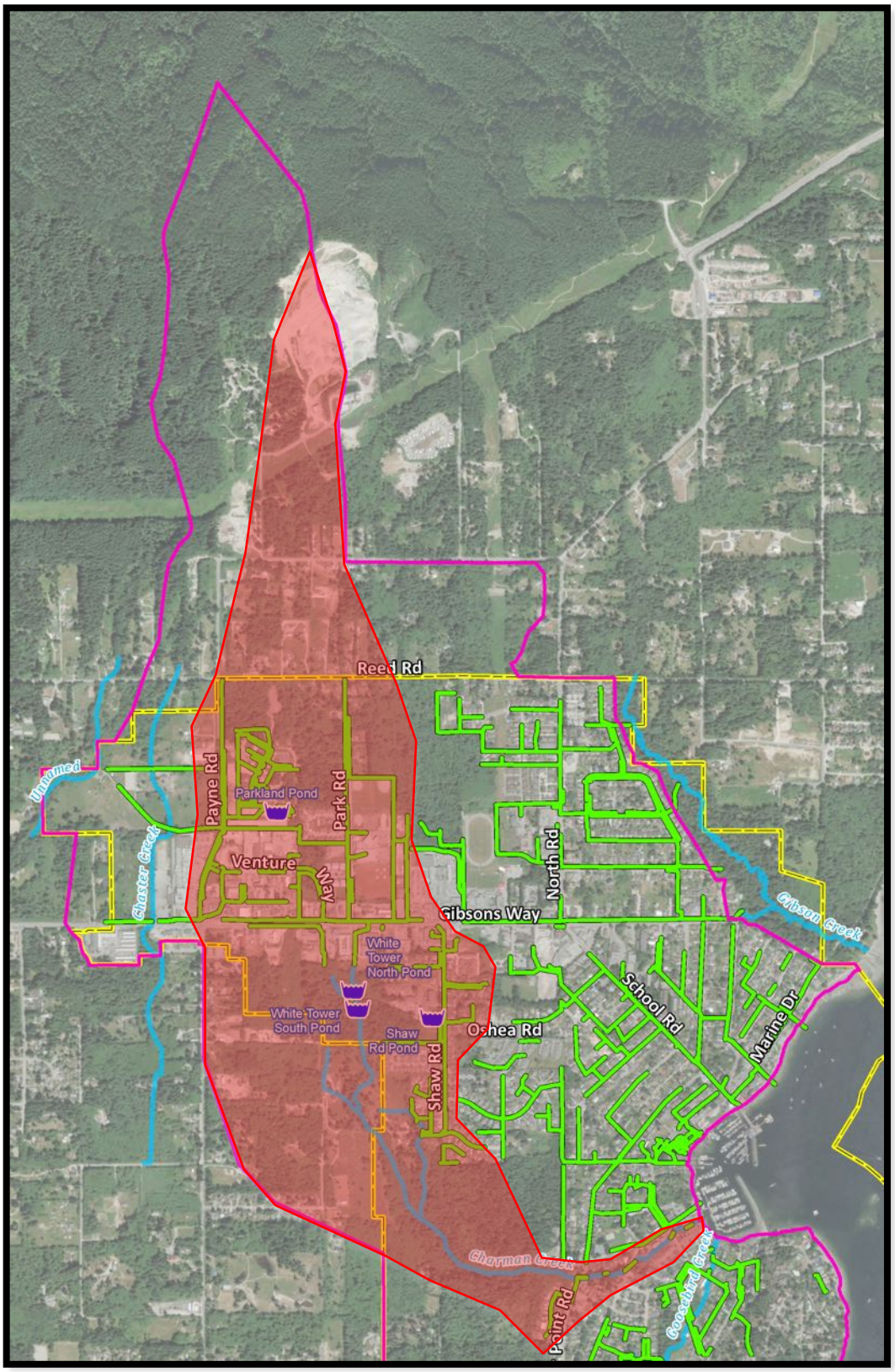


Figure 1: Map of Charman Creek (arrow) showing the approximate catchment area ("watershed") in red[2].

- Reach length,
- Estimated bankfull/channel width,
- Existing wetted width (where water was located),
- Structures considered to be impeding the natural channel forming process and/or impeding access to the stream channel to area salmonids,
- Riparian conditions including areas of encroachment and invasive species.

All features of the stream considered to be impeding or affecting Charman Creeks ability to "function" in a more natural way were documented. Each site was photographed and the location marked geospatially using UTM coordinates. The sites that appear to be candidates for restoration and/or remediation were then summarized into a priority list.

Stream Flows

Any future planned restoration will require an understanding of seasonal flows within Charman Creek. Presently flow data and an understanding of area hydrology is underway by the Town of Gibsons. This data is not presented at this time.

Water Temperature

Water temperature data is important for the general health of aquatic ecosystems and specifically the survival of rearing salmonids. Presently the records of instream, year around, water temperatures in Charman Creek is sporadic. In order to start and build a data set of stream temperatures, an Onset Tidbit temperature data logger was installed at Dougall Road and a second logger in White Tower Park downstream of the stormwater retention ponds (**Figure 1**) Loggers are recording water temperatures continuously every 15-m. Data will be downloaded and using the data the 7-day maximum will be determined.

In order to secure the data loggers, they are installed inside a 15-cm length of 4-cm diameter steel pipe (**Figure 2**). The case was then chained to an anchor holding it in place during high water events. Data loggers were installed in August 2021.

Benthic Invertebrates

Benthic invertebrates were sampled once in June, 2021. The date of the sampling coincides with sampling completed in 2018[1] and provides a recent data set comparison.

Replicate benthic samples were collected from 2 locations in Charman Creek (**Figure 3**) using a 500-micron Surber sampler placed on the bottom in suitable

Figure 2: Onset® Tidbit temperature data logger housed inside steel pipe for protection then anchored to a boulder to prevent it from washing away during high flows.

"riffle" like habitat(s). The sampler area measures 0.09-m^2 and all substrate within this area is washed into a collection net. The net sample is then washed and transferred to a 500-ml Nalgene jar, fixed with 95% Ethanol and shipped to Sandpiper Biological, Victoria, BC for sorting.

Sorting of the samples is completed to the genus/order and each group enumerated. Using these results the EPT taxa and individual richness were determined. These are then compared to the 2018 data.

Fish Presence/Absence

Charman Creek has , in the past supported coastal cutthroat trout and coho salmon. Historically, there are reports of chum salmon (*O. keta*) utilizing the lower reach near tide line.

Fish presence data is not well documented and the upper extent of distribution is poorly defined. Lack of summer base flows limits upstream recruitment and habitat utilization.

In order to confirm salmonids presence, water samples from Charman Creek were collected during low flow in December, 2021. Duplicate 100-ml samples

were collected in disinfected, clean 1000-ml Nalgene bottles. Samples were collected from the thalweg where flow is concentrated. A set of three samples, in duplicate, were collected, first at Dougall Road below the reach with known salmonid presence, above the Town of Gibsons treatment plant and at or near the past distribution point for salmonids, and the last at Inglis Road, an area to reportedly support salmonids (**Figure 3**).

The duplicate samples of water were collected to run eDNA for coastal cutthroat trout and coho salmon. Each sample, collected was processed by filter it through a sterile 47-mm Cellulose Nitrate filter with 0.45um pore size. The collection and processing methods followed BC Government protocols[3].

Once filtered the filter substrate was removed and packaged with silica then shipped to the Bureau Veritas DNA laboratory in Guelph, Ontario. Results of the lab analysis are then forwarded back when complete.

Results of the eDNA analysis will be used to determine an approximate upper extent to salmonid distribution in Charman Creek as of 2021.

Streamside Protection and Enhancement Areas

Streamside Protection and Enhancement Areas (SPEA) or riparian reserves are added to Charman Creek along its length from Gibsons Marina to White Tower park. The SPEA is determined following the process outlined in the Provincial Riparian Area Regulations and the assessment methodology[4], where the buffer width has been determined using the bankfull of channel width and presence of absence of fish and/or fish habitat. The SPEA is added to the map and will be provided as guidance for the Town of Gibsons planning department.

Results and Discussion

Habitat Assessment

The habitat assessment of Charman Creek was modified during the field work in the summer of 2021. The lack of surface flows in the lower reaches prevented the identification and subsequent delineation of macrohabitat features such as pools and riffles. These important habitats were non-existent, with the existence of 2 individual pools with standing water that contained rearing juvenile trout.

In order to provide an opinion on the health of the stream, the mainstem was first broken into reaches. A total of 9 reaches has been delineated using digital spatial

[3] Hobbs, J., Helbing, C.C and Goldberg, C. 2021. Environmental DNA Protocol for Freshwater Aquatic Ecosystems version 3.0. BC Ministry of Environmental, Ecosystems Branch, Victoria, BC.

[4] BC Ministry of Forests, Lands, Natural Resource Operations and Rural Development. 2019. Riparian Area protection Regulation Technical Assessment Manual. Fish and Aquatic Branch, Victoria, BC.

data and ground truthing (**Figure 3**). Once delineated, channel width of each reach was measured and an average width determined. This would be used to determine the SPEA and suggest channel changes in the most heavily impacted stream lengths. **Table I** summarizes the channel features observed in each Reach.

Table I: *Summary of reaches identified in Charman Creek with the reach length, bankfull or channel width, wetted width and channel type (Riffle/Pool=RP, Step Pool=SP, g=gravel, b=boulder, f=fines) represented. Baseflow represents whether surface water connectivity was evident during the assessment in July, 2021.*

Reach	Length (m)	Channel Width (m)	Wetted Width** (m)	Channel Type[5]	Continuous Baseflow (Y or N)
1	280	1.34	0.50	RPc	N
2	185	1.30	0.00	RPc	N
3	200	3.60	0.00	SPb	N
4	450	3.70	0.43	SPb	N
5	310	3.30	0.00	RPb	N
6	125	1.50	0.10	RPb	N
7	280	1.50	0.00	RPg	N
8	170	1.50	0.00	RPs	N
9	115	1.50	0.00	RPs	N
Total	**2115**				

** In areas where there was little of no stream flow pockets of "standing" water was noted. These areas are likely supported by hyporheic baseflows.

In addition to the basic stream features, structures that were influencing the channel processes and conditions of the shoreline including the riparian conditions were documented.

In all, the most significantly impacted and detrimentally altered stream features were found in Reaches 1, and 2. The lack of summer base flows , hardened instream structures, constriction of the channel through channelization and the abundance of invasive and non-indigenous riparian vegetation dominated the results of the habitat review in this length of Charman Creek. All locations that were considered detrimental where numbered using the reach and consecutive numbers. The locations are shown on **Figure 4** and summarized with comments in **Table II**. In total there were 23 locations flagged in the first two reaches.

Each structure has was photographed and is presented in **Figures 5** through **20**.

[5] Hogan, D.L. and Bird, S.A. 1996.Channel Assessment Procedure. BC Ministry of Environment, Lands and Parks. And BC Ministry of Forests. WRP Tech Circ. No. 7, Victoria, BC

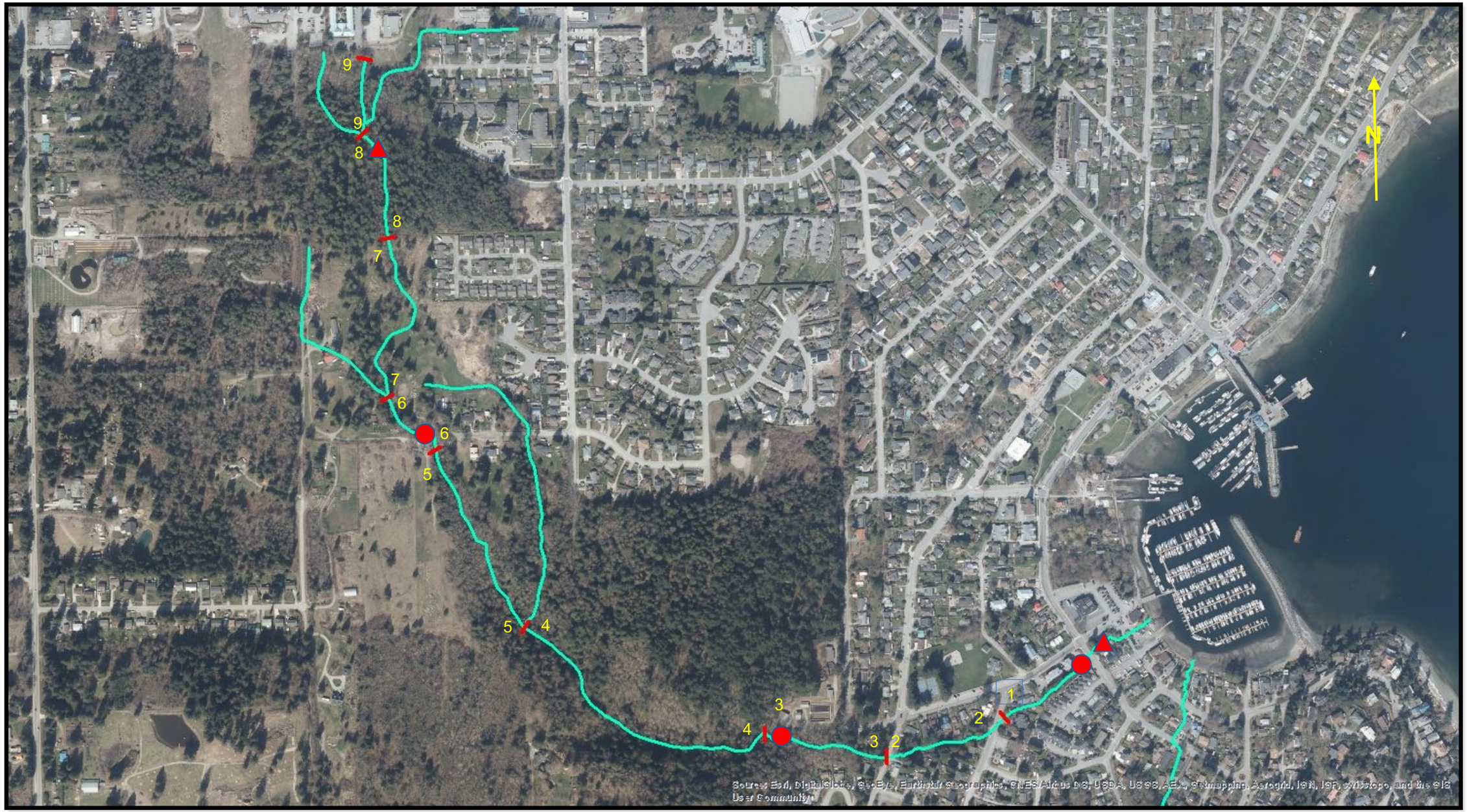


Figure 3: Map of Charman Creek showing the location of reach breaks. A total of 9 reaches have been assigned to the mainstem of Charman Creek. The major tributaries were not delineated. In addition to the reach breaks, the locations of the benthic invertebrate samples is shown as red triangles and the eDNA sample locations in red dots.

Figure 4: Reach 1 and 2 of Charman Creek showing the approximate locations of structure, unauthorized instream works and passage obstructions. Many of the structures have resulted in channel and habitat degradation.

Table II: *Location of descriptions of man-made stream features in Reach 1 and 2, that are considered to be problematic for natural stream processes in Charman Creek. Many of these structures present passage barriers and in some cases appear to accelerating channel failures in the lower reaches of Charman Creek.*

Station	Location		Description	Figure
	Easting	Northing		
R1-001	463210	5471808	Culvert-Closed pipe	5
R1-002	463197	5471800	Culvert-Closed pipe	5
R1-003	463167	5471793	Stacked rock wall confining channel	6
R1-004	463144	5471773	Dougall Road culvert	7
R1-005	463135	5471762	Failing loc-bloc wall and channel constriction. Back watering evident at culvert inlet. Lack of native vegetation.	8
R1-006	463131	5471754	Manmade rock walls constricting the channel. Structures used to confine channel but appears to allow channel to down cut or break over the structures. Lack of native vegetation.	9
R1-007	463128	5471751	Concrete weir redirecting flows to opposite back. Weir is impeding natural channel function.	10
R1-008	463125	5471748	Channel spanning weir that is resulting in downcutting and is a passage barrier at low flow. Structure preventing/influencing channel forming processes.	10
R1-009	463113	5471739	Manmade retaining wall.	-
R1-010	463097	5471725	Concrete weir with scoured plunge pool below. Passage barrier at low flows. Possibly attributing to accelerated bank erosion.	11
R1-011	463084	5471709	Concrete weir impeding passage	11
R1-012	463074	5471704	Series of rock walls, concrete weirs and riprap walls. Bottom has been sealed with concrete preventing any channel forming processes. Numerous barriers to fish distribution and passage at low and medium flows. Length of channel dry. Rock walls and weirs have effectively channelized the stream. This may also create velocity barriers at higher flows.	12
R1-013	463045	5471694	Dry Channel with series of concrete weirs, hardened bottom and banks. No surface connectivity	13
R1-014	463034	5471689	Dry channel. Lengths of armoured banks that may constrict channel.	13
R1-015	463019	5471679	Building structure that is encroaching on channel and constricting lateral movement	14

Table II: *Continued*

R1-016	463016	5471677	Concrete culvert under Glassford Road. Length and materials would potentially make this culvert inaccessible at low water and at high water create a velocity barrier for juveniles.	14
R2-001	462982	5471651	Channel appears to be channelized but invasive plant coverage makes it difficult to identify any structures that might be present. Length dominated by invasive streamside vegetation (Himalayan Blackberry, English Ivy.	15
R2-002	462965	5471646	Rock wall have been built along the stream effectively channelizing the stream. Channel forming features appear impaired by main-made control structures.	16
R2-003	462930	5471641	Old Corrugated Metal Pipe (CMP) with rusted bottom. Impeding channel processes and pre a barrier at low flows.	17
R2-004	462905	5471633	Channel is dry. Rock retaining wall located near bankfull edge. Evasive and ornamental vegetation within riparian corridor.	18
R2-005	462890	5471632	Channelized armoured stream banks presumably constructed by property owners. Channel confined within armoured channel. Poor habitat, dry channel.	19
R2-006	462866	5471620	Concrete and rock retaining (armour) structures that are failing. Downcutting and undermini evident. Channelization of stream resulting in degraded habitats.	19
R2-007	462850	5471620	Culvert under Gower Point Road. Barrier to passage during low flows and potentially high fl by creating a velocity barrier. This is considered the upper extent of fish distribution because of passage issues.	-
R3-001	462796	5471626	“Natural” stream channel begins and Reach 3 and 4 are more indicative of a natural stream channel and processes. This area is steeper that Reaches 1 and 2 so exp habitats differ. No contiguous running surface waters. Expected water in pools or depressio with hyporheic flows would provide contributing flow.	20
R3-002	4627741	5471635	Surface water in pool. No fish observed.	-

Figure 5: Station R1-001 and 002. Culverts under road ways at Gibsons Marina. The concrete pipes are embedded and appear to backwater sufficiently to allow access for migrating adult salmonids. There is a possibility the pipe may be undersized.

Figure 6: Station R1-003 downstream of the Dougall Road culvert. The channel is confined by an old rock and concrete pieces “retaining wall. The channel is confined through this area by the wall and a parking lot.

Figure 7: Station R1-004, the Dougal Road Culvert. This s a concrete pipe under the road that may be undersized during highwater events. The culvert backwaters and there is evidence of increased erosion. The culvert may present a passage barrier at low water.

Figure 8: Station R1-005, loc-bloc retaining wall located upstream of Dougall Road. The wall is collapsing and at risk of blocking the active stream channel. Channel shows evidence of backwatering from under capacity culvert and erosion near toe of wall.

Figure 9: Station R1-006, channelization of Charman Creek using rock hardened banks. Channel confined within the hardened channel resulting in degraded habitat.

Figure 10: Station R1-007 and 008, concrete weirs that “throat” down the channel. Bottom has been hardened with concrete. Weirs prevent natural channel forming functions and have resulted is significant habitat degradation including downcutting of bottom. Structures present passage barriers. Pooled water shave provided limited refuge for juvenile salmonids.

Figure 11: Stations R-010 (top) and 011 (bottom), and concrete weird spanning the channel width preventing natural channel forming functions and impeding passage. Scour at the base of the weirs have created isolated pools (top) used by juvenile salmonids for refuge. Predation in pools is high.

Figure 12: Station R1-012, concrete weirs and corrugated metal pipe used to create weirs and hardened bank protection. Stream has undercut structures and habitat significantly degraded. Scour pool created provides refuge for juvenile salmonids with likely high predation risk. Passage barrier to salmonids.

Figure 13: Station R1-013 (top) and 014 (bottom) where a numerous weirs, concrete bottom and hardened banks channelizes Charman Creek. Habitat is degraded or non-existent (top) and structures present passage barriers, including potential velocity barriers at high flows. Channel was dry during low summer flow period.

Figure 14: Stations R1-0015 (top) and 015 (bottom) where a building appears within or near the active channel bank (top) and the concrete culvert under Glassford Road (bottom). The culvert is not embedded and is dry during the summer months creating a passage barrier. At higher flows this pipe may create a velocity barrier, particularly for juvenile salmonids. Back flooding may be an option.

Figure 15: Station R2-001, channelized stream channel with invasive plants dominating the riparian vegetation. Evidence of locals dumping organic waste into the ditch that will block the channel. Dumping organics waste into the stream channel is an infraction under the Federal Fisheries Act.

Figure 16: Station R2-002, hardened stream bank using stacked boulders. The image illustrates the risk of poorly planned bank protection. The armouring on the wone side has forced the channel to adjust and erode the opposite bank as it adjusts its channel capacity and energy dissipation.

Figure 17: Station R2-003, rusted corrugated metal pipe. Culvert should be removed and replaced with an embedded structure that encourages substrate deposition within the pipe.

Figure 18: Station R2-004, channelized length of creek with non-native plantings within the riparian. Channel is dry and habitat poor.

Figure 19: Stations R2-005 (top) and 006 (bottom) showing channelized stream channel with hardened retaining structures. The structures prevent the channel from developing natural geomorphological processes that would contribute to creation of suitable salmonid habitat.

Figure 20: Examples Charman Creek stream channel and riparian area above Gower Point Road. The upper reaches , with some exceptions, are in better condition and exhibit “better” quality habitat. The limiting factor in these areas is the lack of summer surface baseflows.

In all, the most significant habitat issues and opportunities for restoration, remediation are found in Reach 1 and 2. While the upper reaches also have some areas that have been impacted through development, these upper areas are not directly fish bearing.

The question of what can be done in the lower reaches is difficult. The creek bisects private lands over the length of the reaches. Planned restoration that will involve the removal of hardened structures, establishment of small flood plain areas and encouraging some development of scour and fill with the potential of macrohabitat forming processes is encouraged but will require cooperation from land owners.

In general the entire length presents opportunities for removal of in-channel hardened structures and removal/reconfiguration of hardened banks. Introduction of complex cover features such as engineered woody debris structures may also be possible where the risk to creating downstream issues is low. Planning is important. All documented structures illustrate the impacts to the stream channel from poorly designed structures that do not take into account natural channel geometry or the implications of hardening a channel on downstream properties. Consultation with an knowledgeable stream restoration practitioner is very important.

The riparian area in these reaches present numerous opportunity for removal and replacement of current ground, shrub and tree cover with native species suitable for the area. This will also require cooperation from the property owners and a level of education. In that case informing local land owners that they have a responsibility to ensure the provincial Riparian Area Protection Regulations (RAPR) are followed and that improvements within the Streamside Protection and Enhancement Areas (SPEA) must follow a naturalized process that ensures Charman creek riparian function is both restored and maintained.

While restoring the channel and removal of manmade structures currently impacting Charman creek is encouraged, it must be noted that the current single greatest bottleneck to salmonid health and survival in Charman Creek is summer base flows.

Fish need water and in this case, Charman Creek, a known cutthroat trout and coho salmon stream requires rearing flows throughout the year in order to support these fish. How or if a more consistent flow can be maintained should be discussed. I there is any known water extraction points from Charman, these should be revisited and in that case the process of determining the Environmental Flow Needs (EFN)[6] may be warranted.

[6] Hatfield, T., Perkins, T., Cathcart, J., Faulkner, S., Harwood, A., Alexander, C. and Lewis, A. 2016. Environmental Flow Needs Implementation Guidance for British Columbia, Ministry of Environment, Water protection and Sustainability Branch, Victoria, BC.

The following is a proposed list of issues that require attention in an attempt to improve fish habitat in lower Charman Creek:

- Summer base flow that ensure surface connectivity;
- Removal of in channel hardened structures that prevent channel forming processes and create passage challenges;
- Review structures channelizing the stream and where possible widen the channel providing a bankfull width in line with the more natural widths observed in Reaches 3, 4 and 5.

Stream Flow

Continuous stream flow data is unavailable at this time. Monitoring by the Town of Gibsons in the summer of 2021 reported a base flow of approximately 10-lps July 15 and 16, 2021[7]. This measurement was reported near the top of Reach 1 and provides an indication of how low the base summer flows reached in 2021. While there was a flow reported, field review of the reaches the following week found limited surface connectivity and extensive areas of the channel dry where any flows were subsurface or hyporheic.

Summer base flows remains a critical bottle neck to salmonid production in Charman Creek. Continued monitoring of flows and review of ater source is encouraged.

Water Temperature

Data loggers continue to collect water temperature data in Reach 1 and Reach 8 of Charman Creek. Once data is downloaded the information will be summarized and provided to the ToG in a separate technical note.

Benthic Invertebrates

The benthic invertebrate samples collected, sorted and identified are provided in Appendix I. This data was summarized using the EPT index, an indication of the presence of pollution intolerant invertebrate orders (Ephemeroptera, Plecoptera, and Tricoptera). In addition the presence of more tolerant orders that indicate an increase in pollution or decreasing water quality were also summarized. The results are provided in **Table III**.

In reviewing the results, the EPT in 2021 had increased when compared to 2018. Values or indices greater than 27 are indicative of excellent water quality and those between 0-6% are considered poor. The results show water quality in Upper Charman as poor increasing as we progress downstream t9 the lower fish bearing reaches where the 2018 suggested good water quality but the 2021 EPT index suggests excellent water quality.

[7] Wing, S. 2021. Email on surface water monitoring data to D. Bates.

When the pollution tolerant groups, namely Chironomidae, oligochaetes and Isopods are reviewed they have remained similar to the 2018 results (increase in oligochaete numbers). These results are more open to greater interpretation given the limited samples, but they provide a baseline for future sample comparisons.

Table III: *Summary of benthic invertebrate samples collected in Charman Creek in 2021 and compared to values reported in 2018[1]. The EPT index suggests water quality in the known fish bearing reach is good to excellent while the water quality at White Tower Park is poor.*

Group	Lower Charman (Dougall Road)		Upper Charman (White Tower Park)	
	2018	2021	2018	2021
Total Taxa	36	20	36	13
Total Individuals	253	129	964	1170
EPT Taxa	7	9	1	0
EPT Taxa Index	19.4%	45.0%	2.8%	0.0%
EPT Individuals	64	58	1	0
EPT Individual Index	25.3%	45.0%	0.1%	0.0%
Chironomid Individuals	95	25	54	32
% Chironomids	37.5%	19.4%	5.6%	2.7%
Isopod Individuals	2	0	16	0
% Isopods	0.8%	0.0%	1.7%	0.0%
Oligochaetes Individuals	27	29	221	464
% Oligochaetes	10.7%	22.5%	22.9%	39.7%

Fish Presence/Absence

The use of eDNA was used on samples of water collected from the lower reaches of Charman creek (1 and 2), above the potential upper distribution of salmonids (Reach 3 and mid-stream length in an area where previous reports of trout have been made (Inglis Road).

Results from the duplicate samples using qPCR and 8 technical replicated from each water sample found the presence of eDNA for both coastal cutthroat trout and coho salmon in Reach 1 and 2. This is consistent with observed trout presence during filed work in July 2021 and consistent with previous reported presence of adult coho utilizing lower Charman Creek.

While there presence of trout and coho were found in lower Charman, there was no evidence of salmonid presence above the upstream barrier in Reach 3 near the ToG treatment plant. This is also consistent with earlier reports from works

completed by Fisheries and Oceans Canada (J. Wilson pers com). **Figure 21** shows the current distribution of salmonids in Charman Creek

The results of the lab analysis for the eDNA is attached as **Appendix II**.

Ideally samples would have been collected during the summer but the opportunity to detect coho use was considered greater following the area escapement period for coho.

Streamside Protection and Enhancement Areas

The Streamside Protection and Enhancement Areas (SPEA) are usually delineated following a simple or detailed assessment process outlined by the legislation and methodology guide[4].

The SPEA designation is intended to preserve, protect, restore and enhance fish and wildlife habitat along stream channels. In urban areas this can become challenging. In order to provide guidance and potential expectation for SPEA's along Charman Creek the measured stream channel width, presence of salmonids, presence of flows and the perceived Zones of Sensitivity (ZoS) are used to determine the SPEA width. Exact layout may change following detail review of the area topography and any future development design(s).

The SPEA in this case assumes development along the length of Charman will occur. In that case a detailed assessment would be warranted. In the evn lands are not to be developed, a larger SPEA may result using the simple assessment process. In this case, and as an example for the purposes of illustrating the potential SPEA, we have assumed a detailed assessment would be warranted.

In using the detailed approach the measured bankfull width is required. This measure, used with the channel type was used to determine the possible SPEA for each reach in Charman Creek. **Table IV** provides the possible SPEA using the channel width, channel type and Zones of Sensitivity. **Figure 22** shows the expected SPEA on a watershed map. The 30-m assessment trigger zone is also shown.

Conclusion/Challenges

Initially this project was to focus on existing habitat issues on Charman Creek and attempt to provide areas for stream rehabilitation. The completion of the desktop review and field assessment revealed a number of significant issues and challenges.

As planned works move forward there are a number of potential projects and associated issues that will require solutions. The following list summarizes the

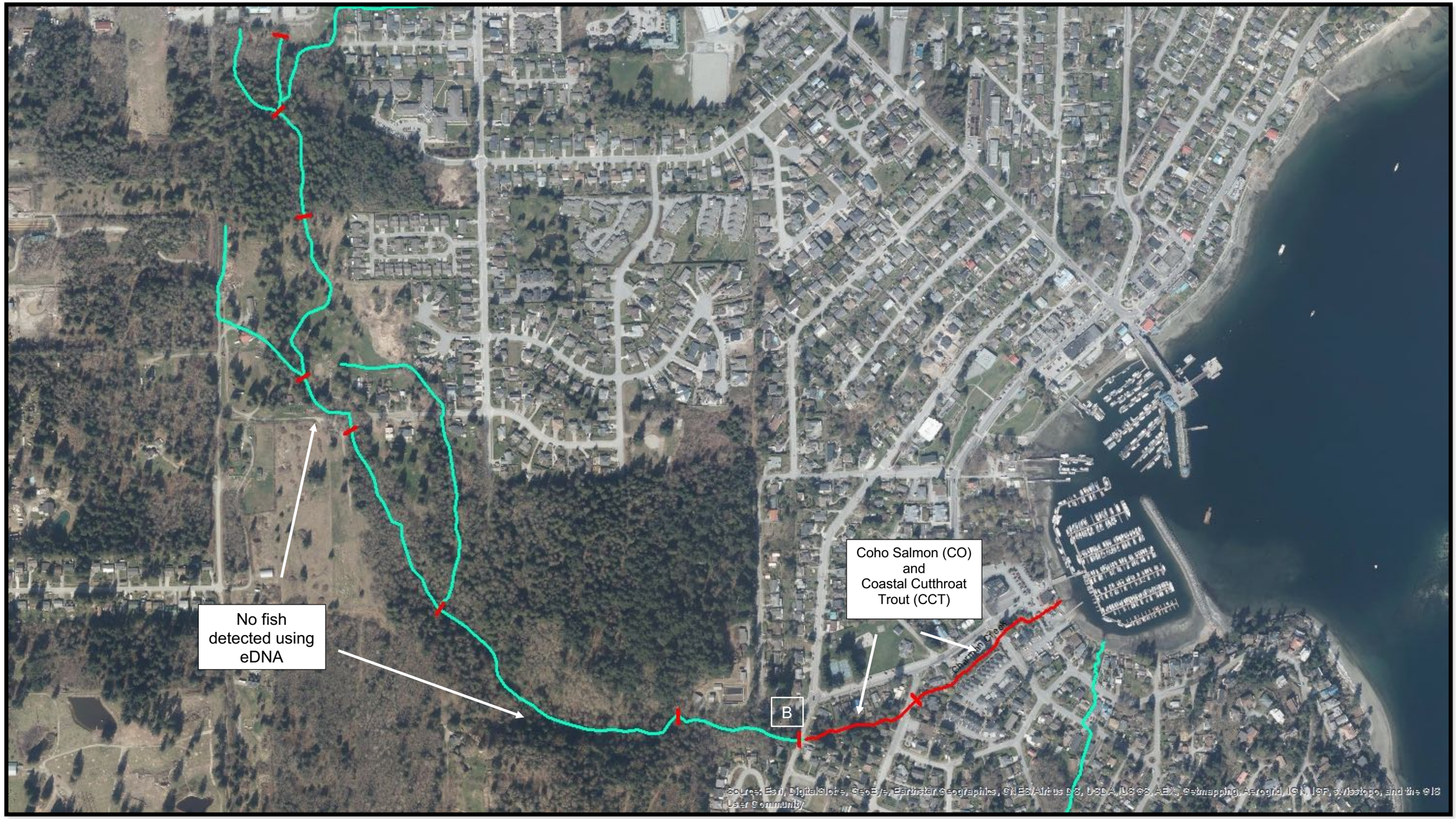


Figure 21: Distribution of salmonids in Charman Creek based on eDNA results and the locations of sample collection for eDNA analysis. The presence of salmonids (CO and CCT) were found in the lower most reaches (1 and 3). The upper extent of distribution has been set at the Gower Point Road culvert (B=barrier).

Table IV: *The possible SPEA setbacks along the reaches in Charman Creek. The final SPEA widths represent 3 x width on reaches comprised of riffle:pool and 2 x width for cascade:pool morphology. Under the RAPR a minimum SPEA width of 10-m applies for reaches contributing to fish habitat. The SPEA presented is provided for planning and would be further refined as development occurred using either the simple of detailed assessment process. In all cases development within 30-m of the channel edge would potentially trigger an assessment.*

Reach	Channel Width (m)**	Channel Type	Zone of Sensitivity			SPEA
			Large Woody Debris/Stability	Litter Fall	Shade	
1	1.34	R/P	4.02	4.02	4.02	10.0
2	1.30	R/P	3.90	3.90	3.60	10.0
3	3.60	C/P	7.20	10.80	10.80	11.0
4	3.70	C/P	7.40	10.40	10.40	11.0
5	3.30	R/P	9.90	9.90	9.90	10.0
6	1.50	R/P	4.50	4.50	4.50	10.0
7	1.50	R/P	4.50	4.50	4.50	10.0
8	1.50	R/P	4.50	4.50	4.50	10.0
9	1.50	R/P	4.50	4.50	4.50	10.0

most important issues/opportunities **and provides comments for consideration in future planning.**

- The greatest bottleneck to long term recovery of Charman Creek is summer and fall base flows. The lack of water and surface connectivity will continue to create challenges in habitat rehabilitation and ultimately salmonid success. Continued water flow monitoring is encouraged in order to determine whether changes in channel structure and vegetative cover could help improve base flows.

- The lower 2 reaches of Charman Creek are important reaches for salmonid survival. These reaches have been channelized, armoured and altered extensively. A planned design that facilitates removal of hardened, restricting structures should be considered. This may not be possible through the entire length, but where the concrete and rock structures fall within the active channel width , removal should be considered.

- There are numerous passage barriers that are all man-made. The culverts at Inglis and Gower Point Road are both a concern. It's unlikely changes here be possible but remediation/retrofit opportunities may exist that would improve passage.

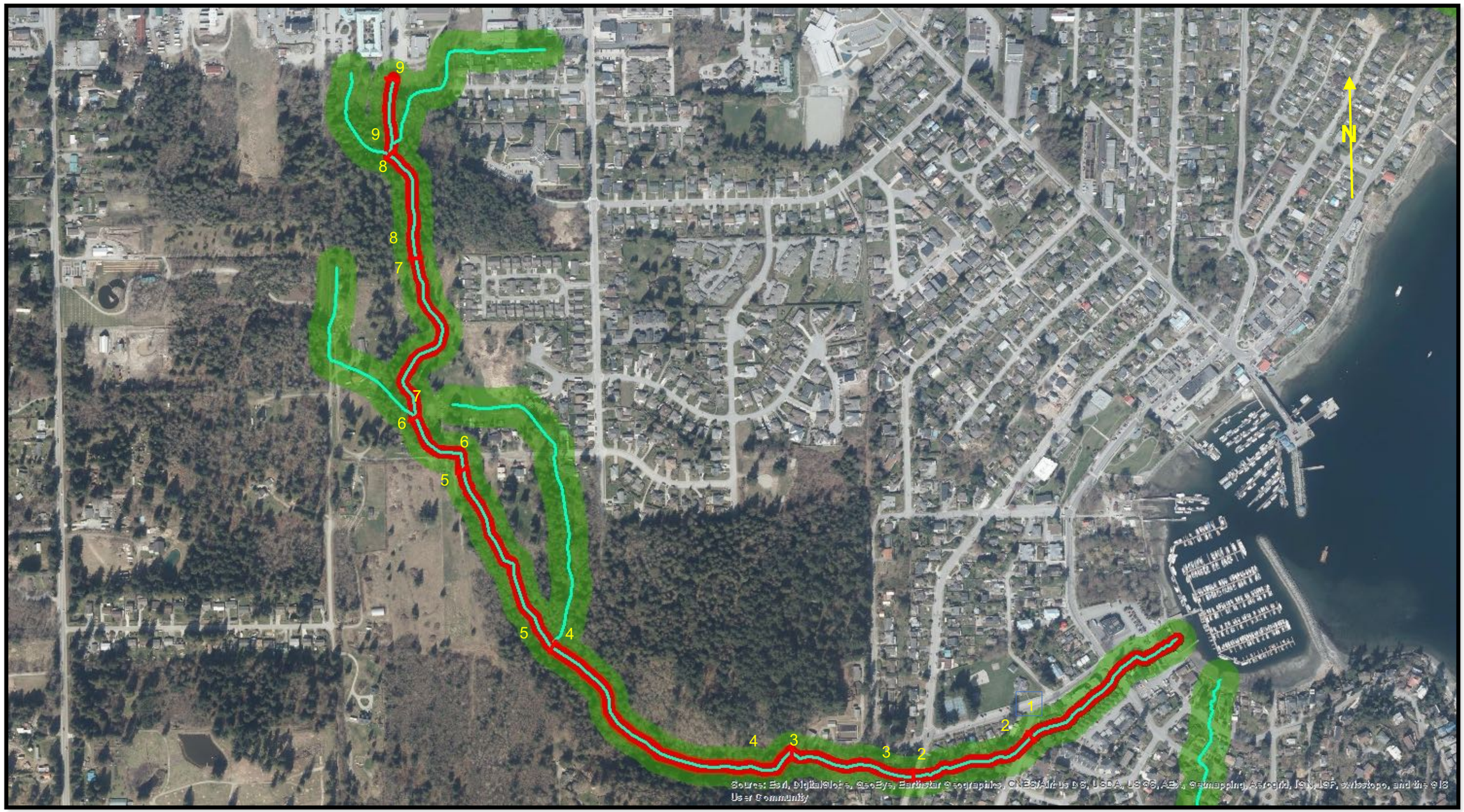


Figure 22: Map of Charman Creek and the possible SPEA setback of 10-m within each reach on the mainstem of the creek. The assessment area (30-m) or trigger zone is shown in green and the possible SPEA in red.

- In Reaches 1 and 2, the riparian area has been extensively altered along the entire stream channel. This is a common issue along urban streams. Education/information through contact with property owners is encouraged. The objective should be to get these owners to work at reverting the riparian vegetation from ornamental and invasive plants to native streamside plantings. This would also help meet the objectives of the RAPR.

- The upper portion of the watershed is non-fish bearing. While it is non fish bearing the RAPR still applies. Conditions in this area was, for the most part good. The challenge in the upper reaches is again the lack of surface water and enforcement of the riparian area regulations that apply to clearing and development.

Closure

This document was prepared by ***FSCI*** Biological Consultants and represents professional judgment based on information available at the time of preparation and appropriate for the scope of the project. This document is for the private information and benefit of the client for whom it was prepared and the specific purpose for which it was developed. Third parties may not use content in this document without the prior written authorization from ***FSCI*** Biological Consultants.

Any use or reliance on this document by third parties is the responsibility of such third parties. ***FSCI*** Biological Consultants and the authors accept no responsibility for damages suffered by any third party as a result of decisions made or action based on this document.

Respectfully

D. Bates, PhD, RPBio
Sr. Biologist

Respectfully

J. Wilson
Sr. Fisheries Technician

Appendix I

Benthic macroinvertebrate data

Town of Gibsons Fresh Water Benthic 20			GM-1	GM-2	GM-3	WT-1	WT-2	WT-3	
All numbers reported are totals									
	Family								
Ephemeroptera									
Ephemeroptera		A							
	Unid J or Damaged	N	1	4					
	Ameletidae	N							
	Baetidae	N	2	5					
	Caenidae	N							
	Ephemerellidae	N		1					
	Heptageniidae	N	2	2	4				
	Leptophlebiidae	N	2	22	5				
Plecoptera									
Plecoptera A		**A**							
	Unid J or Damaged	N							
	Capniidae	N							
	Chloroperlidae	N			1				
	Leuctridae	N							
	Nemouridae	N			1				
	Peltoperlidae	N							
	Perlidae	N							
	Perlodidae	N							
	Pteronarcyidae	N							
	Taeniopterygidae	N							
Trichoptera									
Trichoptera		A							
Trichoptera		P		2					
	Unid J or Damaged	N							
	Apataniidae	N							
	Brachycentridae	N							
	Glossosomatidae	N							
	Hydropsychidae	N							
	Hydroptilidae	N							
	Lepidostomatidae	N							
	Leptoceridae	N							
	Limnephilidae	N	1	3					
	Philopotamidae	N							
	Polycentropodidae	N							
	Rhyacophilidae	N							
	Uenoidae	N							
Coleoptera									
Coleoptera A (terr)		A							
Coleoptera L		L							
Coleptera Unid J		L							
	Carabidae	L							
	Coccinellidae	A							
	Curculionidae	A							
	Dytiscidae	A							

	Dytiscidae		L							
	Elmidae		L							
	Elmidae		A							
	Georissidae		L							
	Haliplidae		L							
	Haliplidae		A							
	Hydrophilidae		L							
	Hydrophilidae		A							
	Scotylidae		A							
	Staphylinidae		L							
	Staphylinidae		A							
Megalopt	Sialidae									
Diptera										
Diptera	Unid J or Damaged		L							
Diptera A			A							
Diptera	P		P							
	Athericidae		L							
	Blephariceridae		L							
	Ceratopogonidae		L							
	Ceratopogonidae		P							
	Chaoboridae		L							
	Chironomidae		A							
	Chironomidae		P		1			1	1	
	Chironomidae		L	4	12	8	22	7	1	
	Deuterophlebiidae		L							
	Dixidae		L							
	Dixidae		P							
	Ephydridae		L							
	Empididae		L							
	Empididae		P							
	Muscidae		L							
	Psychodidae		L							
	Simuliidae		A							
	Simuliidae		L							
	Simuliidae		P							
	Stratiomyidae		L							
	Tabanidae		L							
	Thaumaleidae		L							
	Tipulidae		L		1					
	Tipulidae		A							
Collembola										
	Unid J									
	Hypogastruridae									
	Isotomidae									
	Onychiuridae									
	Sminthuridae									
Hemiptera										
	Unid J									
	Adult									
	Gerridae									
	Corixidae									

Homoptera										
	Unid J or Damaged									
	Adult									
	Aphididae	A								
	Aphididae	N								
	Cicadellidae									
Hymenop	Unid J or Damaged									
	Adult									
	Formicidae									
Lepidoptera										
		A								
		L								
Psocopte	(Terr.)									
Thysanoptera										
Arachnid	terr									
Aranaea	terr									
Mite	terr									
Hydracarina										
	Unid or Damaged									
	Arrenuridae									
	Aturidae									
	Hygrobatidae									
	Feltriidae									
	Lebertiidae									
	Phthiracaridae									
	Oribatidae									
	Oxidae									
	Pionidae									
	Sperchontidae									
	Torrenticolidae									
	Hydryphantidae									
	Unionicolidae									
Amphipoda										
	Unid J									
	Gammaridae									
	Hyalellidae			2	6	11	203	121	229	
Isopoda	Asellidae							4		
Isopoda										
	Assellidae									
	Oniscidea	Terr								
Cladocera										
	Unid J									
	Bosminidae									

	Chydoridae								
	Daphnidae								
	Eurycercinae								
	Polyphemidae								
	Chydoridae								
	Sididae								
Copepoda									
	Calanoida								
	Cyclopoida								
	Harpacticoida								
Ostracoda									
	Unid								
	Candonidae								
	Cyprididae								
	Cypdidodea								
Hirudinea	Unid J								
	Glossiphoniidae								
	Erpobdellidae								
Oligocha	Unid J								
	Enchytraidae		1	4		40	17	6	
	Lumbriculidae								
	Lumbricidae (Terr.)			2					
	Tubificidae			4	5	281	52	45	
	Naididae		1	2		22	1		
Mollusca									
	Acroloxidae								
Bivalva	Unid J			2		8	22	9	
	Pisidiinae		1	1	2	17	44	7	
	Sphaeriidae							1	
Gastropo	Unid J								
	Lymnaeidae								
	Physidae			1					
	Planorbidae					1			
	Valvatidae								
	Ancylidae								
Platyhelminthes									
	Planariidae					3		2	
	Dugesiidae								
	Unid J or Dam								
Hydra									
Nematoda						3			
Odonata									
	Anisoptera	Unid J/D							
	Coengrionidae								
	Gomphidae								

	Corduliidae									
Chilopoda										
Diplopod	terr									
Salmonif	Salmonidae									
Juvenile fish										
Legend										
A=adult										
Unid J = Unidentified Juvenille										
Unid = Unidentified										
L=Larva										
P=pupa										
terr=terrestrial										

Appendix II

Results of eDNA samples

Attention: **Dave Bates**
FSCI Biological Consultants
8-5520 McCourt Road
Sechelt, BC
Canada, V7Z 0K7

Client Project #: **N/A**
Site Location: **Charman Creek**
C.O.C. #: **20211221**
Quote #: **N/A**
PO#: **N/A**

Report Date: **2022/01/06**
Report #: **FS20220106**
Version: **1**

ENVIRONMENTAL DNA - CERTIFICATE OF ANALYSIS

BV JOB #: **E20211221**
Received: **2021/12/21, 10:33 AM**

Sample Type: Cellulose Nitrate (CN) filter, preserved in silica
Samples Received: 6

Analyses (eDNA Isolation - Species)	Test Requested	Test Performed	Date eDNA Extracted	Date Analyzed IntegritE-DNA™	Date Analyzed Target Species	Laboratory Method	Analytical Method (qPCR Primer/Probe set)
eDNA Isolation and IntegritE-DNA™	6	6	2021/12/30	2021/12/31	N/A	GUE SOP-00056	ePlant5
Cutthroat Trout (*Oncorhynchus clarkii*)	6	6	N/A	N/A	2022/01/04	GUE SOP-00056	eONCL4
Coho Salmon (*Oncorhynchus kisutch*)	6	6	N/A	N/A	2022/01/04	GUE SOP-00056	eONKI4

Remarks:

Bureau Veritas Laboratories (Animal DNA Department, DNA Services) is accredited to ISO17025:2017 for eDNA testing.

All work recorded herein has been done in accordance with procedures and practices ordinarily exercised by industry professionals using accepted testing methodologies, quality assurance and quality control procedures (except where otherwise agreed by the client and Bureau Veritas Laboratories in writing). All data has met quality control and method performance criteria unless otherwise noted.

Bureau Veritas Laboratories' liability is limited to the actual cost of the requested analyses, unless otherwise agreed in writing. There is no other warranty expressed or implied. Bureau Veritas Laboratories has been retained to provide analysis of samples provided by the Client using the testing methodology referenced in this report. Interpretation and use of test results are the sole responsibility of the Client and are not within the scope of services provided by Bureau Veritas Laboratories unless otherwise agreed in writing. Bureau Veritas Laboratories is not responsible for the accuracy or any data impacts that result from the information provided by the customer or their agent.

Results relate to supplied samples tested. This Certificate should not be reproduced except in full, without the written approval of the laboratory.

eDNA tests are used to confirm presence of eDNA in samples for the targeted species / species groups.

Collected eDNA samples will contain eDNA at various stages of degradation, being subject to environmental forces that breakdown DNA, including microbial activity, ultraviolet radiation, heat, hydrolysis, and enzymatic activity. eDNA is first evaluated for eDNA quality and presence of qPCR assay inhibitors using the IntegritE-DNA™ assay before testing for target species or genera to confirm that the eDNA is of sufficient quality for testing and to identify and address qPCR inhibition (if present) to avoid false negatives.

SAMPLE RETENTION: Samples and DNA extracts generated from the samples will be retained by Bureau Veritas Laboratories for a period of 90 days after which time they will be discarded unless prearrangement has been made by client with Bureau Veritas Laboratories for longer storage.

Attention: **Dave Bates**
FSCI Biological Consultants
8-5520 McCourt Road
Sechelt, BC
Canada, V7Z 0K7

Client Project #: **N/A**
Site Location: **Charman Creek**
C.O.C. #: **20211221**
Quote #: **N/A**
PO#: **N/A**

Report Date: **2022/01/06**
Report #: **FS20220106**
Version: **1**

ENVIRONMENTAL DNA - CERTIFICATE OF ANALYSIS

BV JOB #: **E20211221**
Received: **2021/12/21, 10:33 AM**

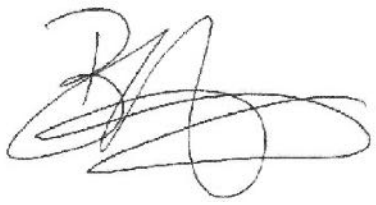
Methodology for Sample Analysis

Samples received to the laboratory are entered into the Laboratory Information Management System (LIMS) upon receipt. Samples were inspected and assessed for amount of silica beads, silica bead saturation level, coin envelope condition and number of coin envelopes in each bag. Samples were stored in freezer until processing in the laboratory. Sample analysis is completed within 10 or 15 business days (as indicated by the client on the COC) following receipt of samples by the testing laboratory.

eDNA isolation is completed using the DNeasy Blood & Tissue Kit™ (QIAGEN). A negative control is included as a blank filter sample with each batch of eDNA isolation to monitor for potential laboratory contamination during the eDNA isolation process.

Following eDNA isolation (150µL) from a quarter of filter, the IntegritE-DNA™ assay[1] is used to avoid the potential of a false negative (Type II error) during target species or genera testing. The IntegritE-DNA™ assay evaluates the integrity of eDNA for suitability for qPCR and for presence of qPCR inhibitors which may reduce the effectiveness of the qPCR assay for target species or genera. This assay evaluates the quality of eDNA to assess whether it is amplifiable using a qPCR assay that targets the chloroplast genome derived from plants/algae that are ubiquitously found in fresh water systems. Four technical replicates per eDNA sample, four technical replicates of negative control (Ultrapure water), and two technical replicates of positive control are used for the IntegritE-DNA™ assay. The cut-off Ct (qPCR cycle threshold) value for the IntegritE-DNA™ assay is 27 due to inhibition. If the IntegritE-DNA™ assay produces a positive detection frequency of ≥ 2 of the 4 technical replicates, this indicates that the eDNA for the target taxa is likely to be of sufficient quality to be detected (if present) with the target assay. If the IntegritE-DNA™ assay produces a positive detection frequency < 2 of the 4 technical replicates (eDNA is degraded or qPCR inhibitors are present), then sample cleanup is completed using the OneStep PCR Inhibitor Removal Kit™ (ZYMO Research) to remove potential qPCR assay inhibitors from the isolated eDNA. Subsequent to inhibitor removal, the IntegritE-DNA™ assay is repeated to re-assess whether the eDNA is of sufficient quality for qPCR. If a sample fails at the IntegritE-DNA™ assay (Ct Value over 30) for the second time the client will be informed that the quality of the sample is insufficient for the qPCR assay. eDNA indicator (IntegritE-DNA™) in the sample suggests that degradation has taken place and therefore the target species assay may be ineffective. Once a sample passes the IntegritE-DNA™ assay, then the target species or genera assay is performed. Eight technical replicates per eDNA sample, eight technical replicates of the negative control (Ultrapure water), and two technical replicates of positive control (total DNA or synthetic DNA) are used for the target species or genera assay to assess the detection or non-detection of DNA of the target species or genera. The cut-off Ct value for target species assay is 50.

[1] Hobbs J, Round JM, Allison MJ, Helbing CC (2019) Expansion of the known distribution of the coastal tailed frog, *Ascaphus truei* , in British Columbia, Canada, using robust eDNA detection methods. PLOS ONE 14(3): e0213849.



BECKY HENDERSON
Senior Customer Service Representative, Bureau Veritas Laboratories, DNA Services
Email: becky-a.henderson@bureauveritas.com
Phone #: (519) 836 2400 Ext. 7067714

Please direct all questions regarding this Certificate of Analysis to your Customer Service Representative above.
==========
For Service Group specific validation please refer to the Validation Signature Page.

Total Cover Pages: 2

BV JOB #: E20211221
Report Date: 2022/01/06
Report #: FS20220106

Client Name: FSCI Biological Consultants
Client Project #: N/A
Site Location: Charman Creek
Sampler Initials: JW

RESULTS

Client Sample ID	BV Case ID	Sampling Date	Preservation Type	IntegritE-DNA™ Positive detection (Ct≤27)[1]	QC Batch	Cleanup required	IntegritE-DNA™ Positive detection (Ct≤30)[1] after cleanup	QC Batch	Analytical Method (qPCR Primer/Probe set)	Target Species eDNA Positive detection (Ct≤50)[2]	QC Batch
CHAR-01	FS202100001	2021/12/16	Silica	4/4	211231Q5	No	N/A	N/A	eONCL4[3]	1/8	220104Q1
CHAR-02	FS202100002	2021/12/16	Silica	4/4	211231Q5	No	N/A	N/A	eONCL4	1/8	220104Q1
CHAR-03	FS202100003	2021/12/16	Silica	4/4	211231Q5	No	N/A	N/A	eONCL4	0/8	220104Q1
CHAR-04	FS202100004	2021/12/16	Silica	4/4	211231Q5	No	N/A	N/A	eONCL4	0/8	220104Q1
CHAR-05	FS202100005	2021/12/16	Silica	4/4	211231Q5	No	N/A	N/A	eONCL4	0/8	220104Q1
CHAR-06	FS202100006	2021/12/16	Silica	4/4	211231Q5	No	N/A	N/A	eONCL4	0/8	220104Q1
CHAR-01	FS202100001	2021/12/16	Silica	4/4	211231Q5	No	N/A	N/A	eONKI4[4]	2/8	220104Q2
CHAR-02	FS202100002	2021/12/16	Silica	4/4	211231Q5	No	N/A	N/A	eONKI4	4/8	220104Q2
CHAR-03	FS202100003	2021/12/16	Silica	4/4	211231Q5	No	N/A	N/A	eONKI4	0/8	220104Q2
CHAR-04	FS202100004	2021/12/16	Silica	4/4	211231Q5	No	N/A	N/A	eONKI4	0/8	220104Q2
CHAR-05	FS202100005	2021/12/16	Silica	4/4	211231Q5	No	N/A	N/A	eONKI4	0/8	220104Q2
CHAR-06	FS202100006	2021/12/16	Silica	4/4	211231Q5	No	N/A	N/A	eONKI4	1/8	220104Q2

[1] **IntegritE-DNA™ Assay:** Four technical replicates were assayed for each eDNA sample. The cut-off Ct value for IntegritE-DNA™ assay was 27 and 30 after clean-up. Results are reported as the number of positive detections (n) out of a total of 4 technical replicates, n/4.

[2] **Target Species Assay:** Eight technical replicates were assayed per eDNA sample. The cut-off Ct value for target species assay was 50. Results are reported as the number of positive detections (n) out of a total of 8 technical replicates, n/8.

[3] eONCL4: qPCR primer/probe assay to assess the presence of Cutthroat Trout (*Oncorhynchus clarkii*) eDNA

[4] eONKI4: qPCR primer/probe assay to assess the presence of Coho Salmon (*Oncorhynchus kisutch*) eDNA

GENERAL COMMENTS

eDNA is extracted (150 µL) from a quarter of filter, and 2 µL is used as a template for each technical replicate.

Results relate only to the items tested.

QUALITY ASSURANCE REPORT

			eDNA Isolation Negative Control[1]		qPCR Positive Controls[2]		qPCR Negative Controls[3]	
QC Batch	Parameter	Date	Detection at: Ct 27 (IntegritE-DNA™) Ct 50 (other assays)	Pass/Fail	Detection at: Ct 27 (IntegritE-DNA™) Ct 50 (other assays)	Pass/Fail	Detection at: Ct 27 (IntegritE-DNA™) Ct 50 (other assays)	Pass/Fail
211231Q5	IntegritE-DNA	2021/12/31	0 of 4 technical replicates	Pass	2 of 2 technical replicates	Pass	0 of 4 technical replicates	Pass
220104Q1	eONCL4	2022/01/04	eDNA Isolation Negative Control is assessed using IntegritE-DNA only once for each extraction batch.	N/A	2 of 2 technical replicates	Pass	0 of 8 technical replicates	Pass
220104Q2	eONKI4	2022/01/04			1 of 2 technical replicates	Pass[4]	0 of 8 technical replicates	Pass

[1] **eDNA Isolation Negative Control:** Blank filters were included for each batch of eDNA extraction to monitor for laboratory contamination during eDNA isolation. eDNA Isolation Negative Control is assessed using IntegritE-DNA™ only. QC results show no eDNA was isolated from the negative control, therefore there was no indication of sample contamination during handling. Acceptance criteria: 0 of 4 technical replicates

[2] **qPCR Positive Controls:** Two technical replicates of isolated eDNA from freshwater sample were used as positive controls for IntegritE-DNA™. Two technical replicates of total DNA or synthetic DNA from the target species were used as positive controls for eDNA assays. Results show that 100% of the technical replicates amplified the positive control eDNA as expected, therefore an observation of negative result in eDNA samples is not related to the qPCR performance. Acceptance criteria: 2 of 2 technical replicates

[3] **qPCR Negative Controls (Ultrapure water):** Four technical replicates for IntegritE-DNA™ and eight technical replicates for target species or genera were used to monitor for laboratory contamination. Results show that 0% of the technical replicates in the negative controls had amplified eDNA, indicating no contamination was detected. Acceptance criteria: 0 of 4 technical replicates for IntegritE-DNA™, and 0 of 8 technical replicates for other assays.

[4] The ONKI qPCR positive control result (1 out of 2) is approved based on the positive detection on field samples on batch 220104Q2.

Unit 2 - 335 Laird Road
Guelph, ON N1G 4P7

Phone: (519) 836-2400
Toll Free: (877) 706-7678
Fax: (519) 836-4218
www.bvlabs.com

BV JOB #: E20211221
Report Date: 2022/01/06
Report #: FS20220106

Client Name: FSCI Biological Consultants
Client Project #: N/A
Site Location: Charman Creek
Sampler Initials: JW

LABORATORY RESULTS VALIDATION SIGNATURE PAGE

The analytical data and all QC contained in this report were reviewed and validated by the following individual(s).

Reporter: ALI MIRABZADEH, M.Sc.
Scientific Specialist, Bureau Veritas Laboratories, DNA Services

==========

Reviewer: HEATHER ALLEN, M.Sc.
Supervisor, Bureau Veritas Laboratories, DNA Services

==========

BV JOB #: E20211221
Report Date: 2022/01/06
Report #: FS20220106

Client Name: FSCI Biological Consultants
Client Project #: N/A
Site Location: Charman Creek
Sampler Initials: JW

Cutthroat Trout (*Oncorhynchus clarkii*) Assay Validation Information

eDNA assay Validation

All eDNA assays are validated through a rigorous multi-step evaluation protocol that includes tests of DNA target specificity and amplification sensitivity. All eDNA tests available at Bureau Veritas Laboratories have been validated for performance using interlaboratory verification.

General eDNA Assay Information

Target Species: Cutthroat Trout (*Oncorhynchus clarkii*)
Species Code: te-ONCL
eDNA qPCR Tool: eONCL4
eDNA qPCR Format: TaqMan
Gene Target: MT-ND1
Published in: N/A

eDNA Assay Sensitivity Test using gBlocks™ synthetic DNA

LOD **0.5** 95% CI **0.3-1.1** Copies LOQ **2** 95% CI **1.3-4.3** Copies
LOB **0** hits/8

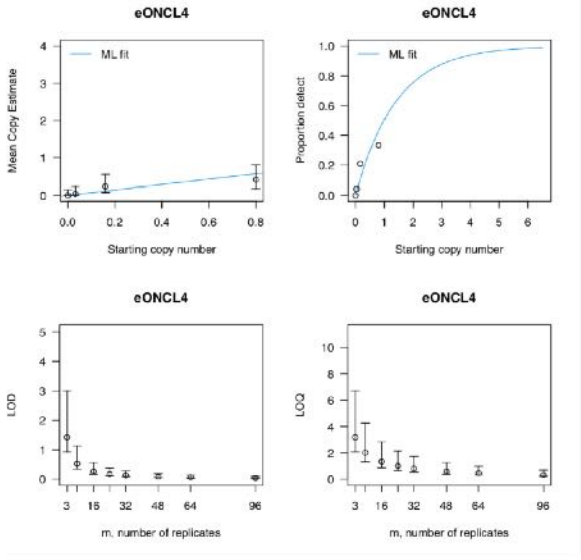
Binomial-Poisson model for 8 technical replicates
Determined using eLowQuant R code[4].

eDNA Assay Specificity Test Information

Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA (n=25 technical replicates)

Species	Common Name (Species)	Detection	Specimens	Sample Sources/Locations
ma-HOSA	Human (*Homo Sapiens*)	No	1	Netherlands
te-ONCLcl	Coastal Cutthroat Trout (*Oncorhynchus clarkii*)	Yes	5	British Columbia
te-ONCLle	Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*)	Yes	9	Alberta
te-ONGO	Pink Salmon (*Oncorhynchus gorbuscha*)	No	1	British Columbia
te-ONKE	Chum Salmon (*Oncorhynchus keta*)	No	1	British Columbia
te-ONKI	Coho Salmon (*Oncorhynchus kisutch*)	No	1	British Columbia
te-ONMY	Rainbow Trout (*Oncorhynchus mykiss*)	No	6	Alberta and British Columbia
te-ONNE	Sockeye Salmon (*Oncorhynchus nerka*)	No	1	British Columbia
te-ONTS	Chinook Salmon (*Oncorhynchus tshawytscha*)	No	1	British Columbia
te-SACO	Bull Trout (*Salvelinus confluentus*)	No	4	Alberta
te-SAFO	Brook Trout (*Salvelinus fontinalis*)	No	4	Alberta
te-SAMA	Dolly Varden (*Salvelinus malma*)	No	1	Alberta
te-SASA	Atlantic Salmon (*Salmo salar*)	No	1	Nova Scotia

eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



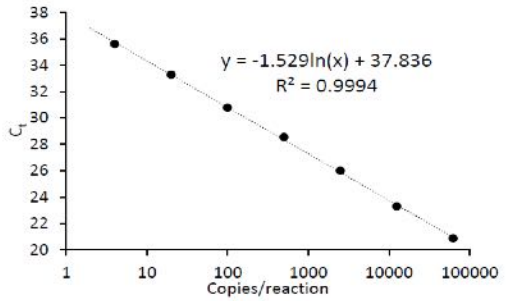
Binomial-Poisson model: No intercept
Determined using eLowQuant R code[4].
Based on a 2 µL DNA input in a total 15 µL reaction

From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.19	0.2
2	0.41	0.31
3	0.67	0.43
4	0.98	0.57
5	1.39	0.75
6	1.97	1.02
7	2.95	1.55

Determined using eLowQuant R code[4].

Applied to reactions with 100% positive hits



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Field Sample Validation

Sample Type	Known presence	# Samples	Detected	Location
Water	Y	18	Y	Southwest Alberta
Water	N	25	Y	Southwest Alberta

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-ND1	Mitochondrial NADH dehydrogenase 1 gene
gDNA	Total genomic DNA extracted from voucher specimen	NTC	qPCR no template control
LOB	Limit of blank	qPCR	Quantitative real-time polymerase chain reaction
LOD	Limit of detection	SE	Standard error

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BV JOB #: E20211221
Report Date: 2022/01/06
Report #: FS20220106

Client Name: FSCI Biological Consultants
Client Project #: N/A
Site Location: Charman Creek
Sampler Initials: JW

Coho Salmon *(Oncorhynchus kisutch)* eDNA Assay Validation Information

eDNA assay Validation

All eDNA assays are validated through a rigorous multi-step evaluation protocol that includes tests of DNA target specificity and amplification sensitivity. All eDNA tests available at Bureau Veritas Laboratories have been validated for performance using interlaboratory verification.

General eDNA Assay Information

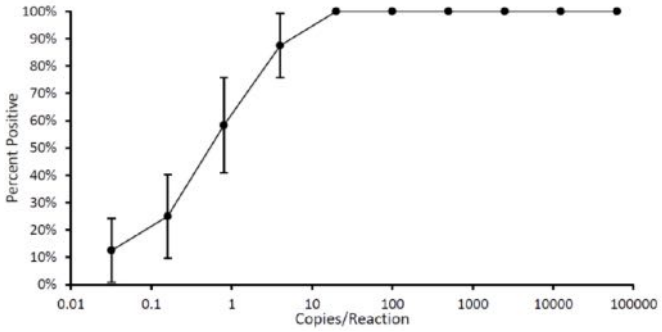
Target Species	Coho Salmon *(Oncorhynchus kisutch)*	eDNA qPCR Primer/Probe	eONKI4
Species Abbreviation	ONKI	eDNA qPCR Format	TaqMan

eDNA Assay Specificity Tests

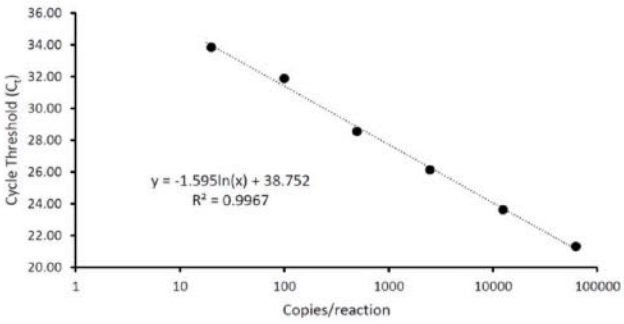
qPCR Activity: Multi-species analysis of eDNA assay efficiency. Multiple qPCR reactions (n=25) performed per target DNA.

Species:	ONTS	ONKI	ONNE	ONGO	ONKE	ONMY	ONCL	THAR	LICA	HOSA	NTC
Detection:	No	Yes	No	No	No	No	No	No	No	No	No

eDNA Assay Sensitivity Test using gBlocks™ synthetic DNA



>100 copies/reaction were tested with n=8 technical replicates.
≤100 copies/reaction were tested with n=24 technical replicates.



The relationship between Cycle Threshold and Copy Number does not necessarily remain linear when fewer than 100% of technical replicates are positive.

Abbreviations

eDNA	environmental DNA
gDNA	Total Genomic DNA extracted from voucher specimen tissue or swabs
HOSA	Human (*Homo sapiens*)
LICA	Bullfrog (*Lithobates (Rana) catesbeiana*)
NTC	qPCR no template control
ONCL	Cutthroat Trout (*Oncorhynchus clarkii*)
ONGO	Pink Salmon (*Oncorhynchus gorbuscha*)
ONKE	Chum Salmon (*Oncorhynchus keta*)
ONKI	Coho Salmon (*Oncorhynchus kisutch*)
ONMY	Rainbow Trout (*Oncorhynchus mykiss*)
ONNE	Sockeye Salmon (*Oncorhynchus nerka*)
ONTS	Chinook Salmon(*Oncorhynchus tshawytscha*)
qPCR	quantitative real-time polymerase chain reaction
THAR	Arctic Grayling (*Thymallus arcticus*)

References

1. Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, Ascaphus montanus, in British Columbia, Canada, using environmental DNA methods. Environmental DNA. 2020; 00: 1– 12. https://doi.org/10.1002/edn3.82
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