

CITY OF IQALUIT
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UNNAMED LAKE FISH AND FISH HABITAT ASSESSMENT TECHNICAL REPORT

FEBRUARY 26, 2021

CONFIDENTIAL





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FINAL VERSION
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SUMMARY

The City of Iqaluit aims to develop a sustainable long-term water supply for its citizens and is currently investigating alternative options for additional water supply for the Lake Geraldine water reservoir. The City of Iqaluit wishes to investigate the option of a lake located approximately 3 km northeast of Lake Geraldine reservoir, referred to as “Unnamed Lake”. If it is confirmed that Unnamed Lake presents a viable long-term solution, it can be assessed together with the other options that have been reviewed from a technical, financial, environmental, and constructability standpoint, to determine the best option to pursue. Therefore, the City of Iqaluit retained WSP Canada Inc. and Qikiqtaaluk Environmental to assess fish and fish habitat for Unnamed Lake. The main goal was to confirm the presence of resident fish populations that may be impacted through the withdrawal of water for supplemental water supply. This project was completed between September 2020 and February 2021.

An initial desktop review of historical and background information on Unnamed Lake was completed. The purpose of this data review was to determine the potential of Unnamed Lake to support aquatic wildlife populations. Different sources of information were collected, such as previous studies on Unnamed Lake and local knowledge. The information gathered suggested that Unnamed Lake could potentially support an aquatic population based on a review of the topography of the area, a bathymetric survey, a water quality survey, and a local resident suggesting the presence of two species of fish (Arctic char and Ninespine stickleback) in Unnamed Lake.

In October 2020, an initial field visit to Unnamed Lake was completed. The purpose of this visit was document existing conditions and observations within the area surrounding the lake. Wolverine and Arctic hare tracks were observed around the lake and caribou bones were found. Wolverine and caribou are listed under the *Species at Risk Act*. Documentation of the Unnamed Lake shoreline substrate was also completed during the initial field visit. Based on those observations, it was determined that certain parts of the lake could potentially offer some spawning ground and juvenile habitat for Arctic char.

Environmental DNA (eDNA) sampling was performed on December 10th in Unnamed Lake. Four water samples were collected from Unnamed Lake in different parts of the lake and at various depths. One sample was also collected from Crazy Lake (Tasirluk), as a positive control. The sample collected in Crazy Lake yielded the successful identification of Arctic char, as anticipated. However, very little fish DNA was identified from the four water samples collected in Unnamed Lake. The largely used 12S rRNA marker for freshwater fish detection did not yield any successful identification for any sample. An additional analysis of the COI gene detected a small amount of *Salvelinus* genus DNA at one station in Unnamed Lake. The findings of this analysis could only allow identification to genus. The COI gene also yielded small amounts of arthropod DNA at each station which could not be specified at a lower taxonomic level.

Therefore, based on the information provided by an Iqaluit resident, the small amount of *Salvelinus* DNA found using the COI gene at one sampling station, and the presence of suitable habitat observed during the October field visit, it is possible to suggest that Arctic char (not listed under the *Species at Risk Act*) are present in low abundance in Unnamed Lake.

Water withdrawal from Unnamed Lake is suspected of having a low to non-existent impact on fish and fish habitat. Furthermore, Unnamed Lake does not provide habitat for aquatic species at risk and does not constitute critical habitat. However, the mitigation and protection measures suggested by the Department Fisheries and Oceans (DFO) to ensure fish and fish habitat protection should be followed to avoid any potential harm to fish or disturbance to fish habitat.

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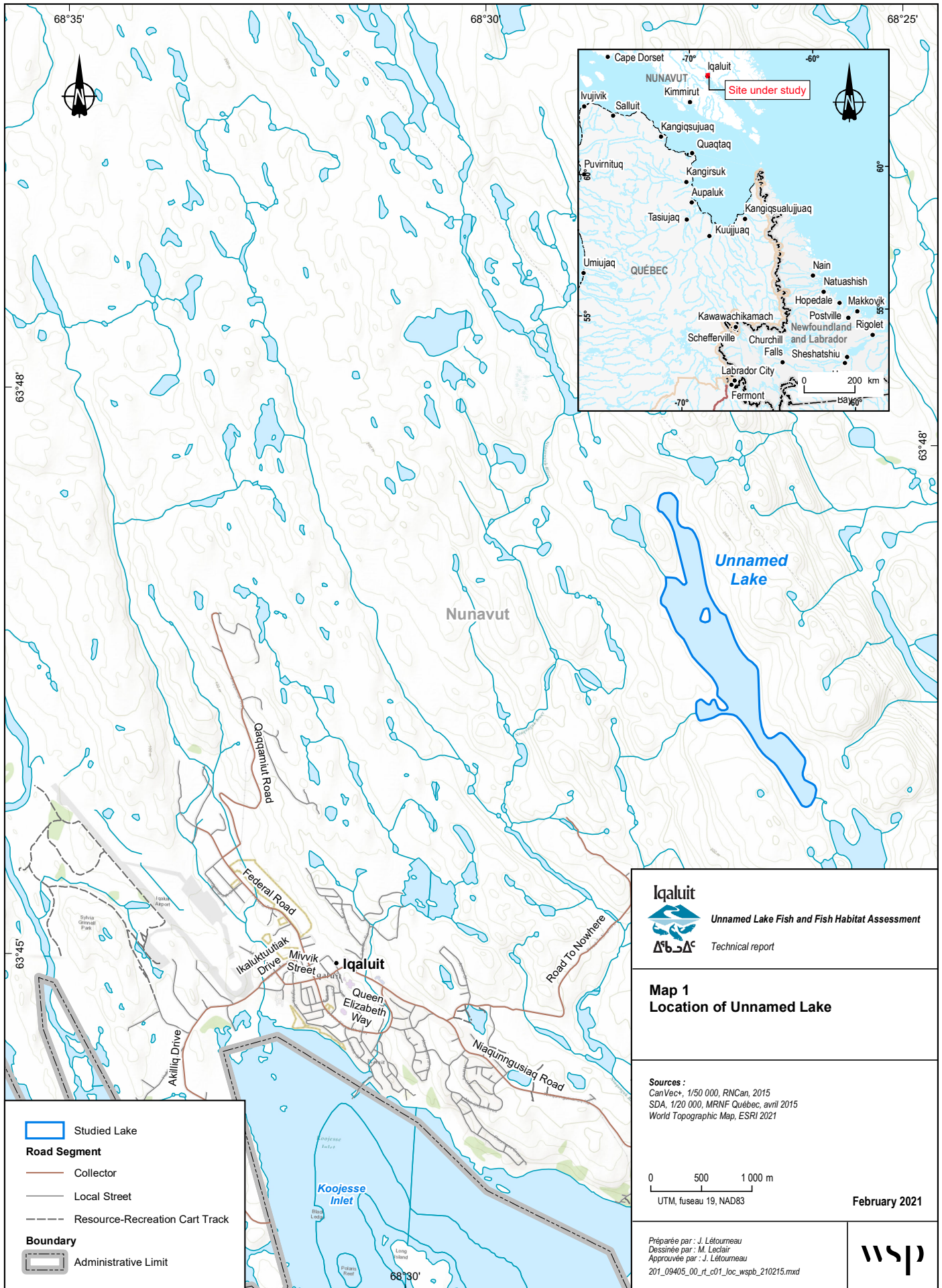
1 INTRODUCTION

The City of Iqaluit (the City) intends to develop a sustainable long-term water supply for its citizen and is currently investigating alternative options for additional water supply for the Lake Geraldine water reservoir. Previous studies have been conducted on the long-term viability of other nearby water sources, such as the Apex River and the Sylvia Grinnell River. The City now wishes to conduct an investigation for a lake, located approximately 3 km northeast of Lake Geraldine reservoir, referred to as “Unnamed Lake” (Map 1). If it is confirmed that Unnamed Lake presents a viable long-term solution, it can be assessed together with the other options that have been reviewed from a technical, financial, environmental, and constructability standpoint, in order to determine which is the best option to pursue. The goal of the long-term water supply and storage mandate is to ensure that a new supply and storage source has been identified, and is implemented for use by 2026, when the City’s current water licence expires.

Therefore, the City retained WSP Canada Inc. (WSP) and Qikiqtaaluk Environmental (QE) to assess fish and fish habitat for Unnamed Lake. This project was completed between September 2020 and February 2021. The purpose of this project was to confirm the presence of resident fish populations that may be impacted through the withdrawal of water for supplemental water supply. The scope of work for this project included the following:

- Desktop review of all historical information on Unnamed Lake;
- Local agency consultation and interviews;
- Site visits to verify information gathered in the interviews and desktop review, and to complete the following:
 - Determination if any fish species at risk (SAR) are present and to identify the species;
 - Fish and fish habitat assessment implemented using environmental DNA (eDNA) sampling method;
 - Evaluation of fish populations, if any are present, and determine how withdrawal of water from Unnamed Lake will impact fish populations;
 - Determination if there are other aquatic (non-fish) populations in Unnamed Lake, including potential for protected species, and analysis and impact of water withdrawal on these aquatic habitats (if any) at Unnamed Lake.
- Analysis of findings, and make determination and recommendations to the City, with regards to fish and fish habitat, general aquatic habitat, in relation to applicable legislation, regulations and scientific evidence, if the Unnamed Lake is a viable water supply source for the City’s residents.

This technical report provides the review of historical and background information on Unnamed Lake, a description of the methodology used to perform the eDNA sampling, including sampling specification, sample size and sample methods, results analysis and discussion, and recommendations.



2 REVIEW OF HISTORICAL AND BACKGROUND INFORMATION

This section presents all the information gathered from a desktop review completed prior to the field sampling in Unnamed Lake. The main goal of this data gathering was to determine the potential of Unnamed Lake to support aquatic wildlife populations. This section describes the geographical and physical aspects of Unnamed Lake, a review of fish species occurring in Nunavut that could potentially be present in Unnamed Lake, and a summary of local knowledge about the fauna potentially using the waterbody and its surroundings.

2.1 TOPOGRAPHY

Unnamed Lake is situated at an elevation of approximately 210 m. The topographic map of this area (Natural Resources Canada, Toporama cartographic tool, 2020) indicated that the lake is connected to two other small unnamed waterbodies (Figure 1). Unnamed Lake is also connected to Niaqunguk (Apex) River, which flows into Koojesse Inlet (Figure 1).

In August and September 2018, Nunami Stantec Limited (Nunami) completed environmental monitoring on Niaqunguk River as the City of Iqaluit undertook an emergency water supply project to withdraw water from the river to supplement Lake Geraldine reservoir. The goal of this monitoring was to support the City's emergency *Fisheries Act* Authorization obtained from Fisheries and Oceans Canada (DFO) in advance of pumping (Nunami Stantec Limited 2019a). A fish habitat survey was performed using an electrofisher and small mesh dip net to identify the presence of fish in the pumping area. No fish (or invertebrates) were captured during this survey, but a local Elder indicated to Nunami's team that, to her knowledge, there are fish throughout the Niaqunguk River system (without specifying the species) (Nunami Stantec Limited 2019a).

Based on this information, fish from Niaqunguk River could access and populate Unnamed Lake, if there are no barriers to fish migration on the river, such as waterfalls or underground flow. No information regarding potential fish populations was found concerning the two other waterbodies connected to Unnamed Lake.



Figure 1. Topographic map of the area of Unnamed Lake

2.2 BATHYMETRIC SURVEY

In July 2019, Tetra Tech Canada Inc. (Tetra Tech) was retained by the City of Iqaluit to perform the bathymetric survey of Unnamed Lake (Tetra Tech 2019; appendix A). It was determined that the surface area of Unnamed Lake is 911,300 m² (91.13 ha) with a total volume of 6,616,900 m³ and an under-ice volume of 4,737,900 m³. The maximum water depth, approximately 22 m, was obtained in the centre of the lake. Tetra Tech could not assess the precise depth of two small bays of Unnamed Lake (Appendix A), due to inaccessibility by boat due to shallow water depths (estimated <0.5 m). Tetra Tech assumed that both bays are entirely frozen during winter.

The shoreline length of the lake was evaluated by WSP using satellite imagery and is approximately 9.35 km. This data and the surface area of the lake estimated by Tetra Tech were used to determine the Shoreline development index (Wetzel 1975). This index is a number that relates the measured shoreline length of a given lake to the shoreline length of a perfectly circular lake of equal area. The formula for the ratio is:

$$D_L = \frac{L}{(2\sqrt{A \times \pi})}$$

D_L = Shoreline development index

L = Shoreline length (perimeter)

A = surface area

A high value Shoreline development index reflects the importance of the shoreline and coastal zone of a lake and indicate the potential for fish populations and activity. A shoreline development index equal to 1 suggests that a lake is circular and doesn't have shallow water bays that usually offer a higher potential for fish populations and activity.

Unnamed Lake has a Shoreline development index of 2.76, indicating its morphometry can be classified as “moderately long”, and suggests that it offers few shallow water bays that could provide moderate aquatic wildlife habitat.

2.3 WATER QUALITY

In October 2019, Nunami submitted a Water Quality Sampling Memorandum for Unnamed Lake to the City. The objective of this report was to confirm the water quality of Unnamed Lake as a source of drinking water for Iqaluit community (Nunami Stantec Limited 2019b; appendix B). Water in Unnamed Lake was sampled at five stations in July and September 2019. Surface water quality results were compared to the Guidelines for Canadian Drinking Water Quality and the Required Water Quality Parameters of the Northern Health Public Health Protection. The main conclusion was that water quality in Unnamed Lake is considered good.

As an additional assessment, these results have been evaluated according to the Canadian Water Quality Guidelines for the Protection of Aquatic Life of the Canadian Council of Ministers of the Environment (CCME). These guidelines are intended to provide protection of freshwater and marine life from anthropogenic stressors such as chemical inputs or changes to physical components (CCME 1999).

The pH of Unnamed Lake is suitable for aquatic fauna with *in situ* values varying between 6.96 and 7.80 in July and September 2019. The electrical conductivity and the concentration of total dissolved solids were also low, potentially indicating oligotrophic conditions within Unnamed Lake. Additionally, the results for alkalinity and the calcium concentration indicate a moderate sensibility to acidification. No volatile organic compounds, such as benzene, toluene and xylene, and petroleum hydrocarbons, were detected in Unnamed Lake. Few metals were detected in Unnamed Lake surface water. The main metals observed in the samples

were aluminum, manganese, silicon and strontium, but none of those were detected in high or problematic concentrations for aquatic fauna.

2.4 FISH SPECIES IN NUNAVUT

In 2001, Richardson *et al.* produced a report for DFO describing the life history characteristics of freshwater fish occurring in the Northwest Territories and Nunavut, with a major emphasis on lake habitat requirements. This document provided, among others, a list of all the freshwater fish species that can be found in Nunavut. This list is included in Table 1. A total of 22 fish species have been identified and could potentially be present in Unnamed Lake. None of these species are registered under the *Species at Risk Act*. However, many are fished extensively and are targeted for sustenance fisheries for northern communities (Reist *et al.* 2006, Hancock 2020).

Table 1. Fish species occurring in freshwaters in Nunavut

Family	Common name	Scientific Name
Cods (<i>Gadidae</i>)	Burbot	<i>Lota lota</i> (Linnaeus, 1758)
Carp and minnows (<i>Cyprinidae</i>)	Lake chub	<i>Couesius plumbeus</i> (Agassiz, 1850)
Perches (<i>Percidae</i>)	Walleye	<i>Stizostedion vitreum</i> (Mitchill, 1818)
Pikes (<i>Esocidae</i>)	Northern pike	<i>Esox lucius</i> (Linnaeus, 1758)
Sculpins (<i>Cottidae</i>)	Fourhorn sculpin	<i>Myoxocephalus quadricornis</i> (Linnaeus, 1758)
	Slimy sculpin	<i>Cottus cognatus</i> (Richardson, 1836)
	Spoonhead sculpin	<i>Cottus ricei</i> (Nelson, 1876)
Smelts (<i>Osmeridae</i>)	Rainbow smelt	<i>Osmerus mordax</i> (Mitchill, 1846)
Sticklebacks (<i>Gasterosteidae</i>)	Ninespine stickleback	<i>Pungitius pungitius</i> (Linnaeus, 1758)
	Threespine stickleback	<i>Gasterosteus aculeatus</i> (Linnaeus, 1758)
Suckers (<i>Catostomidae</i>)	Longnose sucker	<i>Catostomus catostomus</i> (Forster, 1753)
	White sucker	<i>Catostomus commersoni</i> (Lacepede, 1803)
Trouts (<i>Salmonidae</i>)	Arctic char	<i>Salvelinus alpinus</i> (Linnaeus, 1758)
	Arctic cisco	<i>Coregonus autumnalis</i> (Pallas, 1776)
	Arctic grayling	<i>Thymallus arcticus</i> (Pallas, 1776)
	Broad whitefish	<i>Coregonus nasus</i> (Pallas, 1776)
	Lake cisco (lake herring)	<i>Coregonus artedii</i> (Le Sueur, 1818)
	Lake trout	<i>Salvelinus namaycush</i> (Walbaum, 1792)
	Lake whitefish	<i>Coregonus clupeaformis</i> (Mitchill, 1818)
	Least cisco	<i>Coregonus sardinella</i> (Valenciennes, 1848)
	Round whitefish	<i>Prosopium cylindraceum</i> (Pallas, 1784)
Trout-Perches (<i>Percopsidae</i>)	Trout perch	<i>Percopsis omiscomaycus</i> (Walbaum, 1792)

2.5 LOCAL KNOWLEDGE

A list of the main local agencies in Iqaluit that could potentially provide information on Unnamed Lake and its surroundings was developed in collaboration with QE (Table 2). These local agencies were contacted by e-mail and phone.

QE team members present in Iqaluit also tried to establish direct contact with Iqaluit residents and elders but encountered issues due to COVID-19 and could not gather information from this segment of the community as of the time of reporting. Furthermore, representatives of Inukpak Outfitting Inc., a company offering guided services throughout the Southern Baffin region in Nunavut, did not have any information on Unnamed Lake. Iqaluit Hunters and Trappers Association only mentioned that no fishing activities, to their knowledge, were occurring on Unnamed Lake.

Local knowledge was principally obtained from Alexander Flaherty, a Nunavummiut and resident of Iqaluit who owns and operates Polar Outfitting, a company also offering guided trips and expeditions in Nunavut. Mr. Flaherty identified the presence of Arctic char and Ninespine stickleback in Unnamed Lake. He also mentioned that Iqaluit residents occasionally hunt birds and rabbits in the surrounding area. To his knowledge, the primary bird species being hunted within and around Unnamed Lake is Rock ptarmigan (*Lagopus Muta*), a year-round resident species, and Canada goose (*Branta canadensis*), which are present in the area during spring.

Table 2. General information of the local agencies contacted to conduct interviews on Unnamed Lake

Associations	Information	Contact
Iqaluit Hunters and Trappers Association	208 Sinaa Street	Jimmy Akavak
	P. O. Box 629	
	Iqaluit, NU, X0A 0H0	
	Phone: (867) 979-6848	
	amaruq@baffinhto.ca	
Inukpak Outfitting Inc.	3310 Niaqunngusiariaq Street	Martine Dupont
	P. O. Box 11392	
	Iqaluit, NU, X0A 0H0	
	Phone: (867) 222-6489	
	info@inukpakoutfitting.ca	
Polar Outfitting	House 4058	Alexander Flaherty
	Iqaluit, NU, X0A 0H0	
	Phone: (867) 975-1600	
	polaroutfitting@gmail.com	

2.6 SUMMARY

Overall, the information gathered in the context of the desktop review suggested that Unnamed Lake could potentially support an aquatic wildlife population based on the following:

- The hydrographic link between the lake and the Niaqunguk River, as observed on the topographic map, and suggests the potential migration of fish from the river to the lake;
- The bathymetric survey indicating various water depths that could provide multiple fish habitats, such as shallow water bays and deeper pelagic environments;
- The water quality surveys suggest conditions are suitable for aquatic wildlife; and,
- The knowledge of a Nunavummiut suggesting the presence of two species of fish (Arctic char and Ninespine stickleback) in Unnamed Lake, although Iqaluit Hunters and Trappers Association did not report any fishing activities performed by their members on the lake.

3 METHODS

3.1 FIELD OBSERVATIONS

A member of the QE team completed an initial visit to Unnamed Lake on October 13, 2020. The purpose of this visit was to document and photograph existing conditions and observations within and around Unnamed Lake. The observations included:

- The substrate of the shoreline of Unnamed Lake;
 - The presence of notable fish habitat and/or potential spawning ground for fish;
 - The presence of aquatic vegetation;
 - Wildlife tracks in the surrounding terrestrial habitats; and,
 - Incidental observations of fish or aquatic wildlife.
-

3.2 eDNA

eDNA refers to the genetic material shed by organisms in their environment. This genetic material originates from many sources, such as damaged tissue, skin cells, metabolic waste, etc. (Miya *et al.* 2015). Since the first demonstration of the successful use of eDNA for detecting an aquatic vertebrate in 2008 (Ficetola *et al.* 2008), eDNA represents a tool that is increasingly used to detect aquatic organisms in various environments (ponds, streams, rivers, seawater, etc.).

Two main methods are currently available to monitor the presence of fish species in aquatic systems. The first method is based on the detection of a specific species, such as an invasive species or a rare/endangered species that is difficult to catch or observe using traditional practices. The laboratory analysis employed to look for a specific species is called quantitative PCR (qPCR) and is using probe-based chemistry to detect a target species. The second method, which was employed in the context of the fish and fish habitat assessment of Unnamed Lake, is used to monitor fish assemblages with broader taxonomic scopes (Miya *et al.* 2015). This method is called eDNA metabarcoding and is described as the high-throughput multispecies identification using degraded DNA extracted from an environmental sample (Taberlet *et al.* 2012). This allows the detection of the species present in the environment by using primers that targeted specific regions of genes. Those selected regions are hypervariable and contain sufficient information to unambiguously associated a fragment of DNA to a taxonomic family, genus and/or species (Miya *et al.* 2015). To achieve this, the DNA fragment identified is compared to worldwide databases of sequenced DNA of many species.

3.2.1 SAMPLING

eDNA sampling was performed on December 10, 2020 by two members of the QE team. Four water samples were collected at various locations and depths from Unnamed Lake (Map 2; Table 3). One sample was also collected from Crazy Lake (Tasirluk), as a positive control, since this lake is known to support a land-locked population of Arctic char, with Iqaluit residents engaging in subsistence fishing activities throughout the year (Dyck 2005).

At each station, a manual auger was used to create a hole in the ice. Team members waited approximately 15 minutes for the surface water to stabilize and then collected water samples using a bailer (one clean bailer per station). Two litres of water were collected per station and conserved in pre-labeled bottles. Water bottles were placed in individual plastic bags and then into a clean cooler to minimize exposure to sunrays that may degrade

DNA. After the water sampling, a multi-parameters probe (HANNA HI 9829) was used at each station to measure temperature, specific conductivity, and pH at 4 m below the water surface (see Photos 1 and 2).

Table 3. Data collected at each eDNA sampling station

Station ID	Geographic coordinates (DMS; NAD83)		Sampling date	Sampling hour	Approx. depth of sampling (m)	Ice thickness (m)
	Latitude	Longitude				
UNL-ST01	N 63° 47' 09.2"	W 68° 26' 59.2"	2020-12-10	12:40	10	0,55
UNL-ST02	N 63° 46' 59.9"	W 68° 26' 58.3"		12:05	6	0,55
UNL-ST03	N 63° 46' 42.5"	W 68° 26' 34.4"		11:30	20	0,60
UNL-ST04	N 63° 46' 22.1"	W 68° 26' 04.6"		10:20	12	0,55
REF-ST01	N 63° 52' 09.6"	W 68° 29' 29.5"		13:40	18	0,70

3.2.2 FILTRATION

Water samples were brought back to QE office and kept at 4 °C. Water filtration was performed the next day (December 11th) using a peristaltic pump and 1.2 µm self-preserved filters. Prior to the filtration of the water samples, the work space and the filtration unit were cleaned using a 10% bleach solution. After the bleach treatment, the filtration unit was also rinsed with distilled water to remove the excess bleach. The two litres of water sampled per station were then filtered through a 1.2 µm self-preserved filter. The filtration unit used could pump three samples at a time (see Photo 3). The sterilization process (using a 10% bleach solution) was performed between each round of filtration (see Photo 4). After filtration, 1.2 µm self-preserved filters were put back in their individual bag, secured in a cooler and shipped to the analysis laboratory (Precision Biomonitoring Inc) in Guelph, Ontario.

3.2.3 QUALITY CONTROL

Multiple quality control measures were followed during the sampling and filtration process to ensure reliable results:

- One day prior to sampling, the bailers used to sample the water were cleaned using a 10% bleach solution and rinsed with distilled water. The transport bins used to carry the material on the field were also sterilized using the same process;
- QE team members wore clean nitrile gloves to perform the sampling and used a new pair at each station;
- The manual auger used to perform holes in the ice was cleaned between each station;
- The multi-parameter probe used to register the water physicochemical parameters was also cleaned between each station and water sampling was performed before putting the probe in the water at each station;
- The samples were kept away from the sunrays and placed at 4 °C prior to filtration to preserve the DNA present in the water as best as possible;
- Prior to filtration, the work space and filtration unit were sterilized using 10% bleach solution and rinsed with distilled water;
- QE team members wore clean nitrile gloves during the whole filtration process;
- Self-preserved filters were used to minimize manipulation and prevent contamination. The filters developed by Smith-Root are composed of a housing chamber made of hydrophilic plastic that absorbs remaining moisture in the package and preserves eDNA filtered in the membrane at room temperature. Once at the analysis laboratory, the filter housing is removed, and DNA extraction can be performed with the remaining filter membrane (Thomas *et al.* 2019);

- A laboratory blank (BLA-ST01), coming from the filtration of distilled water through a 1.2 µm self-preserved filter, was also sent to the analysis laboratory as a negative control; and,
- As mentioned, a positive control was also sampled in Crazy Lake. The goal of this sampling was to obtain water from a lake where the presence of fish was confirmed, in this case Arctic char, to verify the proper functioning of the eDNA method.

The analysis laboratory (Precision Biomonitoring Inc.) also has its own quality control measures, which are described in the next section.



Photo 1. Auger and multi-parameter probe used during water sampling on Unnamed Lake



Photo 2. A member of the QE team performing water sampling on Unnamed Lake

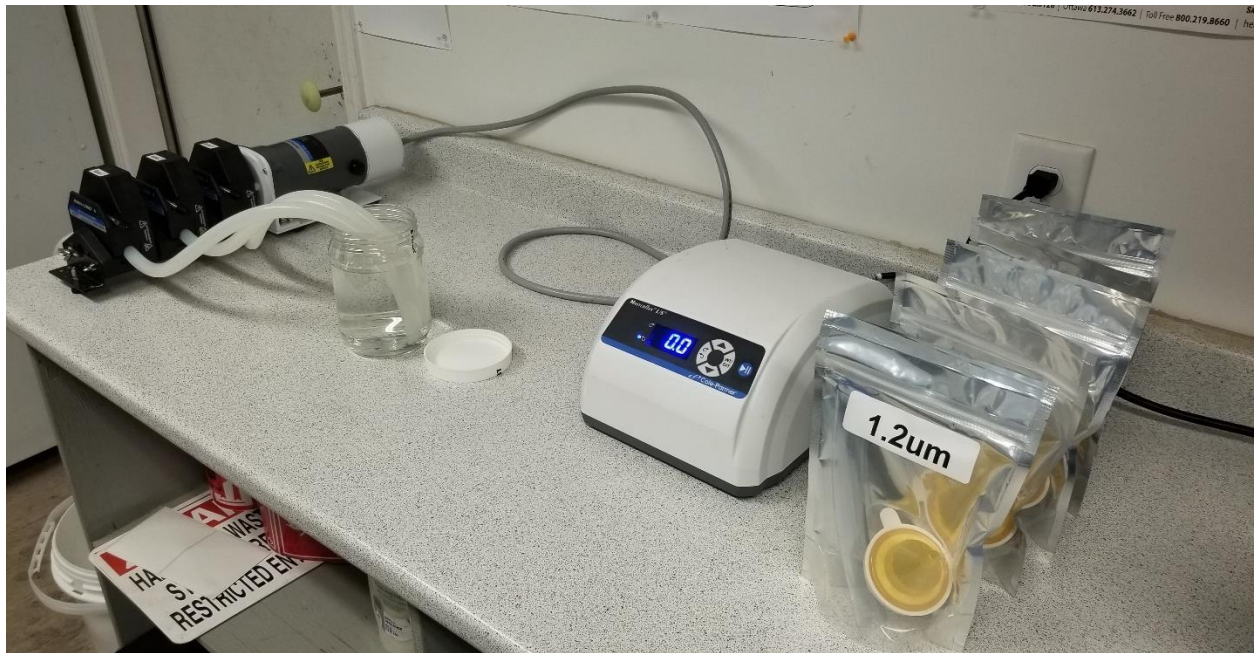
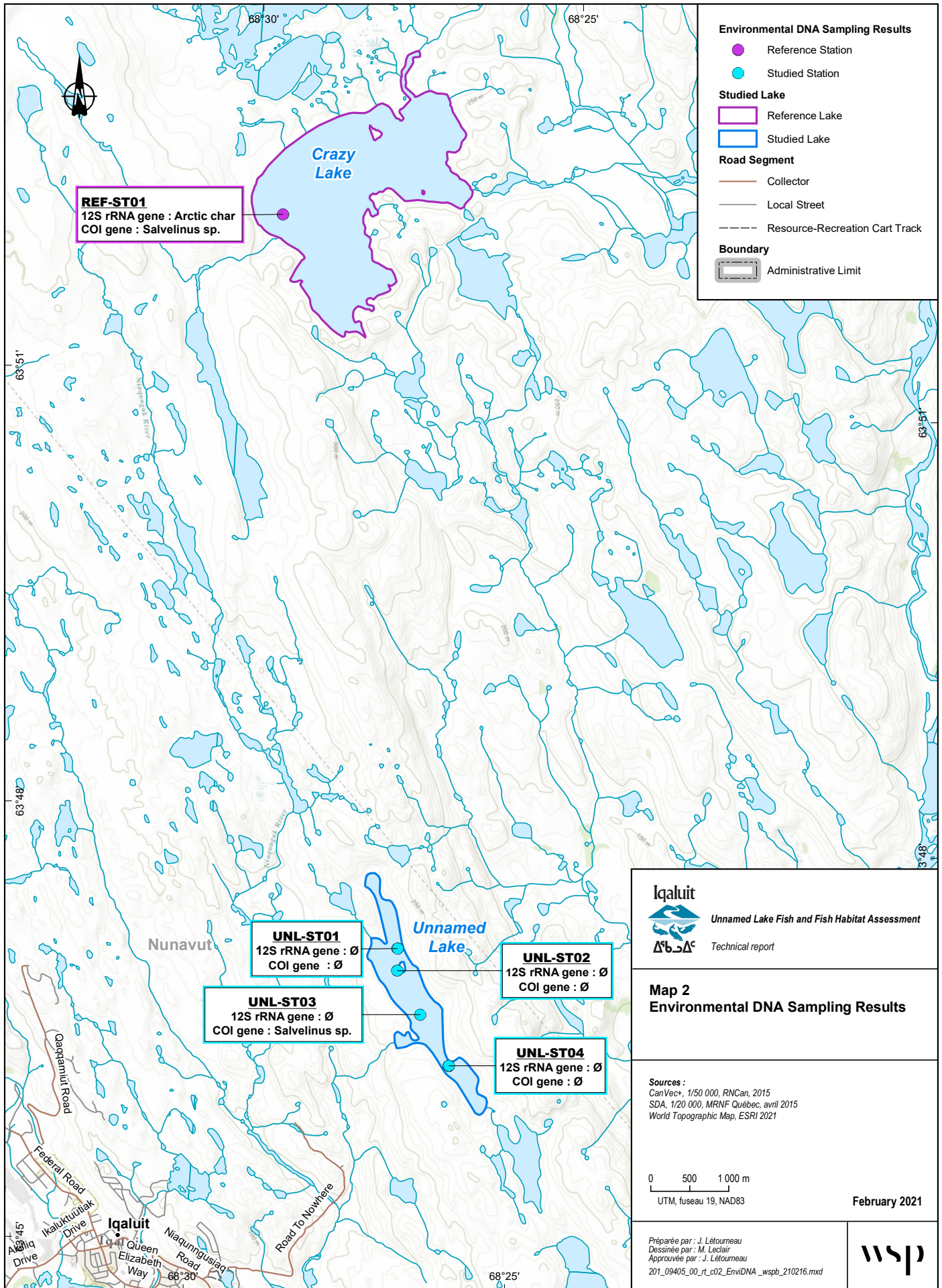


Photo 3. Sterilization of the filtration unit (peristaltic pump and tubing) using a 10% bleach solution



Photo 4. Filtration unit (peristaltic pump and tubing), 1.2 μ m self-preserved filters and water samples



3.2.4 LABORATORY INVESTIGATION

As described in the DNA Metabarcoding Report provided by Precision Biomonitoring Inc. (Appendix C), eDNA from self-preserved filters were extracted using a modified protocol incorporating Qiagen's DNeasy Blood & Tissue Extraction Kit and Qias shredder. Extracted total eDNA was eluted in 200 µL of elution buffer. Each sample was quantified for DNA yield using a Qubit Flex fluorometer (ThermoFisher) to confirm analyzable quantities of DNA.

Amplicon sequencing libraries were prepared from DNA samples following the procedures described in the 16S Metagenomic Sequencing Library Preparation Guide (Illumina). The universal MiFish primers and the PS1 primers were used as the locus-specific sequences to target a hypervariable region of fish mitochondria 12S rRNA gene (approximately 160-190 bp) and COI gene, respectively (approximately 560 bp).

Triplicate PCR reactions were conducted for library preparation. The library quality and quantity were assessed by a Fragment Analyzer Automated CE System with the High Sensitivity Small Fragment Analysis kit (Advanced Analytical) and Qubit® Fluorometer with the Qubit® dsDNA BR Assay kit (Thermo Fisher Scientific). The purified libraries were then normalized and combined in an equal molar ratio for sequencing. PhiX (Illumina) was included to serve as an internal control for sequencing, and a fish control containing 10 different species was included to monitor the entire process. Sequencing was conducted using a MiSeq sequencer with a MiSeq v2 reagent kit (Illumina) and 2x250 paired-end cycles according to the manufacturer's protocol.

Raw sequence reads were filtered using the MiSeq Sequencer System Software (Illumina) to remove low-quality sequences and trimmed to remove adaptor sequences. The sequences were further analyzed using the MiFish pipeline. The sequence database for the fish mitochondria 12S rRNA gene target was MitoFish. The COI gene sequences were further analyzed using Geneious software v10.2.4 (Geneious Biologics) against sequences held in the Barcode of Life Database (BOLD) (<http://www.boldsystems.org>). The analysis software aligns the sequences with the database sequences, identifies fish to taxonomic genus or species and generates data summaries of taxa present.

4 RESULTS

4.1 FIELD OBSERVATIONS

4.1.1 FAUNA AND FLORA

Several wildlife tracks were observed in the snow during the field visit in October 2020 (Photos 5 to 8). The photos of those tracks were identified belonging to two different species, Wolverine (*Gulo gulo*) and Arctic hare (*Lepus arcticus*). Caribou (*Rangifer tarandus*) bones were found on the shore of the lake (Photo 8). Wolverine is listed as “*Special concern*” under the *Species at Risk Act*, meaning that the Wolverine is a wildlife species that may become threatened or endangered due to a combination of biological characteristics and identified threats. The Arctic hare is not listed under the *Species at Risk Act*. Pertaining to Caribou, the *Baffin Island Caribou Management Plan* published in 2018 mentioned that: “*Barren-ground caribou, such as those inhabiting Baffin Island, are known to undergo large cyclical fluctuations in abundance over a 50-90 years period, transitioning from periods of low abundance to high abundance, with phases of increasing and decreasing between. Baffin caribou are currently in the Red Phase, which means they are extremely vulnerable to overharvesting which could cause extirpation or prevent recovery.*” Thus, the barren-ground population of caribou is currently listed as “*Threatened*” under the *Species at Risk Act*, meaning that it is a wildlife species likely to become endangered if nothing is done to reverse the factors leading to its extirpation or extinction. The presence of Caribou bones near Unnamed Lake does not indicate that a herd is actively frequenting the area. The bones may have been brought there by a predator or humans and it is not possible to determine how long the bones have been there. However, in February 2021, a member of QE observed a small herd of three Caribou approximately 10 km north from the Crazy Lake sampling station, and a small herd of five Caribou approximately 8 km north from Iqaluit.

Additionally, despite the late period when the field visit was carried out, some areas surrounding Unnamed Lake were not snow-covered, and plants could be observed. Among those plants, one has been identified as Cowberry or Mountain Cranberry (*Vaccinium vitis-Ida*), based on the presence of fruits (Photos 9 and 10). This plant species is very frequent in alpine, arctic and subarctic regions. The fruits are edible and are usually harvested by residents of northern communities.



Photo 5. Wolverine tracks found in the area of Unnamed Lake



Photo 6. Wolverine tracks found in the area of Unnamed Lake



Photo 7. Arctic hare tracks found in the area of Unnamed Lake



Photo 8. Caribou bones found in the area of Unnamed Lake



Photo 9. *Vaccinium vitis-Ida* observed in the area of Unnamed Lake



Photo 10. *Vaccinium vitis-Ida* observed in the area of Unnamed Lake

4.1.2 HABITAT DESCRIPTION

The terrestrial habitats surrounding Unnamed Lake are characteristic of Canadian arctic/subarctic lacustrine nearshore habitats that are dominated by a boulder substrate with interstitial spaces and little to no vegetation (Callaghan *et al.* 2016; Photos 11 and 12). Based on observations, it was concluded that Unnamed Lake is an oligotrophic lake, like most high-latitude lacustrine environments (Young 2018). Oligotrophic lakes are characterized by very clear water, few nutrients (confirmed by the water quality analysis) and low biomass. No aquatic vegetation was observed in Unnamed Lake during the field visit, but some vegetation may occur during the summer growing season.

The field visit performed in October also allowed the observation of Unnamed Lake shoreline substrate (Photos 13 to 15). The dominant substrate observed was formed by boulders (size >256 mm), cobbles (size between 64-256 mm) and pebbles (size between 16-64 mm). Some areas of the lake were also characterized by the presence of sand. Based on those observations, certain parts of the lake could potentially offer some spawning ground for Arctic char, which usually spawn over gravel (fine pebble) and cobble substrates at depths varying between 2 and 10 m (Richardson *et al.* 2001). This type of littoral habitat could also offer cover for young char hiding between cobbles, rubbles and boulders.



Photo 11. **Representative image of the surroundings of Unnamed Lake**



Photo 12. **Representative image of the surroundings of Unnamed Lake**



Photo 13. Substrate of the littoral area of Unnamed Lake



Photo 14. Substrate of the littoral area of Unnamed Lake



Photo 15. Substrate of the littoral area of Unnamed Lake

4.2 eDNA

4.2.1 QUALITY CONTROL

All samples, except for the laboratory blank (BLA-ST01), yielded DNA quantities over 100 ng mL⁻¹ (range: 584 – 2020 ng mL⁻¹), confirming that extraction generated enough DNA quantities for downstream PCR-based metabarcoding analyses (Table 4).

The very small quantity of DNA found in the laboratory blank indicates that the measures used to avoid contamination between water samples were effective. Indeed, analysis revealed that the DNA found in the laboratory blank came mostly from the Mammal class, likely from unavoidable human DNA shed during the field and filtration operations (Appendix C).

Moreover, the positive control sampled in Crazy Lake yielded the successful identification of Arctic char (Appendix C). No other species of fish were detected. The results obtained validate the known information on Crazy Lake (Dyck 2005).

Table 4. DNA yield obtained from the self-preserved filters for the laboratory blank, Unnamed Lake samples and Crazy lake Samples

Sampling station	Lake	DNA yield (ng mL ⁻¹)
UNL-ST01	Unnamed Lake	584
UNL-ST02		1 780
UNL-ST03		1 610
UNL-ST04		2 020
REF-ST01	Crazy Lake	1 420
BLA-ST01	Laboratory blank	71

4.2.2 UNNAMED LAKE

Table 5 presents the results of the physico-chemical parameters measured at a depth of 4 m at each station in Unnamed Lake prior to water sampling. The specific conductivity was low, with an average of 37.5 $\mu\text{S/m}$, and the pH was slightly basic with values varying between 7.71 and 7.78. These results do not suggest any issues or problems in regard of the water quality for aquatic fauna at the time of sampling.

Table 5. Physico-chemical measurement results in Unnamed Lake

Sampling Station	Temperature ($^{\circ}\text{C}$)	Specific conductivity ($\mu\text{S/m}$)	pH
UNL-ST01	0,66	69,0	7,71
UNL-ST02	0,48	19,0	7,73
UNL-ST03	0,47	17,0	7,73
UNL-ST04	0,51	45,0	7,78

As described in the DNA Metabarcoding Report provided by Precision Biomonitoring Inc. (Appendix C), very little fish DNA was identified from the four water samples collected in Unnamed Lake, despite high levels of DNA derived from the filters (Table 6). The largely used 12S rRNA marker for freshwater fish detection did not yield any successful identification for any sample (except for the reference sample in Crazy Lake, REF-ST01). As a secondary line of analysis, Precision Biomonitoring Inc. conducted metabarcoding of the samples using the COI gene, which detected a small amount of *Salvelinus* in sample UNL-ST03. However, the findings of this analysis could only allow identification to genus. The COI gene also yielded small amounts of arthropod DNA at each station which could not be identified at lower detailed taxonomic level.

Table 6. eDNA results for the water samples coming from Unnamed Lake

Sampling station	12S rRNA gene results	COI gene results
UNL-ST01	Fish species not detected	Arthropods Fish species not detected
UNL-ST02	Fish species not detected	Arthropods Fish species not detected
UNL-ST03	Fish species not detected	Arthropods <u><i>Salvelinus sp.</i></u> detected
UNL-ST04	Fish species not detected	Arthropods Fish species not detected

The eDNA results are discussed in the next section, as they pertain to Unnamed Lake field observations and the background information and literature.

5 DISCUSSION

Based on information provided by Iqaluit residents and field observations, the area of Unnamed Lake is frequently used by wildlife, such as birds (Ptarmigan and Canada goose) and mammals (Arctic hare, Wolverine and potentially Caribou). Caribou and Wolverine are registered under the *Species at Risk Act*. Some local resident also used this land for hunting, but no information confirmed if Unnamed Lake is actively used for fishing. One resident of Iqaluit did confirm, to his knowledge, the presence of Arctic char and Ninespine stickleback in Unnamed Lake, which are not listed as aquatic species at risk by DFO (based on the *Species at Risk Act*).

The eDNA analysis did not completely confirm and validate these observations. The initial analysis, based on the 12S rRNA gene, did not yield any successful identification for any sample in Unnamed Lake, but successfully confirmed the presence of Arctic char in Crazy Lake. However, the results based on the COI gene did indicate the presence of a small amount of the *Salvelinus* genus DNA at one station of Unnamed Lake (UNL-ST03).

Two explanations may explain the eDNA results:

It is known that Arctic Char exhibit many different life-history traits across its distribution range and that different populations show localized adaptations to their environment (Young 2018). This species is known to have evolved an impressive intraspecific genetic biodiversity (Young 2018; Kristjánsson *et al.* 2011), after colonizing geologically young Arctic lakes where environmental conditions are difficult and interspecific competition is very low, as they are often the only fish species present (Young 2018; Klemetsen *et al.* 2003; Kahilainen and Lehtonen 2002). Therefore, the inability to provide a reliable observation or confirmation for this species suggests that more DNA sequence variation needs to be collected in northerly latitudes to sustain further metabarcoding work in these more remote areas. As mentioned by Precision Biomonitoring Inc. in the DNA metabarcoding report (Appendix C), more direct sequencing of fish species in northerly latitude fisheries in Nunavut and other remote Canadian and First Nations territories should be used to populate DNA barcoding databases in order to facilitate more informed metabarcoding efforts.

Secondly, as suggested by Precision Biomonitoring, the lack of detection may also reflect reality, in that northerly waterbodies hold less biomass and biodiversity than lower latitudes, including some lakes in which there are no fish present at all. Therefore, it is possible that sampling at four different stations in Unnamed Lake would not have been sufficient to detect eDNA of fish at low abundance, with attendant highly diluted eDNA. It is possible that fish are in very specific regions of the lake during winter and that their metabolic activity is highly reduced, thus resulting in downregulated eDNA shedding. Based on these suggestions, if further investigation is required, several improvements could be made such as:

- Conducting field sampling during the optimal monitoring period (during suspected spawning periods) to confirm site detection for externally reproducing fish species;
- Sampling greater volumes of water per filtration event;
- Sampling at different depths in the water column to account for stratification of the lake; and,
- Pooling samples to increase the probability of detection.

As for Ninespine stickleback presence, mentioned by an Iqaluit resident, the eDNA method could not confirm this assumption. However, it is theoretically possible that this species could be present in the lake, since it is present in various habitats and is tolerant to difficult conditions. As mentioned by Richardson *et al.* (2001), although sticklebacks are usually found in association with dense vegetation and shallow bays of the lake, they may also frequent open water areas over sand and gravel beaches with sparse vegetation. Since the topography map of the area of Unnamed Lake suggests a hydrographic link between the lake and Niaqunguk River, it is not unlikely that species of fish, such as Ninespine stickleback, could migrate towards Unnamed Lake during certain periods of the year.

6 RECOMMENDATIONS

From the information gathered in the context of the Fish and Fish Habitat Assessment project, Unnamed Lake should be considered as suitable aquatic habitat.

Based on this determination, the water withdrawal project in Unnamed Lake must be regulated under the *Fisheries Act* (2019) and the *Species at Risk Act* (2002), which were implemented following the original guiding principle set out in the federal *Policy for the Management of Fish Habitat* (1986). The principal goal of that legislation is to ensure no-net-loss of fish habitat productivity in Canadian waters (Hancock 2020).

Unnamed Lake does not support a population of aquatic SAR and does not represent a critical habitat according to DFO criteria. However, it likely supports a small population of Arctic char and has the potential to shelter Ninespine stickleback at certain periods of the year, potentially coming from Niaqunguk River. Therefore, the impact on fish and fish habitat in Unnamed Lake should be low or non-existent if DFO's fish and fish habitat protection measures are applied throughout the withdrawal project.

Key mitigation measures to prevent the injury or death of fish should be implemented using the DFO's interim code of practices for end-of-pipe fish protection screens for small water intakes in freshwater (<https://www.dfo-mpo.gc.ca/pnw-ppe/codes/screen-ecran-eng.html>). As determined by DFO, this code of practice can be used if:

- There are no aquatic species at risk present in the affected area (which is the case for Unnamed Lake);
- The water withdrawal is for small-scale water intakes, where the water intake flow rate is up to 0.150 m³/s, or 150 litres per second (L/s). If it is estimated that the water withdrawal from Unnamed Lake will be higher than 0.150 m³/s, a request for a project near water review should be submitted to DFO; and,
- All other applicable measures are put in place to protect fish and fish habitat.

End-of-pipe fish protection includes the design of a suitable fish screen and the maintenance and cleaning of the screen to avoid any entrainment and impingement of adult fish, eggs and larval fish. The following practices, listed by DFO (<https://www.dfo-mpo.gc.ca/pnw-ppe/codes/screen-ecran-eng.html>), should also be followed when installing the fish screen at the end of the pipe:

- Plan in water work, undertaking or activity to respect timing windows to protect fish including their eggs, juveniles, spawning adults and/or the organisms upon which they feed and migrate;
- Place screens away from natural or man-made structures that may attract fish that are migrating, spawning, or in rearing habitat;
- Place screens in waters with low concentrations of fish throughout the year;
- Orient the screen so any natural water flow passes across the surface of the screen material;
- Place screens a minimum of 30 cm above the bottom of the watercourse to prevent the entrainment of sediment and benthos that dwell in the substrate;
- Ensure all openings for guides and seals are smaller than the opening width of the screen material (2.54 mm) so fish cannot pass through;
- Ensure there is enough structural support to prevent sagging or collapsing of the screen panel;
- Account for the areas blocked by supports while meeting the effective screen area recommended in this code of practice;
- Protect large screens with trash racks fabricated of bar (150 mm spacing is typical) or grating in areas where there is debris loading (i.e. woody material, leaves or algae mats);
- Check the approach velocity directly in front of the screen to ensure it does not exceed the designed approach velocity at any location;
- Avoid withdrawing water from the littoral zone when possible; and,

- When possible, avoid withdrawing water, or reduce the rate of water withdrawal, during critical timing windows to diminish the likelihood of entraining eggs and larval fish.

The restricted activity timing windows should be based on the spawning period of Arctic char. As outlined by the DFO, Arctic char spawns during the Fall, and therefore, the period between September 1 and June 30 should be avoided for the installation of the pipe in Unnamed Lake to ensure the protection of fish during spawning and incubation periods when spawning fish, eggs and fry are vulnerable to disturbance or sediment.

Furthermore, the protection of the shores of Unnamed Lake should be assured by maintaining an undisturbed buffer zone between areas of on-land activity and the high-water mark of the lake and using methods to prevent soil compaction, such as swamp mats or pads. The project should also include proper sediment control during the installation of infrastructure.

Finally, it is also highly recommended to avoid the installation of pipes in susceptible Arctic char spawning ground area of Unnamed Lake (see the description of potential Arctic char spawning site, Section 4.1.). The water withdrawal should also be controlled to ensure the replenishment of the lake and avoid any drying up of the littoral and shoreline habitats.

7 CONCLUSION

In summary, it is likely that Arctic char are present in low abundance in Unnamed Lake, based on the information provided by an Iqaluit resident, the small amount of *Salvelinus* DNA found using the COI gene at one sampling station, and the presence of adequate habitat observed during the field visit in October. The presence of Ninespine stickleback, suggested by an Iqaluit resident, could not be proven using eDNA, but it is possible fish from this species could migrate to Unnamed Lake through the Niaqunguk River.

Water withdrawal from Unnamed Lake is suspected of having a low to non-existent impact on fish and fish habitat, since the lake does not provide habitat for aquatic species at risk and is not a defined critical habitat. Nevertheless, the measures suggested by DFO to ensure fish and fish habitat protection should be followed to avoid any potential fish death or fish habitat disturbance.

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APPENDIX

A

BATHYMETRIC SURVEY OF
UNNAMED LAKE

September 3, 2019

City of Iqaluit
City Hall, P.O. Box 460
Iqaluit, NU X0A 0H0

ISSUED FOR USE
FILE: ENG.GEOP03157-01
Via Email: m.hamp@city.iqaluit.nu.ca

Attention: Matthew Hamp, Director of Public Works and Engineering

Subject: Iqaluit DFO Bathymetric Lake Surveys

1.0 INTRODUCTION

Tetra Tech Canada Inc. (Tetra Tech) was retained by the City of Iqaluit (the City) to conduct bathymetric surveys on two lakes. The two lakes surveyed were Unnamed Lake and Lake Geraldine, approximately 5 km and 1.5 km northeast of Iqaluit, NU, respectively.

The City has historically obtained its fresh water supply from the Lake Geraldine water reservoir. In 2018 a shortage of water in the reservoir was experienced following the spring freshet, requiring the City to supplement the drinking water supply from an additional source. The City's objective is to develop a sustainable long-term water supply. Unnamed Lake has been identified as a possible alternative water supply source. A bathymetric survey is required for the lake to provide an accurate map of lake morphometry and a calculation of potential available water volume. Additionally, a bathymetric survey was requested for Lake Geraldine to assess its current conditions.

The City required the bathymetric surveys to be completed in accordance with Scenario E of Application of the NWT Winter Water Withdrawal Protocol with Bathymetric Profiles of Select Small Lakes in the Mackenzie Delta Region (DFO 2005). Data was collected between July 23 and July 25, 2019.

2.0 EQUIPMENT

Tetra Tech utilized an Ohmex SonarMite single beam acoustic echosounder to complete the bathymetric survey. The SonarMite has a 235 kHz active transducer, a beam width of $\pm 4^\circ$ and a measurement accuracy of ± 2.5 cm. The SonarMite unit employs digital signal conditioning and analysis circuitry to digitally output water depths at a rate of 2 Hz. The water depth measurements are recorded to a field laptop computer.

A Topcon HiperXT real-time kinematic (RTK) GPS system was used to provide positioning information. The GPS base station was positioned on shore for each lake and broadcast positioning correction information. The GPS rover was setup on the boat to receive positioning corrections from the base and integrate corrected positions with the echosounder data as the data was recorded. Given appropriate satellite constellations at the time of data collection, the RTK system typically provides 2 cm horizontal accuracy and 2 cm to 3 cm vertical accuracy.

The bathymetric survey for Unnamed Lake was conducted from a 9.5-foot zodiac with a Yamaha 4 HP outboard motor. The bathymetric survey on Lake Geraldine was conducted from a 12-foot Princecraft aluminum boat with a Yamaha 4 HP outboard motor.

3.0 DATA COLLECTION

The bathymetric survey was carried out by James Mickle, P.Geoph. (AB) of Tetra Tech's Calgary office. Data was collected between July 23 and 25, 2019. Survey support was provided by Qairrulik Outfitting, based in Iqaluit. Lake Geraldine was accessible by truck, while Unnamed Lake was only accessible by ATV. Surveys were carried out using the equipment listed in Section 2.0.

Survey navigation was achieved using a navigation tracking software package that displayed the boat location and pre-programmed track lines in real time for the boat operator. Data was collected with three longitudinal profiles and transverse profiles spaced every 100 m on Unnamed Lake with the location of the profiles optimised to account for the shape of the lake. For Lake Geraldine, a single longitudinal profile and transverse lines every 100 m were collected. In addition to the bathymetric lines, circumferential data representing the complete shoreline outline (0 metre water depth) of both lakes was obtained by digitising a Sentinel-2 L1C satellite image acquired on July 23, 2019. To fix the lake surface elevations at the time of the survey, 10 RTK GNSS locations distributed around each lake were collected, the average value of which was used; at both lakes. The maximum and minimum water level elevations for each lake were within +/- 1 cm of the average value. The averaged value for both lakes was further corrected using a temporary field control point that was measured during both the bathymetric survey and the August 2019 LiDAR program

Based on the shape of both lakes and the paper "Application of the NWT Winter Water Withdrawal Protocol with Bathymetric Profiles of Select Small Lakes in the Mackenzie Delta Region, 2005", it is estimated that the volumes calculated potentially underestimate total volumes by 5% in the case of Unnamed Lake and between 5% and 10% at Geraldine Lake at the time of the survey. The larger potential error for Geraldine Lake is due to the presence of an inaccessible lobe at the south end due to very shallow water conditions at the time of the bathymetric survey.

Three sonar depth calibrations were completed for each lake as part of Tetra Tech's standard Quality Control/Quality Assurance (QA/QC) procedures.

3.1 Unnamed Lake

The bathymetric survey on Unnamed Lake was completed between July 24 and 25, 2019. Data collection tracks for the survey are plotted on Figure 1.

Two small bays can be seen in Figure 1 at the north end and west side of the lake. Tetra Tech was able to access a small portion of the north bay, but the water level was so shallow that the sonar system could not provide depth readings. The west bay was inaccessible due to shallow water depths (<0.5 m) and numerous rocks. Based on field observations, it is assumed that both bays will be isolated from the main water body during winter (frozen) conditions. Therefore, the two bays would not affect the under ice water volume.

3.2 Lake Geraldine

The bathymetric survey on Lake Geraldine was completed on July 23, 2019. Data collection tracks for the survey are plotted on Figure 2.

The southeast arm of Lake Geraldine was inaccessible due to a section of dry land approximately 50 m in length preventing the boat from passing. Extremely rough and rocky terrain was noted on the land around the southeast arm, preventing movement of the boat to that area. Small rocks were also noted protruding from the water in the middle of the southeast arm, so it is assumed that the water depth was minimal. This portion of Lake Geraldine was

essentially cut off from the main lake at the time of the survey and thus would not contribute to the immediate water withdrawal capacity of the lake.

Two small islands were present at the time of the survey. These are seen as small gaps in the bathymetry data in Figure 2. Water was extremely shallow (<0.3 m) with numerous rocks in the channel between the larger island and the western shore, preventing a data collection track in this channel. Due to the low water level, this channel is expected to freeze to ground in the winter and therefore not affect the under ice water volume.

4.0 DATA PROCESSING

Data processing consisted of the following steps:

- Submitting static GPS base station observation files to the Natural Resources Canada (NRCAN) online precise point positioning (PPP) correction system;
- Applying PPP horizontal and vertical corrections to all positioning information;
- Applying depth corrections to bathymetry data based on sonar depth calibrations;
- Averaging the ten water level elevations recorded for each lake to establish an elevation for each lake surface at the time of the survey (see Section 5.1);
- Digitizing the shoreline from georeferenced satellite imagery taken within a couple of days of the bathymetric survey to assign the water surface outline (i.e., 0 m depth contour) at the time of the bathymetric survey;
- Contouring the water depth data using a Kriging algorithm;
- Plotting depth contour results on georeferenced air photos;
- Calculating the lake surface areas using the digitized shoreline; and
- Calculating the required lake volumes using the trapezoidal method of calculation and subtracting the required estimated ice thickness (2.0 metres) as per the DFO Protocol (2015) for calculating water withdrawal volumes above the tree line.

4.1 QA/QC

Tetra Tech's QA/QC procedures included conducting sonar depth calibrations in multiple locations on each lake. This was done by manually measuring the lake depth using a weighted tape measure and comparing that number to the depth displayed by the sonar at the same location. Depth calibrations are typically conducted in three locations per lake. Differences between the manually measured depths and the sonar readings are plotted and used to determine what depth corrections are required to be applied to the bathymetric results.

Additionally, crossline consistency is checked as part of the QA/QC procedures. At locations where transverse survey lines intersect longitudinal survey lines, depth values are compared between the two survey passes over the same location. Discrepancies in depth values between the two passes could be indicative of positioning errors. For these surveys, no significant discrepancies between crosslines were noted.

5.0 RESULTS

Survey results for Unnamed Lake and Lake Geraldine are presented in Figures 1 and 2, respectively. Each figure shows a bathymetric colour contour map. A table has been included on each figure summarizing the required information from DFO Protocol for Winter Water Withdrawal in the Northwest Territories. This includes:

- Lake ID;
- Coordinates;
- Surface area;
- Total lake volume;
- Under ice volume;
- Max expected ice thickness value used; and
- Calculated 5% withdrawal volume.

5.1 Vertical Data Positioning

A lake surface elevation has been provided on each figure for the corrected CVD28 water elevation at the time of the survey. The absolute accuracy of this elevation measurement is approximately ± 3 cm. The water surface elevation of both lakes been further corrected using a common survey point measured during both the bathymetry and LiDAR programs, and a vertical shift of -1.78 cm was applied to the GNSS position data collected to align the lake bottom elevations with the LiDAR program results.

An x,y,z DEM based on a 2 metre grid size has been generated for the bathymetric data so that an elevation contour model can be generated for the region by combining the sonar and LiDAR datasets.

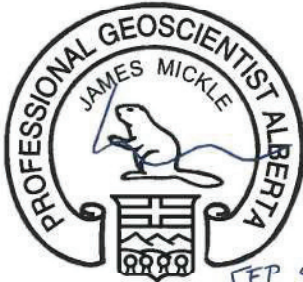
6.0 LIMITATIONS OF REPORT

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We trust this document meets your present requirements. If you have any questions or comments, please contact the undersigned.

Respectfully Submitted,
Tetra Tech Canada Inc.



SEP 4, 2019
FILE: ENG.GEOP03157-01
FILE: ENG.GEOP03157-01
FILE: ENG.GEOP03157-01

Prepared by:
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/jf



SEPTEMBER 4, 2019
FILE: ENG.GEOP03157-01
FILE: ENG.GEOP03157-01
FILE: ENG.GEOP03157-01

Reviewed by:
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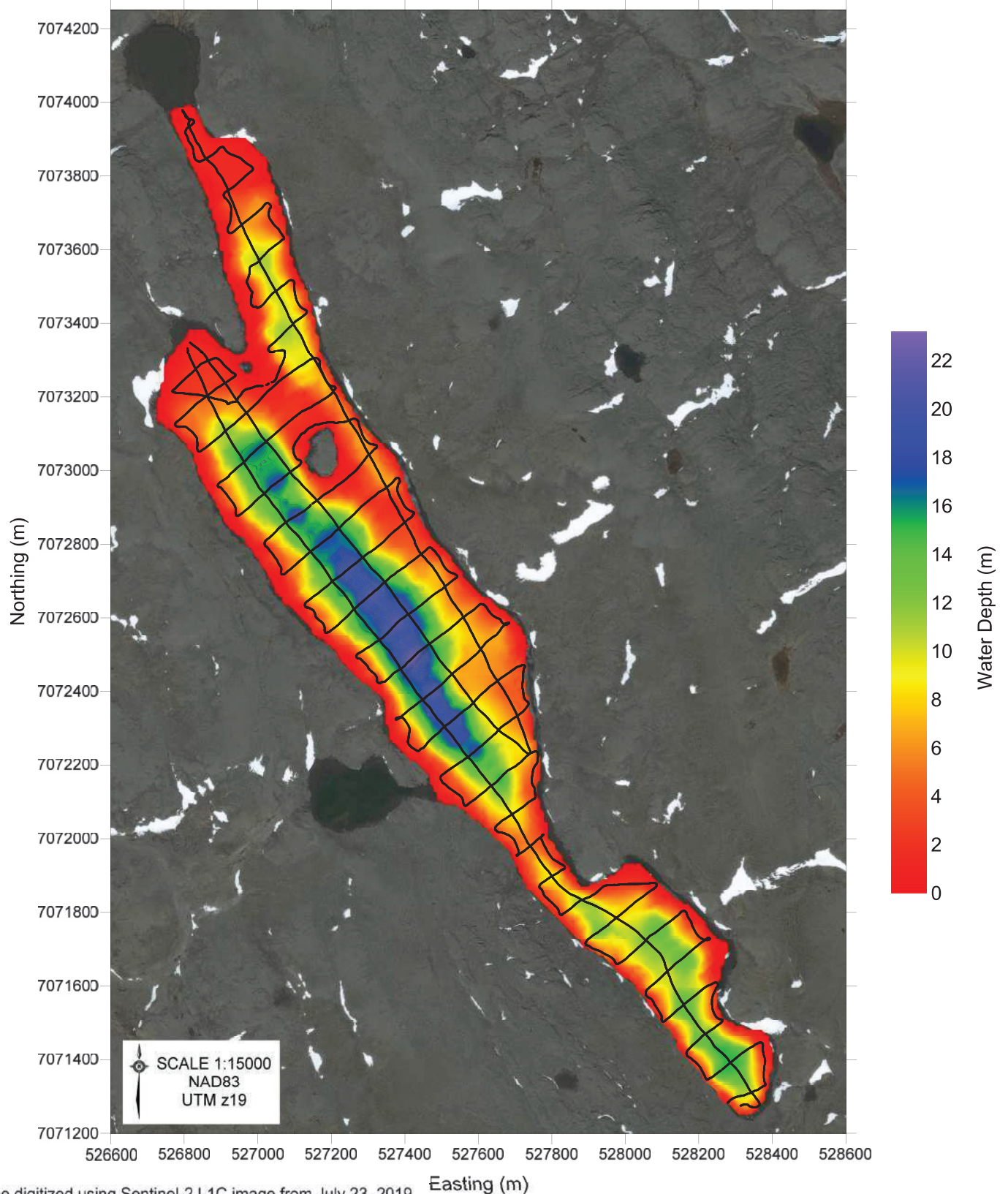
PERMIT TO PRACTICE TETRA TECH CANADA INC.

Signature _____

Date _____

PERMIT NUMBER: P 018

NT/NU Association of Professional
Engineers and Geoscientists



Shoreline digitized using Sentinel-2 L1C image from July 23, 2019
 Displayed background image from HERE
 Water level elevation at time of survey 203.32 m in CVD28 datum using HT2_1997 geoid model

LEGEND

— Data collection tracks

DFO Lake Summary

Lake ID: Unnamed Lake
Centre Coordinates: 527,432 m E 7,072,564 m N
Surface Area: 911,300 m²
Total Lake Volume: 6,616,900 m³
Under Ice Volume: 4,737,900 m³
Maximum Expected Ice Thickness Value Used: 2.0 m
Calculated 5% Withdrawal Volume: 236,895 m³

CLIENT



IQALUIT DFO BATHYMETRIC LAKE SURVEYS

Unnamed Lake Bathymetry Depth Results

Data Collected July 24-25, 2019

PROJECT NO.	DWN	CKD	APVD	REV
ENG.GEOP03157-01	CB	RJM	PIF	0
OFFICE	DATE			
FRA-EDM	July 31, 2019			

Figure 1

LIMITATIONS ON USE OF THIS DOCUMENT

GEOPHYSICAL

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1.7 ENVIRONMENTAL AND REGULATORY ISSUES

Unless stipulated in the report, TETRA TECH has not been retained to explore, address, or consider and has not explored, addressed, or considered any environmental or regulatory issues associated with the development of the site.

1.8 NATURE AND EXACTNESS OF SOIL AND ROCK DESCRIPTIONS

Classification and identification of soils and rocks are based upon commonly accepted systems and methods employed in professional geotechnical practice. This report contains descriptions of the systems and methods used. Where deviations from the system or method prevail, they are specifically mentioned.

Classification and identification of geological units are judgemental in nature as to both type and condition. TETRA TECH does not warrant conditions represented herein as exact, but infers accuracy only to the extent that is common in practice.

Where subsurface conditions encountered during development are different from those described in this report, qualified geotechnical personnel should revisit the site and review recommendations in light of the actual conditions encountered.

1.9 LOGS OF TESTHOLES

The testhole logs are a compilation of conditions and classification of soils and rocks as obtained from field observations and laboratory testing of selected samples. Soil and rock zones have been interpreted. Change from one geological zone to the other, indicated on the logs as a distinct line, can be, in fact, transitional. The extent of transition is interpretive. Any circumstance which requires precise definition of soil or rock zone transition elevations may require further investigation and review.

1.10 STRATIGRAPHIC AND GEOLOGICAL INFORMATION

The stratigraphic and geological information indicated on drawings contained in this report are inferred from logs of test holes and/or soil/rock exposures. Stratigraphy is known only at the locations of the test hole or exposure. Actual geology and stratigraphy between test holes and/or exposures may vary from that shown on these drawings. Natural variations in geological conditions are inherent and are a function of the historic environment. TETRA TECH does not represent the conditions illustrated as exact but recognizes that variations will exist. Where knowledge of more precise locations of geological units is necessary, additional investigation and review may be necessary.

1.11 SURFACE WATER AND GROUNDWATER CONDITIONS

Surface and groundwater conditions mentioned in this report are those observed at the times recorded in the report. These conditions vary with geological detail between observation sites; annual, seasonal and special meteorological conditions; and with development activity. Interpretation of water conditions from observations and records is judgmental and constitutes an evaluation of circumstances as influenced by geology, meteorology and development activity. Deviations from these observations may occur during the course of development activities.

APPENDIX

B

WATER QUALITY SAMPLING
MEMORANDUM FOR UNNAMED
LAKE

To:	Josip Deronja, Engineering Manager City of Iqaluit	From:	Erica Bonhomme, Project Manager Nunami Stantec
File:	144902884	Date:	October 1, 2019

Reference: City of Iqaluit 2019 Emergency Water Supplementation Program – Water Quality Sampling Unnamed Lake

INTRODUCTION

Nunami Stantec Limited (Nunami) is pleased to submit this Water Quality Sampling Memorandum (Memo) for the City of Iqaluit's Unnamed Lake to Apex River Water Withdrawal Program in 2019 (herein referred to as the "2019 Emergency Water Supplementation Program"). This Memo addresses the baseline water quality parameters of Table 1 of the Northern Health guidance for water source approval (Northern Health, 2012), provided to Nunami by A.Gill August 15, 2019, and additionally as required based on correspondence between A.Gill and E.Bonhomme of Nunami up to August 21, 2019. Water quality samples were collected on July 4 and September 12, 2019 at five locations within Unnamed Lake, as shown on Figure A-1 (attached). Water quality sampling was outside of the scope outlined in Nunami's Operational Monitoring Plan (Nunami Stantec 2019).

The objective of water quality monitoring activities was to establish confirm suitable water quality in Unnamed Lake as a source of drinking water for the City of Iqaluit, prior to transfer to Lake Geraldine reservoir. This memo presents the methodology, regulatory framework, results and conclusions.

REGULATORY FRAMEWORK

Surface water chemical analytical results are compared to the following specific standards that are considered applicable to Unnamed Lake:

- Guidelines for Canadian Drinking Water Quality – Summary Table (Health Canada 2019),
- Northern Health Public Health Protection – Table 1. Required Water Quality Parameters (Northern Health 2019).

METHODS

Field staff conducted surface water sampling in accordance with Stantec Consulting Ltd. (Stantec)'s Standard Operating Procedures. Special care was taken at the sampling locations to not disturb sediment to minimize the amount that entered sample containers. In-situ physical water quality parameters (temperature, pH, dissolved oxygen, and conductivity) were measured using a YSI 556 multi meter.

All surface water samples were collected in laboratory-supplied containers with appropriate preservative and placed in insulated coolers. Samples were uniquely labeled, and control was maintained using chain of custody forms. Sample locations and the analyses performed for each sample are shown in Table 1 below.

Reference: City of Iqaluit 2019 Emergency Water Supplementation Program – Water Quality Sampling Unnamed Lake

Table 1 Sample location summary table

Location ID	Parameters Sampled	Date Sampled	Latitude	Longitude
SW19-01	General Chemistry, benzene/ toluene/ ethylbenzene/ xylene (BTEX) and Petroleum Hydrocarbons (PHCs), Metals, and Microbiology	July 4, 2019 and September 12, 2019 (BTEX, PHC, and mercury)	63.781474	-68.45223
SW19-02	General Chemistry, BTEX and PHCs, Metals, and Microbiology	July 4, 2019 and September 12, 2019 (BTEX, PHC, and mercury)	63.77787	-68.44533
SW19-03	General Chemistry, BTEX and PHCs, Metals, and Microbiology	July 4, 2019 and September 12, 2019 (BTEX, PHC, and mercury)	63.77353	-68.43791
SW19-04	General Chemistry, BTEX and PHCs, Metals, and Microbiology	July 4, 2019 and September 12, 2019 (BTEX, PHC, and mercury)	63.77522	-68.44123
SW19-05	General Chemistry, BTEX and Petroleum Hydrocarbons, Metals, and Microbiology	July 4, 2019 and September 12, 2019 (BTEX, PHC, and mercury)	63.77502	-68.44905

RESULTS

Analytical results for surface water are provided in Table A-1, attached. All water quality parameters were reported to be below the applicable standards with the exception of Total Alkalinity at all five locations and Lagelier Index (at 4°C) at four locations. Total alkalinity was below the lower threshold put forth in the Northern Health Public Health Protection Table 1, but it is noted that the lower limit of 30 identified in this standard is listed as approximate. Additionally, results for Langelier Index were slightly below the lower limit given by the Northern Health Public Health Protection Table 1, which is also listed as approximate.

Results for BTEX and PHC were all below the laboratory's detection limit. Total coliforms and Escherichia coli (E.Coli) were reported as zero colony-forming units (cfu) for all sample locations. Nutrient and metals analytical results were generally low, with many parameters reporting values below the laboratories detection limit.

Field parameters were measured using a YSI multi-parameter probe and are summarized in Table 2 below.

October 1, 2019

Josip Deronja, Engineering Manager

Page 3 of 4



Reference: City of Iqaluit 2019 Emergency Water Supplementation Program – Water Quality Sampling Unnamed Lake

Table 2 Field parameters summary table

Location ID	Temperature (°C)	pH	Conductivity (µs/cm)
SW19-01	9.03	7.03	30
SW19-02	9.04	7.05	30
SW19-03	9.00	6.96	29
SW19-04	8.62	7.06	29
SW19-05	12.22	7.8	34

CONCLUSIONS

Based on the results of the water quality sampling program conducted at Unnamed Lake, the following conclusions can be made:

- Water quality in Unnamed lake is considered to be good based on the reported analytical results,
- BTEX and PHC were below laboratory detection limits,
- Total Coliforms and E.Coli were reported at 0 cfu/100ml.

REFERENCES

Health Canada. 2019. Guidelines for Canadian Drinking Water Quality – Summary Table. Water and Air Quality Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario.

Northern Health. 2012. PHP Guideline of Required water quality parameters for Water Source Approval. Accessed September 21, 2019 at:

https://www.northernhealth.ca/sites/northern_health/files/services/environmental-health/documents/guidelines-required-water-quality-parameters.pdf

Nunami Stantec. 2019. "City of Iqaluit 2019 Emergency Water Supplementation Program - Operational Monitoring Plan." Iqaluit.

Nunami Stantec Ltd.

Digitally signed by
Andrew Sullivan
Date: 2019.10.01
09:59:19 -03'00'

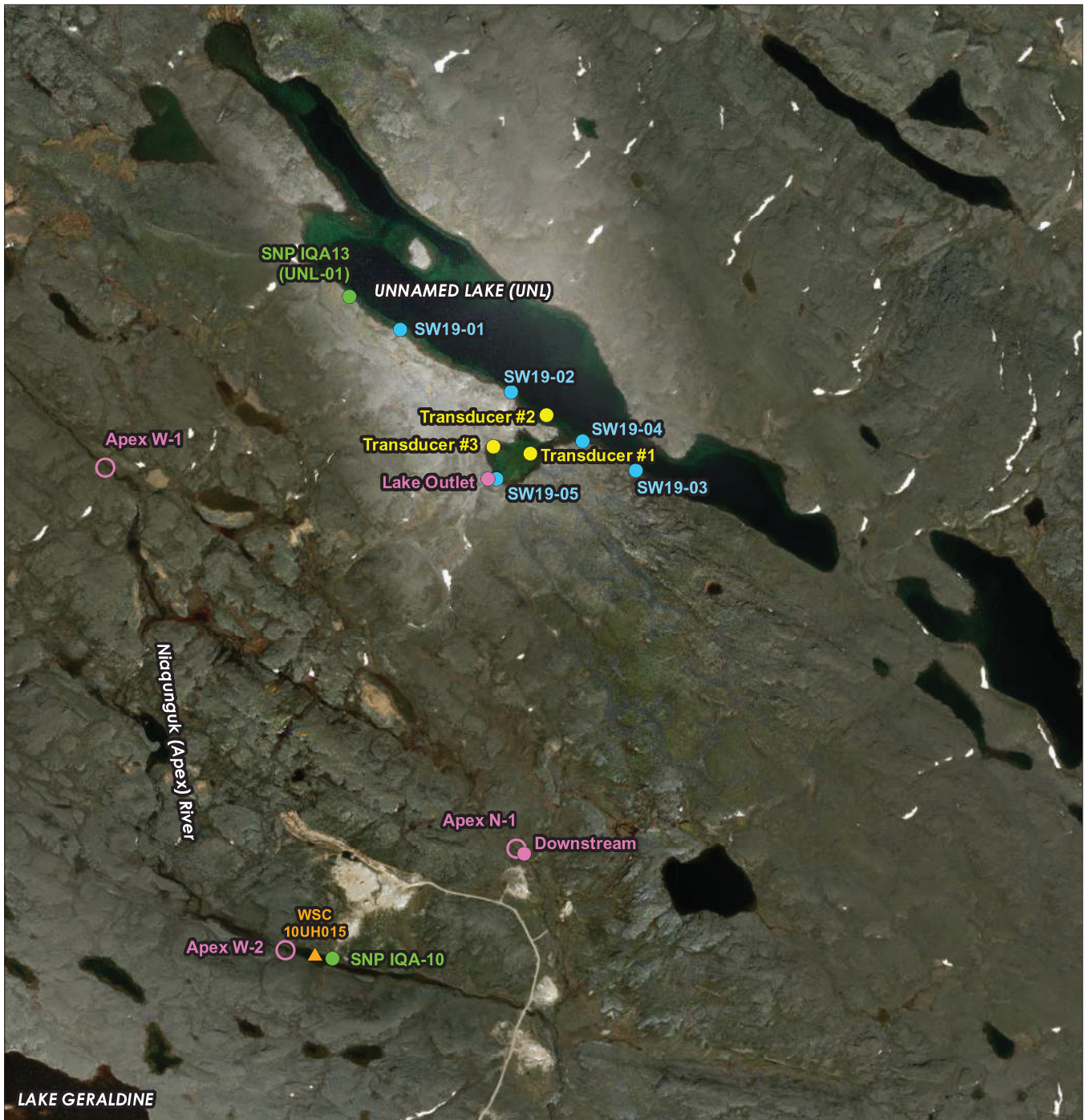
Andrew Sullivan P.Eng. (NS); NT/NU registration pending
Environmental Engineer
Phone: (902) 468-7777
Andrew.sullivan@stantec.com

2019.09.30
13:19:26
-06'00'

Erica Bonhomme M.Sc., P.Geo.
Team Lead, Environmental Services
Phone: 867-920-2882
erica.bonhomme@stantec.com

Attachment:

Figure A-1 – Sample locations
Table A-1 – Analytical Water Quality Results



Legend

- Water Quality Sampling Location 2019
- Water Level Monitoring Location
- Flow Measurement July 2019
- SNP Location
- ▲ Water Survey of Canada Station
- 2019 Emergency Pumping Project Flow Monitoring Location

0 200 400 Metres
(At original document size of 8.5x11)
1:25,000



Project Location
Iqaluit,
Nunavut

Prepared by ACampigotto on 2019-09-23
Reviewed by EBonhomme on 2019-09-23

Client/Project
City of Iqaluit 2019 Emergency Water
Supplementation Project

144902884

Figure No.
1

DRAFT

Title
**Apex River and Unnamed Lake Data
Collection and Monitoring Locations**

Notes

1. Coordinate System: NAD 1983 UTM Zone 19N
2. Imagery Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Table A-1
Summary of Surface Water Analytical Results
Lake Geraldine Water Supply
Nunam Stantec Limited

Sample Location		Units	Health Canada	Northern Health	SW19-01		SW19-02		SW19-03		SW19-04		SW19-05	
Sample Date	Sample ID				4-Jul-19	12-Sep-19	4-Jul-19	12-Sep-19	4-Jul-19	12-Sep-19	4-Jul-19	12-Sep-19	4-Jul-19	12-Sep-19
Sampling Company	Laboratory				SW19-01	SW19-01	SW19-02	SW19-02	SW19-03	SW19-03	SW19-04	SW19-04	SW19-05	SW19-05
Laboratory Work Order	Laboratory Name				STANTEC	STANTEC	STANTEC	STANTEC	STANTEC	STANTEC	STANTEC	STANTEC	STANTEC	STANTEC
Laboratory Sample ID	Laboratory Sample ID				BV	BV	BV	BV	BV	BV	BV	BV	BV	BV
					B07022	B07022	B07022	B07022	B07022	B07022	B07022	B07022	B07022	B07022
General Chemistry														
Alkalinity, Carbonate (as CaCO3)	mg/L	n/v	n/v	n/v	<1.0	-	<1.0	-	<1.0	-	<1.0	-	<1.0	-
Alkalinity, Total (as CaCO3)	mg/L	n/v	n/v	n/v	167	-	167	-	167	-	167	-	177	-
Ammonia (as N)	mg/L	n/v	n/v	n/v	1.5 ₁₀	-	0.25	-	0.072	-	<0.060	-	<0.060	-
Bicarbonate(as CaCO3, Calculated)	mg/L	n/v	n/v	n/v	16	-	16	-	16	-	16	-	17	-
Chloride	mg/L	s250 ^a	n/v	n/v	250 ^a	-	1.3	-	1.5	-	1.2	-	1.4	-
Electrical Conductivity, Lab	µmhos/cm	n/v	n/v	n/v	46	-	45	-	45	-	45	-	47	-
Hardness (as CaCO3)	mg/L	n/v	n/v	n/v	250 ^a	-	19	-	19	-	19	-	20	-
Langelier Index (at 20 C)	none	n/v	n/v	n/v	-2 to +2	-	-	-	-	-	-	-	-	-
Langelier Index (at 4 C)	none	n/v	n/v	n/v	-2 to +2	-	-	-	-	-	-	-	-	-
Nitrate (as N)	mg/L	10 ³	10 ³	10 ³	<0.10	-	<0.10	-	<0.10	-	<0.10	-	<0.10	-
Nitrite (as N)	mg/L	1 ³	1 ³	1 ³	<0.010	-	<0.010	-	<0.010	-	<0.010	-	<0.010	-
Orthophosphate (as P)	mg/L	n/v	n/v	n/v	<0.010	-	<0.010	-	<0.010	-	<0.010	-	<0.010	-
pH, Lab	S.U.	7.0-10.5 ^a	6.5-8.5 ^a	7.48	7.48	-	7.43	-	7.48	-	7.47	-	7.54	-
Phosphorus, Total	mg/L	n/v	n/v	n/v	0.006	-	0.013	-	0.006	-	0.005	-	0.008	-
Saturation pH (at 20 C)	none	n/v	n/v	n/v	9.25	-	9.25	-	9.27	-	9.26	-	9.22	-
Saturation pH (at 4 C)	none	n/v	n/v	n/v	9.50	-	9.53	-	9.52	-	9.52	-	9.47	-
Sulfate	mg/L	s500 ^a	500 ^a	2.7	2.7	-	2.5	-	2.4	-	2.4	-	2.8	-
Total Dissolved Solids (Calculated)	mg/L	s500 ^a	500 ^a	23	23	-	22	-	22	-	22	-	24	-
Total Organic Carbon	mg/L	n/v	n/v	1.6	1.6	-	1.4	-	1.4	-	1.3	-	1.4	-
Turbidity, Lab	NTU	<0.31,0.01 ^c	1 ³	<0.1	<0.1	-	<0.1	-	<0.1	-	<0.1	-	<0.1	-
BTX and Petroleum Hydrocarbons														
Benzene	µg/L	5 ³	5 ₁₀ ³	-	<0.20	-	<0.20	-	<0.20	-	<0.20	-	<0.20	-
Toluene	µg/L	24 ³ 60 ³	24 ₁₀ ³	-	<0.20	-	<0.20	-	<0.20	-	<0.20	-	<0.20	-
Ethylbenzene	µg/L	1.6 ³ 140 ³	2 ₁₀ ³	-	<0.20	-	<0.20	-	<0.20	-	<0.20	-	<0.20	-
Xylene, m & p-	µg/L	n/v	n/v	-	<0.40	-	<0.40	-	<0.40	-	<0.40	-	<0.40	-
Xylene, o-	µg/L	n/v	n/v	-	<0.20	-	<0.20	-	<0.20	-	<0.20	-	<0.20	-
Xylenes, Total	µg/L	20 ³ 90 ³	300 ₁₀ ³	-	<0.40	-	<0.40	-	<0.40	-	<0.40	-	<0.40	-
PhC F2 (n-C10-C16 range)	µg/L	n/v	n/v	-	<100	-	<100	-	<100	-	<100	-	<100	-
PhC F3 (n-C10-C24 range)	µg/L	n/v	n/v	-	<200	-	<200	-	<200	-	<200	-	<200	-
PhC F4 (n-C10-C20 range)	µg/L	n/v	n/v	-	<200	-	<200	-	<200	-	<200	-	<200	-
Chromatogram to baseline at C50	none	n/v	n/v	-	YES	-	YES	-	YES	-	YES	-	YES	-
Metals, Dissolved														
Calcium	mg/L	n/v	100 ₁₀ ³	6.6	-	6.4	-	6.5	-	6.5	-	6.7	-	-
Magnesium	mg/L	n/v	30 ₁₀ ³	0.80	-	0.77	-	0.74	-	0.76	-	0.81	-	-
Potassium	mg/L	n/v	400 ₁₀ ³	<1	-	<1	-	<1	-	<1	-	<1	-	-
Sodium	mg/L	s200 ^a	1,000 ₁₀ ³	0.7	-	0.7	-	0.7	-	0.7	-	0.7	-	-
Metals, Total														
Aluminum	µg/L	<100,200 ^a	n/v	5.5	-	5.5	-	5.1	-	5.1	-	8.0	-	-
Antimony	µg/L	0 ³	6 ₁₀ ³	<0.50	-	<0.50	-	<0.50	-	<0.50	-	<0.50	-	-
Arsenic	µg/L	10 ³	20 ₁₀ ³	<1.0	-	<1.0	-	<1.0	-	<1.0	-	<1.0	-	-
Barium	µg/L	1,000 ³	1,000 ₁₀ ³	<2.0	-	<2.0	-	<2.0	-	<2.0	-	<2.0	-	-
Beryllium	µg/L	n/v	n/v	<0.50	-	<0.50	-	<0.50	-	<0.50	-	<0.50	-	-
Boron	µg/L	s500 ^a	5,000 ₁₀ ³	<10	-	<10	-	<10	-	<10	-	<10	-	-
Cadmium	µg/L	5 ³	5 ₁₀ ³	<0.10	-	<0.10	-	<0.10	-	<0.10	-	<0.10	-	-
Calcium	µg/L	n/v	100,000 ₁₀ ³	6,700	-	6,800	-	6,700	-	6,900	-	7,400	-	-
Chromium	µg/L	s6 ^a	50 ₁₀ ³	<5.0	-	<5.0	-	<5.0	-	<5.0	-	<5.0	-	-
Cobalt	µg/L	n/v	n/v	<0.50	-	<0.50	-	<0.50	-	<0.50	-	<0.50	-	-
Copper	µg/L	s1000 ^a 2,300 ^a	1,000 ₁₀ ³	<1.0	-	<1.0	-	<1.0	-	<1.0	-	<1.0	-	-
Iron	µg/L	s200 ^a	300 ₁₀ ³	<100	-	<100	-	<100	-	<100	-	<100	-	-
Lead	µg/L	5 ³	10 ₁₀ ³	<0.50	-	<0.50	-	<0.50	-	<0.50	-	<0.50	-	-
Magnesium	µg/L	n/v	30,000 ₁₀ ³	760	-	710	-	740	-	750	-	820	-	-
Manganese	µg/L	s20 ^a 120 ^a	50 ₁₀ ³	3.1	-	2.6	-	2.9	-	3.5	-	2.8	-	-
Mercury	µg/L	1 ³	1 ₁₀ ³	-	<0.01	-	<0.01	-	<0.01	-	<0.01	-	<0.01	-
Molybdenum	µg/L	n/v	n/v	<0.50	-	<0.50	-	<0.50	-	<0.50	-	<0.50	-	-
Nickel	µg/L	n/v	n/v	<1.0	-	<1.0	-	<1.0	-	<1.0	-	<1.0	-	-
Potassium	µg/L	n/v	400,000 ₁₀ ³	<200	-	<200	-	<200	-	<200	-	<200	-	-
Selenium	µg/L	50 ^a	10 ₁₀ ³	<2.0	-	<2.0	-	<2.0	-	<2.0	-	<2.0	-	-
Silicon	µg/L	n/v	n/v	460	-	460	-	460	-	460	-	540	-	-
Silver	µg/L	n/v	n/v	<0.10	-	<0.10	-	<0.10	-	<0.10	-	<0.10	-	-
Sodium	µg/L	s200,000 ^a	660	-	670	-	660	-	650	-	730	-	-	-
Strontium	µg/L	n/v	n/v	10	-	9.7	-	9.9	-	9.9	-	10	-	-
Thallium	µg/L	n/v	n/v	<0.050	-	<0.050	-	<0.050	-	<0.050	-	<0.050	-	-
Titanium	µg/L	n/v	n/v	<5.0	-	<5.0	-	<5.0	-	<5.0	-	<5.0	-	-
Vanadium	µg/L	n/v	n/v	<0.50	-	<0.50	-	<0.50	-	<0.50	-	<0.50	-	-
Zinc	µg/L	s500 ^a	5,000 ₁₀ ³	<5.0	-	<5.0	-	<5.0	-	<5.0	-	<5.0	-	-
Microbiological Analysis														
Total Coliform Background	cfu/100mL	n/v	n/v	0	-	7	-	8	-	0	-	2	-	-
Total Coliforms	cfu/100mL	0 ^c	0 ^c	0	-	0	-	0	-	0	-	0	-	-
Escherichia coli (E. Coli)	cfu/100mL	0 ^c	0 ^c	0	-	0	-	0	-	0	-	0	-	-
See notes on last page.														



Table A-1
Summary of Surface Water Analytical Results
Lake Geraldine Water Supply
Nunami Stantec Limited

Notes:	
Health Canada	Health Canada (June 2016). Guidelines for Canadian Drinking Water Quality – Summary Table. Water and Air Quality Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Ontario.
A	Guidelines for Canadian Drinking Water Quality – Aesthetic Objectives/ Operational Guidelines
B	Guidelines for Canadian Drinking Water Quality – Maximum Acceptable Concentration
C	Guidelines for Canadian Drinking Water Quality – Microbiological Parameters
Northern Health	Public Health Protection, Environmental Health
P	Table 1. Required Water Quality Parameters
15.5	Concentration exceeds the indicated standard.
15.2	Measured concentration did not exceed the indicated standard.
<0.50	Laboratory reporting limit was greater than the applicable standard.
<0.03	Analyte was not detected at a concentration greater than the laboratory reporting limit.
NV	No standard/guideline value.
-	Parameter not analyzed / not available.
(i)	Total metals required. Dissolved metals optional, but recommended if turbidity is elevated. Scan to include both high and low level metals.
(ii)	Required for source water characterisation. If all are < 1 mg/L as N, later samples may be analysed for Total N only.
(iii)	Required if hydrocarbon/gasoline type contamination is suspected. Corral laboratory for sampling procedure.
+	This is an operational guidance value, designed to apply only to drinking water treatment plants using aluminum-based coagulants; it does not apply to naturally occurring aluminum found in groundwater. The operational guidance values of 0.1 mg/L applies to conventional treatment plants, and 0.2 mg/L applies to other types of treatment systems.
-	High levels (above 500 mg/L) can cause physiological effects such as diarrhea or dehydration.

APPENDIX

C

DNA METABARCODING
REPORT PROVIDED BY
PRECISION BIOMONITORING

DNA Metabarcoding Report

Report Contents

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References	6
Disclaimer	7

Report Issued:

Prepared By: Steve Crookes	Date: 02/02/2021	Email: steve.crookes@precisionbiomonitoring.com
Validated by: Jay Cashubec	Date: 02/04/2021	Email: jay.cashubec@precisionbiomonitoring.com

Prepared for

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Contact Name	Email Address	Phone
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Sample Information (Table below.)

Sample Collection Conducted by: Catalin Cenar, Qiqiktaaluk Environmental (for WSP Canada Inc.)
Sampling Period: December 2020
Storage condition before shipment: Shipped on ice
Sample Shipment Format: eDNA self-preserving filters (1.2 µm pore size)
Date Shipped: December 11th 2020
Date Received: December 18th 2020
Samples Received By: Steve Crookes
Condition of Samples Upon Receipt: Intact
DNA Extracted by Steve Crookes
Sample storage: Insulated during shipment (self-preserved filters). Samples processed immediately after DNA extraction.

Sample Submission Table

Lab Sample ID	Client Sample ID	Sample Type	DNA Yield (ng mL ⁻¹)
21-001844-0001	UNL-ST01	eDNA	584
21-001844-0002	UNL-ST02	eDNA	1780*
21-001844-0003	UNL-ST03	eDNA	1610
21-001844-0004	UNL-ST04	eDNA	2020*
21-001844-0005	REF-ST01	eDNA	1420
21-001844-0006	BLA-ST01	eDNA	70.8

* Required 5x dilution to mitigate PCR inhibition.

Analysis

Analysis applied: Dual locus metabarcoding for fishes (12S rRNA and COI).

Analysis Conducted By: Shu Chen

Analysis Location: Metabarcoding lab

Date of Analysis: 6th January 2021 – 26th January 2021

Methods

eDNA from self-preserved filters were extracted using a modified protocol incorporating Qiagen's DNeasy Blood & Tissue Extraction Kit and Qias shredder. Extracted total eDNA was eluted in 200 μ L of elution buffer. Each sample was quantified for DNA yield using a Qubit Flex fluorometer (ThermoFisher) to confirm analyzable quantities of DNA (see Sample Summary Table). All samples, except for the presumed field blank (BLA-ST01), yielded quantities over 100 ng mL⁻¹ (range: 584 – 2020 ng mL⁻¹), confirming that extraction generated sufficient DNA quantities for downstream PCR-based metabarcoding analyses.

Amplicon sequencing libraries were prepared from DNA samples following the procedures described in the 16S Metagenomic Sequencing Library Preparation Guide (Illumina) (1) and by Miya et al (2). The universal MiFish primers (2) and the PS1 primers (3) were used as the locus-specific sequences to target a hypervariable region of fish mitochondria 12S rRNA gene (approximately 160-190 bp) and COI gene, respectively (approximately 560 bp). Triplicate PCR reactions were conducted for library preparation. The library quality and quantity were assessed by a Fragment Analyzer Automated CE System with the High Sensitivity Small Fragment Analysis kit (Advanced Analytical) and Qubit® Fluorometer with the Qubit® dsDNA BR Assay kit (Thermo Fisher Scientific). The purified libraries were then normalized and combined in an equal molar ratio for sequencing. PhiX (Illumina) was included to serve as an internal control for sequencing, and a fish control containing 10 different species was included to monitor the entire process. Sequencing was conducted using a MiSeq sequencer with a MiSeq v2 reagent kit (Illumina) and 2x250 paired-end cycles according to the manufacturer's protocol.

Raw sequence reads were filtered using the MiSeq Sequencer System Software (Illumina) to remove low quality sequences and trimmed to remove adaptor sequences. The sequences were further analyzed using the MiFish pipeline as described by Sato et al (3). The sequence database for the fish mitochondria 12S rRNA gene target was MitoFish (4). The COI gene sequences were further analyzed using Geneious software v10.2.4 (Geneious Biologics) against sequences held in the Barcode of Life Database (BOLD) (<http://www.boldsystems.org>). The analysis software programs align the sequences with the database sequences, identifies fish to taxonomic genus or species and generates data summaries of taxa present.

Results

The results are summarized in the attached Excel tables. The results tabulate species identified in each of the samples and their relative abundance based on DNA barcode read frequencies for both 12S and COI, respectively for fishes.

Despite high levels of DNA derived from the samples, very little fish DNA was identified from the samples (see attached Excel data sheets indicating per sample DNA read strength and taxonomic IDs for each sample). The widely used 12S rRNA marker for freshwater fishes did not yield any successful identifications for any sample except for the reference sample (REF-ST01), in which the Arctic char (*Salvelinus alpinus*) was the sole representative. As a secondary line of analysis, we metabarcoded the COI locus and although the resolution of the *Salvelinus* barcodes could only be ascertained to genus, a signal of *Salvelinus* was corroborated for the reference sample. However, a small amount of *Salvelinus* DNA was also detected in sample UNL-ST03 (30 reads). However, the COI locus also yielded small amounts of arthropod and mammalian DNA, which could not be specified at a more granular taxonomic level.

The reference samples yielded *Salvelinus* DNA for both loci in this presumptive control sample, indicated that the metabarcoding reagents and informatics pipeline were functioning correctly. However, that COI could not be identified to species, whereas 12S could, suggests discrepancies of information content (i.e., barcode sequences) between the two databases. Although the iBOLD database has 57 COI barcode sequences (22 collected within Canada), the inability of COI to provide resolution to species for the Arctic char suggests that more COI DNA sequence variation needs to be collected in northerly latitudes to sustain further metabarcoding work in these more remote areas. As a corollary, more direct sequencing of fish species in northerly latitude fisheries in Nunavut and other remote Canadian and First Nations territories should be used to populate DNA barcoding databases to facilitate more informed metabarcoding efforts.

The lack of detection, however, may also be reflect reality, in that northerly waterbodies hold less biomass and biodiversity than lower latitudes, including some lakes in which there are no fishes present at all. Therefore, five field samples (excluding the reference and blank) may not be sufficient to detect eDNA of fishes at low abundance, with attendant highly diluted eDNA. If species are expected to be at low abundance, then optimizing where and when to take samples is imperative. Although samples were taken at depth, the specific time of year (late Fall) during when samples were collected would likely depress metabolic activity and thus downregulate eDNA shedding. The optimal monitoring period for inferring site detection for outwardly reproducing fish species would be during suspected spawning periods. Therefore, for northerly latitude lakes a more granular approach may yield better results – sampling more water per filtration event, sampling at different depths in the water column to account for stratification of the lake and attendant eDNA molecules and aggregates, pooling samples to increase

probability of detection, and to filter during opportune spawning period(s) in which eDNA is released as gamete plumes.

Further considerations include tailoring the bioinformatics of metabarcoding to the aquatic system under consideration. DNA yields were high, but seemingly only a small fraction of this was fish DNA. If the extracts contain a large amount of microbial and non-target (i.e., non-fish) DNA, special considerations may need to be implemented in future analyses of these systems (e.g., the use of 'blocking primers', or development of more specific fish barcoding primer sets).

Attachment: two Excel files

References

1. 16S Metagenomic Sequencing Library Preparation Guide - Preparing 16S Ribosomal RNA Gene Amplicons for the Illumina MiSeq System (Part 15044223 Rev. B).
2. Miya *et al.* 2015. MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. *R. Soc. open sci.* **2**: 150088.
3. Balasingham *et al.* 2018. Environmental DNA detection of rare and invasive fish species in two Great Lakes tributaries. *Mol. Ecol.* **27**: 112-127.
4. Sato *et al.* 2018. MitoFish and MiFish Pipeline: A Mitochondrial Genome Database of Fish with an Analysis Pipeline for Environmental DNA Metabarcoding. *Mol. Biol. Evol.* **35**(6): 1553–1555.

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Metabarcoding Analysis of eDNA - MiFish Target

Sample ID	21-001844-0001 (UNL-ST01)			
	Species (scientific name)	Species (common name)	# of Reads	% RA*
	Fish species not detected	Fish species not detected	0	0,00
Total number of reported reads			0	
Total percent of reads occupied by the reported species				0,00
Total number of raw reads (R1+R2)			177 847	
Total number of assembled reads			80 593	

*%RA: Relative abundance of the reported species within a sample

Sample ID	21-001844-0002 (UNL-ST02)			
	Species (scientific name)	Species (common name)	# of Reads	% RA*
	Fish species not detected	Fish species not detected	0	0,00
Total number of reported reads			0	
Total percent of reads occupied by the reported species				0,00
Total number of raw reads (R1+R2)			260 928	
Total number of assembled reads			116 869	

Sample ID	21-001844-0003 (UNL-ST03)			
	Species (scientific name)	Species (common name)	# of Reads	% RA*
	Fish species not detected	Fish species not detected	0	0,00
Total number of reported reads			0	
Total percent of reads occupied by the reported species				0,00
Total number of raw reads (R1+R2)			177 118	
Total number of assembled reads			81 565	

Sample ID	21-001844-0004 (UNL-ST04)			
	Species (scientific name)	Species (common name)	# of Reads	% RA*
	Fish species not detected	Fish species not detected		
Total number of reported reads			0	
Total percent of reads occupied by the reported species				
Total number of raw reads (R1+R2)			274 919	
Total number of assembled reads			123 020	

Sample ID	21-001844-0005 (REF-ST01)			
	Species (scientific name)	Species (common name)	# of Reads	% RA*
	Salvelinus alpinus	Arctic char	2 580	100,00
Total number of reported reads			2 580	
Total percent of reads occupied by the reported species				100,00
Total number of raw reads (R1+R2)			212 859	
Total number of assembled reads			95 993	

Sample ID	21-001844-0006 (BLA-ST01)			
	Species (scientific name)	Species (common name)	# of Reads	% RA*
	Fish species not detected	Fish species not detected	0	0,00
Total number of reported reads			0	
Total percent of reads occupied by the reported species				0,00
Total number of raw reads (R1+R2)			141 290	
Total number of assembled reads			45 090	

Metabarcoding Analysis of eDNA - PS1-COI Target

Sample ID	21-001844-0001 (UNL-ST01)			
	Species (scientific name)	Species (common name)	# of Reads	% RA*
	Phylum Arthropoda		47	61,04
	Class Mammalia		30	38,96
	Fish species		0	0,00
Total number of reported reads			77	
Total percent of reads occupied by the reported species				100,00
Total number of raw reads (R1+R2)			71 420	
Total number of assembled reads			1 421	

*%RA: Relative abundance of the reported species within a sample

Sample ID	21-001844-0002 (UNL-ST02)			
	Species (scientific name)	Species (common name)	# of Reads	% RA*
	Phylum Arthropoda		69	86,25
	Class Mammalia		11	13,75
	Fish species		0	0,00
Total number of reported reads			80	
Total percent of reads occupied by the reported species				100,00
Total number of raw reads (R1+R2)			57 686	
Total number of assembled reads			794	

Sample ID	21-001844-0003 (UNL-ST03)			
	Species (scientific name)	Species (common name)	# of Reads	% RA*
	Phylum Arthropoda		665	95,14
	Class Mammalia		4	0,57
	Salvelinus sp		30	4,29
Total number of reported reads			699	
Total percent of reads occupied by the reported species				100,00
Total number of raw reads (R1+R2)			73 278	
Total number of assembled reads			2 177	

Sample ID	21-001844-0004 (UNL-ST04)			
	Species (scientific name)	Species (common name)	# of Reads	% RA*
	Phylum Arthropoda		22	100,00
	Fish species		0	0,00
Total number of reported reads			22	
Total percent of reads occupied by the reported species				100,00
Total number of raw reads (R1+R2)			65 982	
Total number of assembled reads			814	

Sample ID	21-001844-0005 (REF-ST01)			
	Species (scientific name)	Species (common name)	# of Reads	% RA*
	Phylum Arthropoda		102	90,27
	Salvelinus sp		11	9,73
Total number of reported reads			113	
Total percent of reads occupied by the reported species				100,00
Total number of raw reads (R1+R2)			69 498	
Total number of assembled reads			1 953	

Sample ID	21-001844-0006 (BLA-ST01)			
	Species (scientific name)	Species (common name)	# of Reads	% RA*
	Class Mammalia		115	100,00
	Fish species		0	0,00
Total number of reported reads			115	
Total percent of reads occupied by the reported species				100,00
Total number of raw reads (R1+R2)			92 098	
Total number of assembled reads			156	