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VIA E-MAIL

November 15, 2018
File No.: 144930086

Matthew Hamp, Director of Engineering and Sustainability
City of Iqaluit
Building, 901, P.O. Box 460
Iqaluit, NU X0A 0H0

Attention: **Matthew Hamp, Director of Engineering and Sustainability**

Dear Mr. Hamp:

Reference: **Environmental DNA Sampling Program for Arctic Char in the Lake Geraldine Reservoir, Iqaluit, NU**

1 INTRODUCTION

The City of Iqaluit (the City) is currently planning a drinking water supplementation project whereby water from a nearby source would be used to supplement the City's current drinking water supply from the Lake Geraldine reservoir. Since Lake Geraldine will receive supplemental water from a secondary source, outside its watershed, Fisheries and Oceans Canada (DFO) expressed concern about the potential impact of changing water levels, and potentially introduced species, on fish and fish habitat in Lake Geraldine. In August 2017, Nunami Stantec Ltd. (Nunami) was retained by the City to conduct an environmental DNA (eDNA) sampling program at the Lake Geraldine reservoir to provide additional evidence to assess fish presence within the reservoir. This letter report provides a summary of the 2017 Lake Geraldine eDNA sampling program for Arctic char (*Salvelinus alpinus*), the results and conclusions.

1.1 Background

The Lake Geraldine reservoir impoundment was initially constructed in 1958 by the Department of National Defence when Iqaluit was a hub for DEW Line[1] construction operations (Concentric 2014). The impoundment has been raised several times since its initial construction (Concentric 2014) however fish and fish habitat studies have not been completed previously.

[1] DEW Line refers to the Distant Early Warning Line radar system that was constructed in the 1940s and 1950s across the Canadian Arctic

Reference: Environmental DNA Sampling Program for Arctic Char in the Lake Geraldine Reservoir, Iqaluit, NU

In July 2016, Nunami was initially retained by the City to complete fish and fish habitat assessments in three Iqaluit waterbodies, including the Lake Geraldine reservoir. Nunami conducted a fish capture program at Lake Geraldine in early September 2016 and used a variety of methods to identify potential fish presence; these included angling (2 hours), minnow trapping (57 hours), fyke netting (19 hours), and multi-mesh gillnetting (3 hours) (Nunami 2017). No fish were captured during the field program but fishing effort, at the time, was limited due to high winds that restricted safe boat access to the lake (Nunami 2017). Collected bathymetric (Budkewitsch et al. 2011) and habitat (Nunami 2017) data for Lake Geraldine (e.g., maximum depth 12 m, with up to 93% of the lake less than 8 m) suggests it could support Arctic char and stickleback species but anecdotal evidence, from water treatment plant operators and community members, suggested that fish are not present in Lake Geraldine (e.g., fish have never been caught in the unscreened drinking water intake over the past 30 years). Actual sampling effort (as completed in September 2016) was considered to be insufficient and therefore did not provide conclusive evidence to assess fish presence. As noted, DFO has expressed concern about the potential effects that drinking water withdrawals could have on fish and fish habitat in Lake Geraldine and in secondary sources used to supplement Lake Geraldine.

Therefore, to provide additional evidence to assess fish presence in Lake Geraldine, Nunami conducted an eDNA sampling program. The use of eDNA was proposed as an additional protocol to further assess the potential presence of Arctic char in Lake Geraldine. Environmental DNA (eDNA) is DNA shed by organisms into the environment through skin cells, mucus cells, gamete (eggs and sperm) production, and feces. If Arctic char eDNA was detected in Lake Geraldine, it could be used to infer its presence in the aquatic system.

2 METHODS

2.1 Sampling Sites

Arctic char is native to Arctic and subarctic coastal waters and has the most northerly distribution of any freshwater fish. There are both resident freshwater populations and anadromous populations that spawn in freshwater. Arctic char spawns in September or October over gravel beds and over rocky shoals in lakes with heavy wave action (DFO 2014). In lake habitats, adult Arctic char is known to spawn in water approximately 1 to 11 m deep (Richardson et al. 2001), redds may be found in water 3 to 6 m deep, and Arctic char can be found in water up to 70 m deep. This understanding of Arctic char life cycle and habitat use was used in the study design to optimize the collection of Arctic char eDNA.

Arctic char eDNA samples were collected from habitats where, and at a time when, this species should be if present in the habitat. Arctic char eDNA samples were collected by Nunami personnel, trained in water sampling, between September 27 to 29, 2017, during the spawning season (which is from September to October; Richardson et al. 2001). Eleven samples were collected from Lake Geraldine in habitats that

would be suitable for spawning, rearing, and deep-water refuge, if the species is present, and from tributaries entering the lake (see Figure 1 in Appendix A). The following was completed:

- When a tributary did not have sufficient flow to collect a sample, a sample was collected in Lake Geraldine, immediately below the stream discharge site or as near as possible given shoreline conditions.
- A deep-water sample was collected at the drinking water treatment plant, from the raw water sample port. The raw water sample port is on the raw water intake line that collects drinking water from the intake, which is at an estimated 9.7 m depth[2] in the reservoir.
- A positive control field sample was collected from the Sylvia Grinnell River where Arctic char is known to occur and therefore eDNA presence should be confirmed.
- Two negative field controls were included: a field blank and a filtering blank. Negative field controls are used to assess for potential contamination (i.e., eDNA introduction) during sample collection, handling, and filtration, to verify the integrity of clean sampling and filtering protocols. For this program, negative field controls included treated tap water collected from the City's water treatment plant and from Nunami's Iqaluit office.
    - As noted, potable water for Iqaluit does come from the Lake Geraldine reservoir, which was the sampled waterbody in this study. However, potable water is disinfected with ultraviolet light, filtered, and chlorinated at the City's water treatment plant prior to distribution to the community. The use of UV light and chlorination are very common practices for disinfection of drinking water as they damage or destroy residual microorganisms and DNA in the water. Given the treatment process, treated tap water was considered acceptable for use as negative controls in this study but, pending results, this may be a potential uncertainty.

Sampling sites in Lake Geraldine are summarized in Table 1 while photos of each site are provided in Appendix B.

[2] In the Lake Geraldine reservoir, the intake elevation is estimated to be at 101.6 m above sea-level (mASL) while the reservoir spillway is at 111.3 mASL (Golder 2013).

Reference: Environmental DNA Sampling Program for Arctic Char in the Lake Geraldine Reservoir, Iqaluit, NU

Table 1: Environmental DNA Sampling Sites in September 2017; Iqaluit, NU

Sample Site	Location	Sample Date	Site Coordinates		Sample Depth (m)	Comments
			Latitude (N)	Longitude (W)		
L1	Lake Geraldine	28-Sept-2017	63º 45.353'	68º 29.765'	0.60	Sampled directly in Lake Geraldine.
L2	Lake Geraldine	28-Sept-2017	63º 45.650'	68º 30.108'	0.60	Sampled directly in Lake Geraldine.
L3	Lake Geraldine	28-Sept-2017	63º 45.714'	68º 30.309'	0.60	Sampled directly in Lake Geraldine.
L4	Water Treatment Plant (raw water sample port)	29-Sept-2017	N/A	N/A	9.7	Sampled from the raw water sample port in the Water Treatment Plant.
L5	Lake Geraldine	28-Sept-2017	63º 45.405'	68º 30.307'	0.60	Sampled directly in Lake Geraldine, on the Lake Geraldine side of the impoundment/spillway.
L6	Lake Geraldine	28-Sept-2017	63º 45.405'	68º 30.307'	1.50	Sampled directly in Lake Geraldine, on the Lake Geraldine side of the impoundment/spillway.
L7	Lake Geraldine	28-Sept-2017	63º 45.625'	68º 30.059'	1.50	Sampled directly in Lake Geraldine.
L8	Lake Geraldine Tributary	28-Sept-2017	63º 45.826'	68º 30.672'	0.60	Sample collected in the active inflow stream.
L9	Lake Geraldine	28-Sept-2017	63º 45.764'	68º 30.381'	0.60	Not possible to capture water in the inflow stream, so sampled in Lake Geraldine at discharge location for this tributary to capture influence of inflow stream.

Reference: Environmental DNA Sampling Program for Arctic Char in the Lake Geraldine Reservoir, Iqaluit, NU

Table 1: Environmental DNA Sampling Sites in September 2017; Iqaluit, NU

Sample Site	Location	Sample Date	Site Coordinates		Sample Depth (m)	Comments
			Latitude (N)	Longitude (W)		
L10	Lake Geraldine	27-Sept-2017	63º 45.312'	68º 29.428'	0.60	Not possible to capture water in the inflow stream, so sampled in Lake Geraldine at discharge location for this tributary to capture influence of inflow stream.
L11	Lake Geraldine	27-Sept-2017	63º 45.220'	68º 29.251'	0.60	Not possible to capture water in the inflow stream, so sampled in Lake Geraldine at discharge location for this tributary to capture influence of inflow stream.
L12a	Treated Tap Water (negative control)	28-Sept-2017	N/A	N/A	N/A	Chlorinated tap water collected from City's drinking water supply system; used to assess potential sample contamination and verify integrity of clean sampling and filtration procedures
L12b		28-Sept-2017	N/A	N/A	N/A	Chlorinated tap water collected from City's drinking water supply system; used to assess potential sample contamination and verify integrity of clean sampling and filtration procedures
L13	Sylvia Grinnell River (positive control)	28-Sept-2017	63º 45.884', W	68º 34.841'	0.60	At location identified for holding Arctic char

Reference: Environmental DNA Sampling Program for Arctic Char in the Lake Geraldine Reservoir, Iqaluit, NU

Table 1: Environmental DNA Sampling Sites in September 2017; Iqaluit, NU

Sample Site	Location	Sample Date	Site Coordinates		Sample Depth (m)	Comments
			Latitude (N)	Longitude (W)		
Notes: N/A = not applicable						

2.2 Sampling Methods

The eDNA sampling methods used for the Lake Geraldine assessment were based on eDNA sampling protocols from British Columbia (Hobbs et al. 2017) and United States Geological Survey (Matthew et al. 2012).

Field personnel collected one two-litre (2 L) composite water sample at each sample site. The bottles were new and composed of high-density polyethylene (HDPE). Bottles were labeled with the site identification, collection date and time, UTM coordinates, and collector identification. Samples were collected using a sample bottle extended on a pole or a Van Dorn water sampler, which were cleaned with a bleach solution before sampling and between sample sites. When collecting a sample, field personnel wore clean nitrile gloves, triple-rinsed the bottle and Van Dorn sampler with site water, and did not enter the water. The eDNA samples were placed in a cooler with ice to keep cool until filtration.

The samples were filtered in the City's Water Treatment Plant laboratory within 24 hours of sample collection to reduce the likelihood of eDNA degradation in the sample. Each sample was filtered through a new 47 mm GF/C (1.2 µm) filter using a peristaltic pump. Filters were obtained from the eDNA analytical laboratory and were specifically for eDNA sample collection. During filtration, personnel wore clean nitrile gloves for each sample and non-disposable filtration equipment (e.g., filter flask, funnel and collar, forceps) was cleaned with a bleach solution before filtration and between samples. Filtration tubing was replaced between samples. Following filtration, the filter was removed while wearing clean nitrile gloves and using tweezers sterilized in a 50% bleach solution. The filter was placed in a pre-labeled sterile polypropylene vial and frozen.

The frozen filters were shipped on ice from Iqaluit to the Institut de Biologie Intégrative et des Systèmes at the Université Laval, in Québec City, QC, for eDNA analysis in the laboratory of Dr. Louis Bernatchez. Dr. Bernatchez holds the Canadian Research Chair for Genomics and Conservation of Aquatic Resources and has conducted eDNA studies on Arctic char.

2.2.1 Quality Assurance/Quality Control

Quality assurance/quality control (QA/QC) protocols for the eDNA sampling program included:

- Use of a protocol for sampling
- Decontamination of sampling equipment, using a 50% bleach solution, between sample sites and between sample filtrations
- Avoidance of contact with site water other than with clean gloves and supplies
- Use of field and filtration blank samples as negative controls
- Collection of the positive field control sample after other samples to prevent cross-contamination

- Filtration of samples in the same order they were collected in the field
- Use of field and filtration blank samples (L12a, L12b) as negative controls
- Use of chain of custody forms to track sample collection, filtering, and laboratory analysis.

2.3 Sample Analysis

The sample eDNA extraction and analysis was performed at the laboratory of Dr. Louis Bernatchez in the Institut de Biologie Intégrative et des Systèmes at the Université Laval, in Québec, QC. Appendix C provides a laboratory report that describes the methods used to extract eDNA from the sample filters, analyze the extracts for the presence of Arctic char eDNA, results of analysis, and their QA/QC measures applied. A summary of the laboratory methods and results are provided herein and sample analysis included the following.

1. Samples were submitted “blind” to the laboratory in that sample source was not indicated and only sample identifier was used.
2. The eDNA samples were analyzed on arrival at the laboratory for quality, in terms of the presence of viable DNA, using a bioanalyzer high-sensitivity DNA assay chip.
3. Primers and probes used to test for Arctic char eDNA were designed be highly specific such that they amplified the eDNA of Arctic char and not close relatives, such as rainbow trout (*Oncorhynchus mykiss*), brook charr (*Salvelinus fontinalis*), Atlantic salmon (*Salmo salar*), and brown trout (*Salmo trutta*).
4. The presence of Arctic char eDNA was tested using quantitative PCR (qPCR) in six replicates for each collected eDNA sample including the negative and positive controls. A negative extraction control was used as an additional QA/QC measure to account for potential contamination (i.e., eDNA introduction) in the lab. An eDNA detection threshold was pre-determined to be 40 qPCR amplification cycles (40 C_T). Therefore, any positive signals achieved after 40 C_T were considered potential artefacts of sample analysis and were not considered positive for eDNA detection.

3 RESULTS

Between September 27 and 29, 2017, 14 eDNA samples were collected to assess the presence of Arctic char in Lake Geraldine. Eleven of the 14 samples were from Lake Geraldine and tributaries, two samples were negative field and filtering control samples, and one sample was a positive control sample from the Sylvia Grinnell River where Arctic char are known to occur.

Analysis of the QA/QC samples confirmed the presence of Arctic char eDNA in the positive field control sample from the Sylvia Grinnell River (L13), no Arctic char eDNA in the two negative control samples (L12a, L12b) of treated tap water. These results were in line with expectations, providing confidence in

application of sampling methods and in interpreting results for the test samples from Lake Geraldine and its tributaries.

Arctic char eDNA was not detected in the samples from Lake Geraldine and its tributaries. Table 2 provides a summary of the qPCR results for the six replicates analyzed for each eDNA sample. As noted, positive amplifications, where the number of cycles were above the established detection threshold (40 C_T), are not considered positive results. Table 3 summarizes the results for the eDNA sample from the Sylvia Grinnell River in which Arctic char eDNA was detected in two of the six replicate subsamples.

Table 2: qPCR Results obtained from 14 samples analyzed for Arctic char eDNA, Iqaluit, NU; September 2017[a]

Sample ID	Location	Replicate No.	C_T	Number of DNA Molecules	Interpretation of Result for Presence of Arctic char eDNA
L1	Lake Geraldine	1	—	—	Negative
		2	—	—	Negative
		3	—	—	Negative
		4	—	—	Negative
		5	—	—	Negative
		6	—	—	Negative
L2	Lake Geraldine	1	—	—	Negative
		2	—	—	Negative
		3	—	—	Negative
		4	—	—	Negative
		5	—	—	Negative
		6	—	—	Negative
L3	Lake Geraldine	1	—	—	Negative
		2	—	—	Negative
		3	—	—	Negative
		4	—	—	Negative
		5	—	—	Negative
		6	—	—	Negative
L4	Water Treatment Plant (raw water sample port)	1	—	—	Negative
		2	—	—	Negative
		3	—	—	Negative
		4	—	—	Negative
		5	—	—	Negative
		6	—	—	Negative
L5	Lake Geraldine	1	—	—	Negative
		2	—	—	Negative

Reference: Environmental DNA Sampling Program for Arctic Char in the Lake Geraldine Reservoir, Iqaluit, NU

Table 2: qPCR Results obtained from 14 samples analyzed for Arctic char eDNA, Iqaluit, NU; September 2017[a]

Sample ID	Location	Replicate No.	C_T	Number of DNA Molecules	Interpretation of Result for Presence of Arctic char eDNA
		3	—	—	Negative
		4	—	—	Negative
		5	—	—	Negative
		6	—	—	Negative
L6	Lake Geraldine	1	—	—	Negative
		2	—	—	Negative
		3	—	—	Negative
		4	—	—	Negative
		5	—	—	Negative
		6	—	—	Negative
L7	Lake Geraldine	1	—	—	Negative
		2	—	—	Negative
		3	—	—	Negative
		4	—	—	Negative
		5	—	—	Negative
		6	—	—	Negative
L8	Lake Geraldine	1	—	—	Negative
		2	—	—	Negative
		3	—	—	Negative
		4	—	—	Negative
		5	—	—	Negative
		6	—	—	Negative
L9	Lake Geraldine	1	—	—	Negative
		2	—	—	Negative
		3	—	—	Negative
		4	—	—	Negative
		5	—	—	Negative
		6	—	—	Negative
L10	Lake Geraldine	1	—	—	Negative
		2	—	—	Negative
		3	—	—	Negative
		4	40.91[b]	0.34	Negative – potential artefact
		5	—	—	Negative
		6	—	—	Negative

Reference: Environmental DNA Sampling Program for Arctic Char in the Lake Geraldine Reservoir, Iqaluit, NU

Table 2: qPCR Results obtained from 14 samples analyzed for Arctic char eDNA, Iqaluit, NU; September 2017[a]

Sample ID	Location	Replicate No.	C_T	Number of DNA Molecules	Interpretation of Result for Presence of Arctic char eDNA
L11	Lake Geraldine	1	—	—	Negative
		2	—	—	Negative
		3	—	—	Negative
		4	—	—	Negative
		5	—	—	Negative
		6	—	—	Negative
L12a	Tap Water (negative control)	1	—	—	Negative
		2	—	—	Negative
		3	—	—	Negative
		4	—	—	Negative
		5	—	—	Negative
		6	—	—	Negative
L12b	Tap Water (negative control)	1	—	—	Negative
		2	—	—	Negative
		3	—	—	Negative
		4	—	—	Negative
		5	—	—	Negative
		6	—	—	Negative
L13	Sylvia Grinnell River (positive control)	1	39.72	1.06	Positive
		2	—	—	Negative
		3	40.56[b]	0.43	Negative – potential artefact
		4	—	—	Negative
		5	—	—	Negative
		6	38.45	1.87	Positive

Notes:

'—' implies no result/detection of eDNA

a. Data presented includes the threshold cycle (C_T), number of DNA molecules, and overall detection results (negative or positive) for the target species, Arctic char

b. Positive amplification above the established detection threshold (40 C_T) that is not considered a positive result but rather a potential artefact of analysis.

Table 3: Summary of qPCR Results for Positive Amplification Samples from the Sylvia Grinnell River, Iqaluit, NU; September 2017

Sample ID	C_T (mean ± SD)	Number of DNA Molecules (mean ± SD)	Number of Replicates with Positive Amplifications
L13 (Sylvia Grinnell River)	39.1 ± 0.9	1.5 ± 0.6	2 of 6

Notes:

[a.] Data presented include the mean and standard deviation (SD) of the threshold cycle (C_T) and number of DNA molecules for Arctic char

4 CONCLUSIONS

The qPCR analysis of the Arctic char eDNA samples confirmed the presence of Arctic char in the Sylvia Grinnell River (the positive control) where there is a known run of Arctic char, and no detection in the negative control samples of treated tap water. These QA/QC results, along with the due diligence used in the laboratory to store, extract and test the samples for the presence of Arctic char eDNA, provides confidence for interpreting the results from Lake Geraldine and its tributaries.

The qPCR analysis did not detect the presence of Arctic char DNA in the samples from Lake Geraldine or its tributaries. There is the potential for false negative results (i.e., not detecting Arctic char when they are present) in the samples due to sampling design or due to very low concentrations of Arctic char eDNA in the sampled water. To mitigate for the potential for false negatives, the eDNA samples were collected at a time of year, and at sites, where Arctic char, if it was present, would be expected to be present (potentially spawning) and shedding eDNA into the water. Further, standardized sampling and analysis protocols for eDNA were used along with quality control checks. As such, we consider the sampling design and analysis to be robust for the detection of Arctic char eDNA if it was present.

The eDNA results from 2017 sampling provide further evidence alongside conventional sampling in 2016 to suggest that Arctic char is not currently present in Lake Geraldine and its tributaries.

Reference: Environmental DNA Sampling Program for Arctic Char in the Lake Geraldine Reservoir, Iqaluit, NU

5 CLOSURE

We trust that the above letter report and accompanying attachments provides the City with sufficient information on the eDNA sampling program for Arctic char in Lake Geraldine. Should you have any questions on the results or conclusions of the sampling program, please contact the undersigned.

This document entitled “Environmental DNA Sampling Program for Arctic Char in the Lake Geraldine Reservoir, Iqaluit, NT” was prepared by Nunami Stantec Ltd. for the account of the City of Iqaluit (the “Client”). Any reliance on this document by any third party is strictly prohibited. The material in it reflects Stantec’s professional judgment considering the scope, schedule and other limitations stated in the document and in the contract between Stantec and the Client. The opinions in the document are based on conditions and information existing at the time the document was published and do not consider any subsequent changes. In preparing the document, Stantec did not verify information supplied to it by others. Any use which a third party makes of this document is the responsibility of such third party. Such third party agrees that Stantec shall not be responsible for costs or damages of any kind, if any, suffered by it or any other third party because of decisions made or actions taken based on this document.

Sincerely,

NUNAMI STANTEC LIMITED

Pam Reece
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Date: 2018.11.21 11:46:29 -08'00'

Digitally signed by Carey Sibbald, M.Sc., R.P.Bio.
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PR/CS/MM/

Attachment: Appendix A – Sampling Site Figure
Appendix B – Photographs
Appendix C – Laboratory Report

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6 REFERENCES

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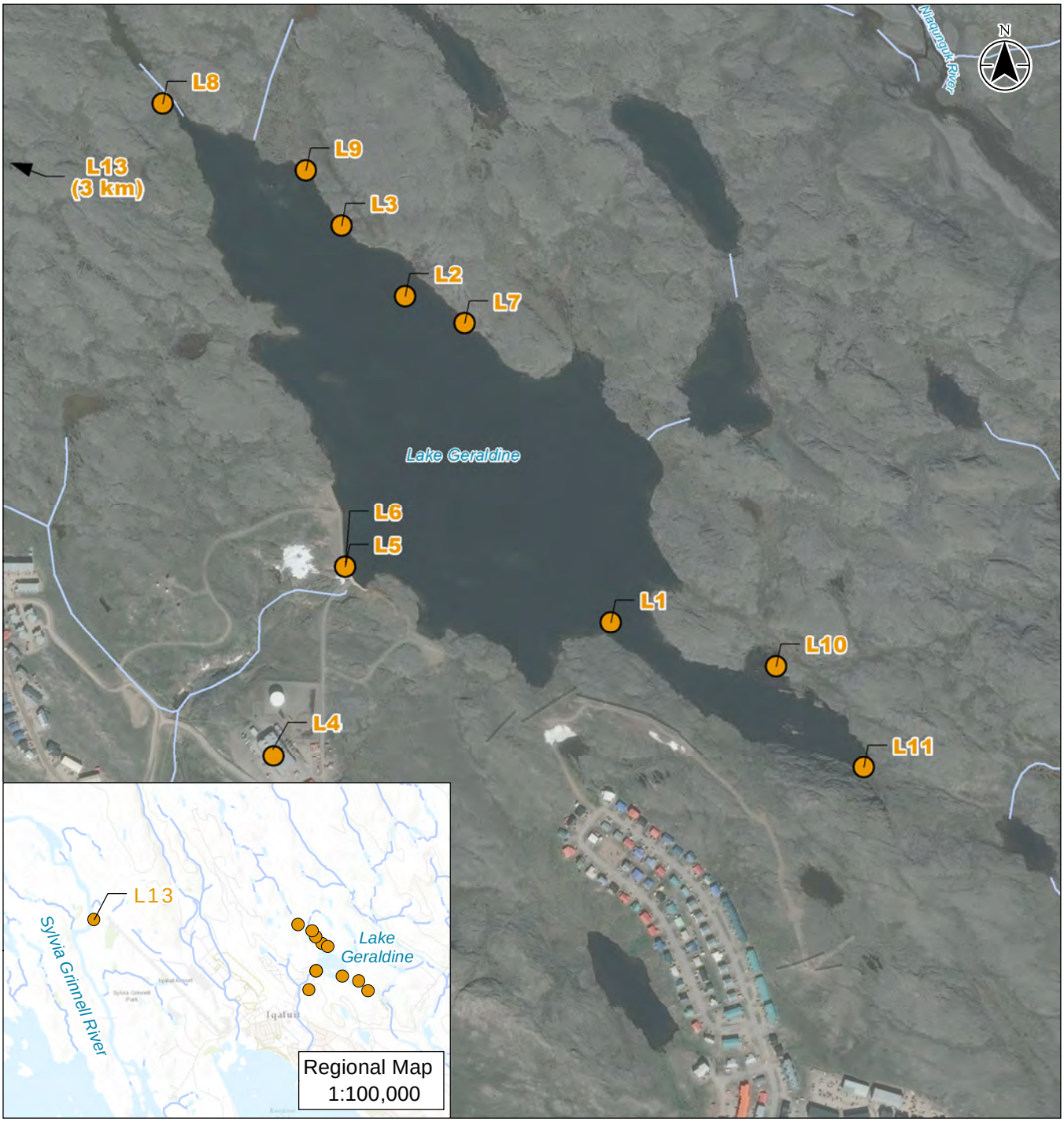
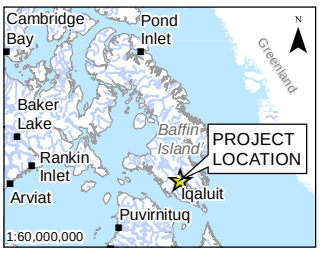
APPENDIX A Sampling Site Figure





Notes
1. Coordinate System: NAD 1983 UTM Zone 19N
2. Data Sources: Natural Resources Canada; ESRI World Imagery

eDNA Sample Site

Watercourse

0 100 200 300 400 500 m

1:10,000 (at original document size of 8.5x11)

Stantec

Iqaluit

Project Location
Iqaluit, Nunavut

Project Number 144930086
Prepared by LTRUDELL on 20180628
Discipline Review by PREECE on 20180628
GIS Review by RCOATTA on 20180927

Client/Project/Report
City of Iqaluit
Sylvia Grinnell River Feasibility Assessment
Environmental DNA Sampling Program
for Arctic Char in Lake Geraldine

Figure No.
1

DRAFT

Title
Lake Geraldine Environmental DNA Sampling Sites, September 2017

APPENDIX B Photographs

Stantec

Photographic Log

Client:	**City of Iqaluit**	**Project:**	**Environmental DNA Sampling Program for Arctic Char in the Lake Geraldine Reservoir**
Site Name:	**Lake Geraldine**	**Site Location:**	**Iqaluit, NU**

Photograph ID: 1 **Photo Location:** Sample Location L1 at Lake Geraldine (south shore) **Direction:** North **Survey Date:** 9/27/2017 **Comments:**	
Photograph ID: 2 **Photo Location:** Sample Location L2 at Lake Geraldine (north shore) **Direction:** Southwest **Survey Date:** 9/28/2017 **Comments:**	

Client:	**City of Iqaluit**	**Project:**	**Environmental DNA Sampling Program for Arctic Char in the Lake Geraldine Reservoir**
Site Name:	**Lake Geraldine**	**Site Location:**	**Iqaluit, NU**

Photograph ID: 3	
Photo Location: Sample Location L3 at Lake Geraldine (north shore)	
Direction: Southwest	
Survey Date: 9/28/2017	
Comments:	

Photograph ID: 4	
Photo Location: Sample Locations L5 and L6 at the Lake Geraldine impoundment/spillway (south shore)	
Direction: North	
Survey Date: 9/28/2017	
Comments:	

Client:	**City of Iqaluit**	**Project:**	**Environmental DNA Sampling Program for Arctic Char in the Lake Geraldine Reservoir**
Site Name:	**Lake Geraldine**	**Site Location:**	**Iqaluit, NU**

Photograph ID: 5 **Photo Location:** Sample Location L7 at Lake Geraldine (north shore) **Direction:** South **Survey Date:** 9/28/2017 **Comments:**	
Photograph ID: 6 **Photo Location:** Sample Location L8, in a tributary to Lake Geraldine **Direction:** Southeast **Survey Date:** 9/28/2017 **Comments:**	

Client:	**City of Iqaluit**	**Project:**	**Environmental DNA Sampling Program for Arctic Char in the Lake Geraldine Reservoir**
Site Name:	**Lake Geraldine**	**Site Location:**	**Iqaluit, NU**

Photograph ID: 7 **Photo Location:** Sample Location L9 at Lake Geraldine (north shore) **Direction:** Northwest **Survey Date:** 9/28/2017 **Comments:**	
Photograph ID: 8 **Photo Location:** Sample Location L10 at Lake Geraldine (north shore in east bay) **Direction:** South **Survey Date:** 9/27/2017 **Comments:**	

Client:	**City of Iqaluit**	**Project:**	**Environmental DNA Sampling Program for Arctic Char in the Lake Geraldine Reservoir**
Site Name:	**Lake Geraldine**	**Site Location:**	**Iqaluit, NU**

Photograph ID: 9 **Photo Location:** Sample Location L11 at Lake Geraldine (east end of east bay) **Direction:** West **Survey Date:** 9/27/2017 **Comments:**	

Photograph ID: 10 **Photo Location:** Sample Location L13 at the Sylvia Grinnell River (positive control sample) **Direction:** West **Survey Date:** 9/28/2017 **Comments:**	

Reference: Environmental DNA Sampling Program for Arctic Char in the Lake Geraldine Reservoir, Iqaluit, NU

APPENDIX C Laboratory Report

Validation of the presence of Arctic Charr (*Salvelinus alpinus*) from thirteen locations

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CONTEXT

DNA is released by organisms in the environment from skin cells, mucus, metabolic waste or gonads (Taberlet et al. 2012; Rees et al. 2014). Thereby, eDNA is a signature of the organism's presence in the environment. Therefore, eDNA studies are used to detect DNA from organisms without the need to capture them by collecting samples from their environment (Lodge et al. 2012).

FULL PROJECT DELIVRABLES

1- DNA quality check of eDNA samples
2- Validate the presence or absence of the Arctic Charr from 14 samples in total
   Location1
   Location2
   Location3
   Location4
   Location5
   Location6
   Location7
   Location8
   Location9
   Location10
   Location11
   Location12a
   Location12b
   Location13

METHODS

Filtration and eDNA extractions

Water was sampled and filtered by the Stantec team. In total, upon receipt at Ulaval laboratory, 14 filters were frozen and stored at -20°C until eDNA extraction.

The eDNA was extracted using the Goldberg *et al.* (2011) protocol (see Annexe 1 for detailed protocol). The extracted eDNA was stored at -20°C until amplification by quantitative PCR (qPCR) method. For each extraction batch, an extraction negative control (no filter) was added and treated as the other samples to account for possible contamination.

Primers and probes specificity for detection of Arctic Charr by qPCR methods

In order to identify specific fish species, here the Arctic Charr (*Salvelinus alpinus*), DNA sequences from mitochondrial genes such as the cytochrome c oxidase sub-unit 1 (COI) is universally used (Hebert, Ratnasingham & deWaard 2003; April *et al.* 2011).

The primers and probes specificities are crucial in order to identify the species of interest and these were beforehand developed in the Dr. L. Bernatchez's laboratory. The COI sequences from these species were obtained from the bold system (Barcode of Life Database http://www.boldsystems.org/index.php/). The

COI sequences from related species present in the same environment were also used to design the specific primers. Primers and probe were developed to maximise the number of mismatches between the targeted species and the related species. The sequences were aligned using the software Geneious (https://www.geneious.com/). From aligned COI gene sequences, specific primers and probe were successfully designed using the software Geneious (https://www.geneious.com/) and verified using the software Primer Express 3.0 (Life Technologies). In addition, the primer blast tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) was used to verify the specificity of the amplification on other species that could be present in the same environment.

The specific primers and probes designed for Arctic Charr were tested by qPCR method on DNA extracted from tissues of 1) the targeted species, Arctic Charr and 2) related species separately: rainbow trout (*Oncorhynchus mykiss*), brook charr (*Salvelinus fontinalis*), Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*). Primers and probes were also tested with 3) a mix containing DNA of only the four related species (*S. fontinalis, S. trutta, S. salar, O. mykiss*); 4) a mix containing DNA of the four related species including the targeted species (*S. alpinus, S. fontinalis, S. trutta, S. salar, O. mykiss*) and 5) a mix containing the targeted species and a close relative (*S. alpinus, S. fontinalis*) to verify the competitive interaction of the primers and probes between the targeted and related species in cases of co-occurrence of the different species. The amplification was performed on the PCR 7500 Fast Real-Time (Life Technologies) in a final volume of reaction of 20µl including 1.8µl of each primer (10µM), 0.5µl of probe (10µM), 10 µl of Environmental Master Mix 2.0 (Life Technologies), 3.9µl of H2O and 2µl of DNA following these conditions: 2 min at 50 °C, 10 min at 95 °C, 50 cycles of 15 s at 95 °C and 60 s at 60 °C.

During qPCR, the degradation of the probe is accompanied by fluorescence and the level of fluorescence is measured in real time during each PCR cycle. Thus, it is possible to determine the PCR cycle where the detection threshold of fluorescence is reached (C_T); higher is the number of DNA copies, faster the threshold is reached and lower is the C_T value. The presence of DNA from the targeted species is confirmed when amplification is detected before the detection threshold of the fluorescence is reached. For the specific primer tests two methods were used i) FAST SYBR Green method for testing specificity and efficiency of primers and ii) TaqMan method for testing specificity of primers and probe. Specificity and efficiency of all primer pairs were first tested with FAST SYBR Green method, once amplification of targeted species only was achieved, the Taqman assay was performed with the species-specific probes for further specificity enhancement.

eDNA sample quality check

The eDNA sample quality was checked on the Bioanalyzer (Agilent®) using the high sensitivity DNA assay chip. This method measures the length, in base pairs, of the DNA fragments present in a sample by electrophoresis and is compared to a known ladder. The latter verify the presence or absence of DNA fragments in a sample as well as identifying their length which can be used as a proxy for DNA quality (longer are the DNA fragments, higher is the quality).

eDNA sample analysis by qPCR method

To analyse the collected eDNA samples, the TaqMan qPCR method was used with the addition of the SPUD to the reaction as well as a standard curve. DNA presence of each targeted species was tested on

six replicates for each eDNA sample and the negative extraction control (as described in the method section).

The SPUD is used as an internal positive control to evaluate the efficiency of reaction and to identify the presence of inhibitors in the samples. The amplification was performed in a final volume reaction of 20µl including 1.8µl of each primer (10µM), 0.5µl of probe (10µM), 10 µl of Environmental Master Mix 2.0 (Life Technologies), 3.9µl of SPUD and 2µl of DNA following these conditions: 2 min at 50 °C, 10 min at 95 °C, 50 cycles of 15 s at 95 °C and 60 s at 60 °C.

For the targeted species, a synthetic DNA template of 500 base pairs (gBlocks, IDT) was designed from the COI sequence to be used as a standard curve for quantification. Using the gBlocks, the detection threshold for each primer pair was determined by serial dilution until the fluorescence signal corresponding to one molecule was reached (Forootan et al. 2017). The threshold of one molecule was detected at 40 Ct for the primer pair. Therefore, all signals exceeding 40 Ct were considered as potential artefacts of PCR and were eliminated from the analysis.

Finally, all qPCR results were quantified using a standard curve of known DNA quantities. The latter, allowed us to quantify positive PCR amplification in number of molecules to quantify the relative quantity of DNA from the targeted species. The number of molecules of the six replicates were averaged based only on positive amplifications.

RESULTS

eDNA samples quality check by Bioanalyser

The eDNA samples have been checked using the Bioanalyzer method (14 samples) and the DNA profile results showed that most DNA samples were above 10 000 base pairs and no short fragments (degraded) were detected. However, three samples (Location 9, 12a and 12b) seemed to have very few or no DNA (no long fragments or only short fragments detected).

Specificity of primers and probes

Results of the specific amplification by qPCR are presented in Figure 1. Specific primers amplified the targeted species *Salvelinus alpinus* and no amplification was detected for the related species *Salvelinus fontinalis*, *Salmo salar* and *Salmo trutta*. A low but positive amplification was observed for *Oncorhynchus mykiss*. Nevertheless, this positive amplification is detected 8 C_T after the amplification of the desired species, that corresponds to an amplification 2^8 (256) times lower than the targeted species. Therefore, all positive amplifications are sequenced for species validation. In addition, these primers were successfully used to detect the presence of Arctic Charr in lakes from southern Quebec where *O. mykiss* occurs.

eDNA samples analysis by qPCR

Positive amplifications were detected for Location 13 (Table 1 and Table 2). Two replicates on a total of six showed a positive amplification.

Two other positive amplifications were also detected (one replicate from Location 10 and one replicate from Location 13). However, as the signal exceeded the 40 C_T, they are eliminated from the analysis because these signals were considered as potential artefacts of PCR.

Negative controls and positive amplifications sequencing

All extraction negative controls showed no positive amplification indicating the absence of contamination during sample extraction.

The field negative controls ensure that the field material was initially exempt from residual DNA, which is a possibility when the same material is used to sample several sites. Because we did not know if field negative controls have been taken or which samples are the negatives controls we can not conclude on the possible contamination in the field. Consequently, we cannot assert that the positive detection of Arctic Charr come from the sampled lake and not from the residual DNA.

To confirm that positive detections represent actual targeted species, several positive amplifications were sequenced by Sanger sequencing. The sequencing results showed that positive amplifications came from the targeted species, Arctic Charr.

CONCLUSION

The analysis by qPCR method has confirmed the presence of Arctic Charr (*Salvelinus alpinus*) in one sample (location 13). However, we can not assert that the positive detection of Arctic Charr come from the sampled lake and not from the residual DNA because no field control was identified among the samples.

Table 1. qPCR results obtained for positive amplification samples. Results of the mean threshold cycle (C_T), the average number of DNA molecules of all samples with a positive detection for each replicate (6 replicates) and the number of positive amplifications (Amp.), Sd: standard deviation.

Sample Name	C_T (Mean ±sd)	Nb. Mol. (Mean ±sd)	Amp.
Location 13	39.1 ± 0.9	1.5 ± 0.6	2

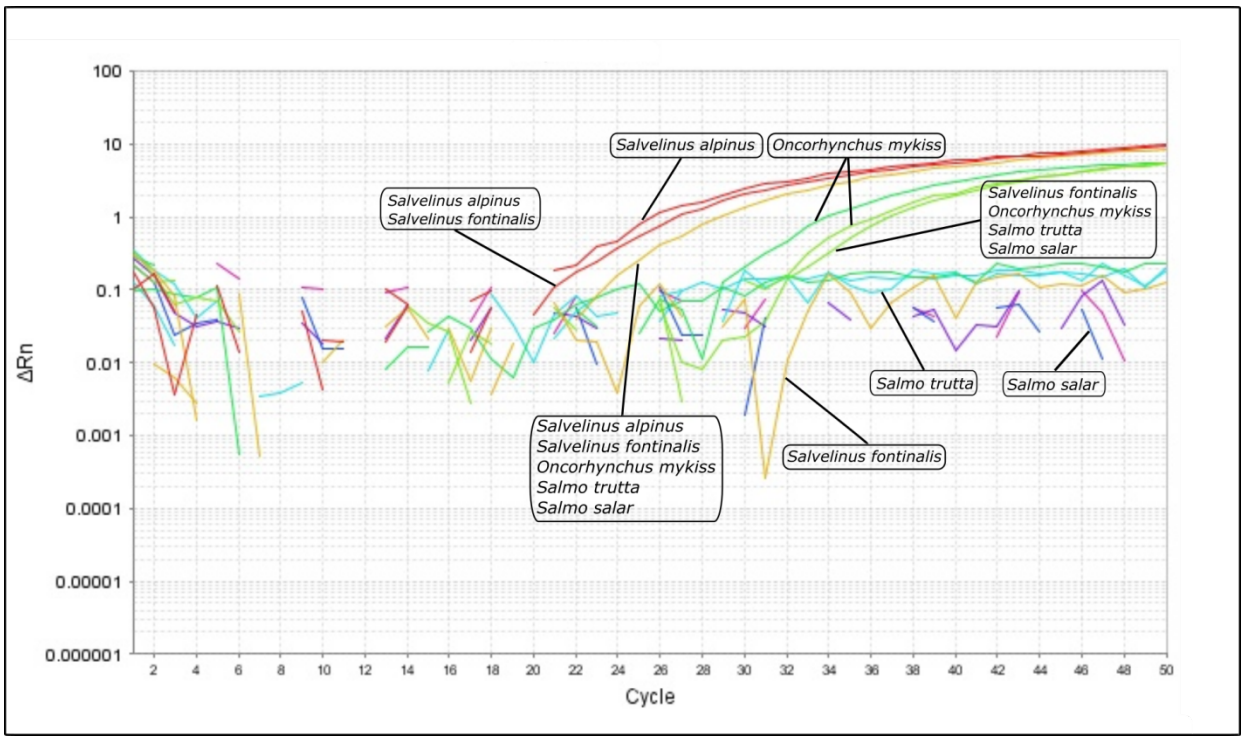
Table 2. qPCR results obtained for all analysed samples. Results of the threshold cycle (C_T), the number of DNA molecules are presented as well as the detection results (negative or positive) for the target species (Arctic charr). Positive amplifications with an asterisk (*) are above the detection threshold determined (40 C_T) and are removed from the analysis.

Sample	Replicate #	C_T	Nb. Mol.	Results
Location 01	1	--	--	Negative
	2	--	--	Negative
	3	--	--	Negative
	4	--	--	Negative
	5	--	--	Negative
	6	--	--	Negative
Location 02	1	--	--	Negative
	2	--	--	Negative
	3	--	--	Negative
	4	--	--	Negative
	5	--	--	Negative
	6	--	--	Negative
Location 03	1	--	--	Negative
	2	--	--	Negative
	3	--	--	Negative
	4	--	--	Negative
	5	--	--	Negative
	6	--	--	Negative
Location 04	1	--	--	Negative
	2	--	--	Negative
	3	--	--	Negative
	4	--	--	Negative
	5	--	--	Negative
	6	--	--	Negative
Location 05	1	--	--	Negative
	2	--	--	Negative

	3	--	--	Negative
	4	--	--	Negative
	5	--	--	Negative
	6	--	--	Negative
Location 06	1	--	--	Negative
	2	--	--	Negative
	3	--	--	Negative
	4	--	--	Negative
	5	--	--	Negative
	6	--	--	Negative
Location 07	1	--	--	Negative
	2	--	--	Negative
	3	--	--	Negative
	4	--	--	Negative
	5	--	--	Negative
	6	--	--	Negative
Location 08	1	--	--	Negative
	2	--	--	Negative
	3	--	--	Negative
	4	--	--	Negative
	5	--	--	Negative
	6	--	--	Negative
Location 09	1	--	--	Negative
	2	--	--	Negative
	3	--	--	Negative
	4	--	--	Negative
	5	--	--	Negative
	6	--	--	Negative
Location 10	1	--	--	Negative
	2	--	--	Negative
	3	--	--	Negative
	4	40.91*	0.34	Negative
	5	--	--	Negative
	6	--	--	Negative
Location 11	1	--	--	Negative
	2	--	--	Negative
	3	--	--	Negative
	4	--	--	Negative
	5	--	--	Negative
	6	--	--	Negative
Location 12a	1	--	--	Negative
	2	--	--	Negative
	3	--	--	Negative

	4	--	--	Negative
	5	--	--	Negative
	6	--	--	Negative
Location 12b	1	--	--	Negative
	2	--	--	Negative
	3	--	--	Negative
	4	--	--	Negative
	5	--	--	Negative
	6	--	--	Negative
Location 13	1	**39.72**	**1.06**	Positive
	2	--	--	Negative
	3	40.56*	0.43	Negative
	4	--	--	Negative
	5	--	--	Negative
	6	**38.45**	**1.87**	Positive

Figure 1. Amplification plots by qPCR for the specificity test of the *Salvelinus alpinus* primers.



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ANNEXE 1 Protocol for eDNA extraction

** Maximum of 11 filters and add 1 extraction control

Always use ART filter tips
Keep all equipment inside the extraction zone
Keep your lab coat inside the extraction zone (take off if needed to move outside)
Change gloves when moving outside the extraction zone
Keep space between the tubes on the rack (don't put tubes side by side)
** Close the tube before opening its bag **

Materials

Bleach (not diluted)
Sterilised forceps
Sterilised scissors
Individually sealed tubes (Fisher Scientific Cat.no. 02-681-271)
QIAshredder tubes (Qiagen Cat.no. 79656)
DNeasy Mini spin column (Qiagen Cat.no. 69506; DNeasy Blood & tissue kit)
Beaker for liquid waste
Falcon tube 50ml

Solutions

Proteinase K (Qiagen Cat.no. 19133)
Ethanol 95% (Alcool du Commerce Cat.no.1011C)
Buffer AW1 (Qiagen Cat.no. 19081)
Buffer AW2 (Qiagen Cat.no. 19072)
Buffer AL (Qiagen Cat.no. 19075)
Buffer ATL (Qiagen Cat.no. 939011)
Ultrapure water (Life Technology Cat.no. 10977-015)
DNA-away (VWR Cat.no. 53509-506)

Preparation:

** Close the tube before opening the bag **

I. Ultrapure water :
    a. Put 2ml of ultrapure water in individually sealed tubes (~10 tubes).
    b. Sterilise at second floor (put them in beaker sealed by foil).
    c. Put under UV 30 minutes (Can be do at the sterilisation step).
    d. Keep them under UV station.
II. Prepare 95% EtOH:
    a. Fill a Falcon tube of 50 ml with 95% EtOH
    b. Put under UV 30 minutes (Can be do at the sterilisation step).
    c. Keep it under UV station.

Sterilisation (steps I to II could be done 24h in advance)

Bench 1133 section B

I. Water bath: change water and clean inside and outside with DNA away. Put back new Millipore/nanopure water (lab 1132).
II. Clean oven using DNA away (inside and outside).

Sink at laboratory 1133 section C

III. Bleach in Javel 10% for 15 minutes and rinse with distilled water:
    a. Tube rack
    b. Scissors (1 by sample filter)
    c. Forceps (1 by sample filter)
    d. Rack from water bath

pcr bench under the UV station

IV. Clean with DNA away:
    a. Bench
    b. Pipettes
    c. Vortex
V. Put under UV light for 30 minutes (the same day):
    a. Pipettes
    b. Vortex
    c. Tube rack (x2)
    d. Scissors on a large kimwipe (1 by sample filter)
    e. Forceps on a large kimwipe (1 by sample filter)
    f. Tube rack from water bath

<u>Step 1 : Digestion (maximum 11 filters and 1 extraction control)</u>

**Close the tube before opening its bag

1. Prepare and identify 23 tubes (2 tubes by 1 sample filter).
    a. Close the tube before opening its bag.
2. Put the sample filter on ice
3. Put the foil pocket on kimwipe on the bench (1 by 1).
4. Open the foil pocket.
5. Change gloves or clean with DNA away after each sample filter
    a. Do not touch the tube/forceps with the same gloves that were used to take the foil pocket.
6. Change gloves and use sterilised forceps to open the sample filter and to cut in 2 parts
    a. Put the two parts of the filter in two separated tubes et cut each in small pieces
7. Discard the foil and sterilise the bench where the foil was drop off.
8. Repeat step 3 to 7 for all filters

9. Add 500 ul Buffer ATL in each tube including the control tube (change your tip each time).
10. Add 50 ul proteinase K in each tube including the control tube. Make sure that all parts of filter are immersed (change your tip each time).
11. Vortex
12. Incubate at 56^{o}C overnight in oven (bench 1133 section B).

Prepare for the next day:

<u>2.1 :</u> Prepare tubes:

I. Prepare 12 identified tubes x 4.

a. Close the tube before opening its bag.

2.2 : Sink at Laboratory 1133 section C

1. Bleach in Javel 10% for 15 minutes and rinse with distilled water:
    a. Tube rack (x2)
    b. Tube rack for water bath
    c. Forceps (2 / filter)
    d. Styrofoam box
2. Put them under UV station

Étape 2. Extraction

**Close the tube before opening the bag

2.3 : Bench 1133 section B

3. Heat water bath at 70ºC (be sure that water bath is clean and sterilised).
    a. If not, change water and clean inside and outside with DNA away. Put back new Millipore water.
4. Heat Ultrapure water at 37ºC in oven (be sure that oven is clean and sterilised).
    a. If not, clean the oven using DNA away.

2.4 : pcr bench under UV station

5. Clean with DNA away :
    a. bench
    b. Pipettes
    c. Vortex
    d. Centrifuge (exterior and interior) (rotor)
        i. Clean the rotor bores with cut-tips soaked in DNA away.
6. Put under UV light for 30 minutes (the same day):
    a. Pipettes
    b. Vortex
    c. Tube rack (x2)
    d. Forceps on a big kimwipe (x2 by filter)
    e. Styrofoam box (openned)
    f. 95% EtOH
    g. Ultrapure water
    h. 48 identified tubes

7. Identify and prepare 12 QIAshredder tubes
8. Put the first part of filter in QIAshredder tube using clean forceps.
9. Change gloves or wash them using DNA away after to proceed each filter.
10. Centrifuge 3 min at 13 000rpm.
11. Put 400 ul of supernatant in one of four identified tubes
12. Discard the pieces of filter using clean forceps.
13. Repeat steps 8 to 12 for the second half of the filter in the same QIAshredder but put the supernatant in a new separated tube each time (for example use the second tube of the fourth identified tube).
14. Put the liquid from the digestion step (1.9) in the same QIAshredder (change your tip each time).
15. Centrifuge 30 seconds at 13 000rpm.
16. Put 400 ul of supernatant in one of four identified tubes.

17. Repeat steps 14 to 16 with the liquid from the digestion step (1.9) from the second tube. At the end, you have 4 tubes of digested DNA for 1 sample filter (2 tubes for sample filter and 2 tubes for digested buffer with filter from step 1.9.
18. Change gloves or wash them using DNA away.
19. Add 400 ul Buffer AL in each tube (change your tip each time).
20. Vortex.
21. Incubate at 70ºC in water bath for 10 min (bench 1133 section B).
22. Wipe each tube using a Kimwipe.
23. Centrifuge few seconds.
24. Add 400 ul 95% EtOH in each tube (change your tip each time).
25. Vortex.
26. Centrifuge few seconds.
27. Prepare and identify 12 DNeasy Min spin column.
28. Transfer 625 ul of liquid from one of four tube on identified DNeasy Min spin column (change your tip each time).
29. Centrifuge 30 seconds at 13 000 rpm.
30. Discard the flow-through (DNA is in the column).
    a. Touch the bottom of the column on a new kimwipe in order to eliminate the last drop of flow-through.
31. Repeat steps 28 to 30 for each four tubesb 625 ul by 625 ul for a total of 8 times by filter (change your tip each time).
32. Transfer the column in a new collection tube.
33. Add 500 ul Buffer AW1 (change your tip each time)
34. Centrifuge 1 min to 13 000rpm.
35. Transfer the column in a new collection tube and discard the old collection tube with the flow-through.
36. Add 500 ul Buffer AW2 (change your tip each time)
37. Centrifuge 1 min à 13 000rpm.
38. Transfer the column in a new collection tube and discard the old collection tube with the flow-through.
39. Add 500 ul Buffer AW2 (change your tip each time)
40. Centrifuge 3 min at 13 000rpm.
41. Discard the flow-through (DNA is on the column).
    a. Touch the bottom of the column on a new kimwipe in order to eliminate the last drop of flow-through.
42. Prepare and identify 12 individually sealed tubes.
    a. Close the tube before opening its bag.
43. Discard the collection tube.
44. Transfer the column on a new identified sealed tube and discard the collection tube.
    a. Touch the bottom of the column on a new kimwipe in order to eliminate the last drop of flow-through if necessary.
45. Add 80 ul ultrapure water heated to 37ºC directly to the center of column membrane to the DNA elution (change your tip each time).
46. Incubate 10 min at room temperature.
47. Centrifuge 1 min à 13 000 rpm.
48. Add a label on each tube.
49. Put in a sterilized Styrofoam box and close using a tape. Label your box.
50. Put at -20ºC in the freezer #1 at level 0.