June 2017

LUPIN MINE

Enviornmental Effects Montioring Phase 5

Submitted to:
Environment Canada by:
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Toronto, ON

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REPORT

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1.0 INTRODUCTION

The *Metal Mining Effluent Regulations* (MMER) under the *Fisheries Act* were registered in June 2002, and came into force in December 2002 (EC 2002a). Amendments were made in October 2006 and November 2012. The MMER stipulate the conditions under which deleterious substances may be discharged to the aquatic environment by metal mines (EC 2012a). Specifically, these regulations impose limits on the release of deleterious substances, which include cyanide, arsenic, copper, lead, nickel, zinc, radium-226, and total suspended solids (TSS), as well as prohibit the discharge of effluent that is acutely lethal to fish.

The Lupin Mine (the Mine) is a gold mine located on the west shore of Contwoyto Lake, Nunavut, approximately 300 kilometres (km) southeast of Kugluktuk. While operations at the Mine ceased in 2005, it has not been officially designated as having "closed mine status" under the MMER. Therefore, the regulatory requirements outlined by the MMER are applicable to the Mine.

1.1 Metal Mining Effluent Regulations – Environmental Effects Monitoring

The MMER requires three distinct programs that all metal mines discharging an effluent as of 2002 must undertake:

- effluent monitoring
- emergency preparedness
- EEM

The objective of the EEM program, as defined in the Metal Mining Technical Guidance for EEM document (TGD; EC 2012a), is to evaluate the effects of mine effluent on fish, fish habitat, and use of fisheries resources by humans. The guiding principles of the EEM program are that it be scientifically defensible, cost-effective, and flexible around site-specific requirements, without subjecting field crews to unsafe sampling conditions. The EEM program is comprised of two parts:

- Part I – effluent and water quality monitoring:
  - effluent characterization (chemical characterization and toxicity testing)
  - surface water quality monitoring in the receiving environment (exposure area) and in the reference area(s)
- Part II – biological monitoring studies:
  - Biological monitoring of fish and fish habitat (i.e., benthic invertebrate communities) in the receiving environment (exposure area) and in the reference area(s). More specifically, an EEM program consists of four key elements:
    - effluent and water quality monitoring and reporting
    - development and submission of a study design for biological monitoring
    - implementation of the study design in the field
    - data assessment, interpretation, and submission of an interpretative report

1.2 Report Outline

Three phases of EEM have been conducted at Lupin Mine. Phase 1 was conducted as a Periodic Monitoring – Surveillance program in 2005. Phase 2 EEM was conducted as a Periodic Monitoring – Confirmation program in 2008. Both the Phase 1 and Phase 2 EEM programs identified significant differences in fish growth endpoints between the exposure and reference areas. As such, Phase 3 was conducted as an Investigation of Cause (IOC) study in 2010 to determine whether temperature was the causal factor of size differences; this hypothesis was not verified. Following IOC, the next phase of EEM is conducted as a Periodic Monitoring – Surveillance program. A Phase 4 EEM Study Design was developed and submitted to Environment Canada, but the biological investigation was not completed as all activities at the Mine were suspended in August 2013.

Mandalay Resources Corporation (Mandalay) initiated the Phase 5 EEM program for the Mine with the submission of a study design document (Golder 2016a). The program was designed as a Periodic Monitoring – Surveillance program involving fish and benthic invertebrate community surveys, and is a minor update to the Phase 4 EEM Study Design, while taking into account the 2015 water quality monitoring and 2015 sub-lethal toxicity testing of the effluent. The Phase 5 field program was conducted late-August/early-September 2016. This final interpretative report is organized as follows:

- Section 2 – Study Area and Setting
- Section 3 – EEM Studies at Lupin
- Section 4 – Supporting Environmental Data
- Section 5 – Benthic Invertebrate Community Survey
- Section 6 – Fish Survey
- Section 7 – General Findings
- Section 8 – Recommendations for Phase 6
- Section 9 – Closure

2.0 STUDY AREA AND SETTING

Under Schedule 5, Section 19 of the MMER (EC 2002a), a summary of the site characterization for the Mine with detailed descriptions of any changes to the characterization from the previous study design is required. The sub-sections below summarize information presented in the site characterization section of previous study design documents (Golder 2004, 2013, 2016a) and the final interpretative reports (Golder 2006; AECOM 2009, 2011), with updates where applicable.

2.1 Mine History

2.1.1 Background

Mine construction began in August 1980 and was completed in March 1982. The Mine operated continuously between 1982 and 1998, at which time operations were suspended and the Mine entered into care and maintenance status. Production resumed in April 2000 and continued until 2003 when the Mine returned to care and maintenance status following a merger between Echo Bay Mine and Kinross Gold Corporation. Operations began again in 2004, but the Mine once again entered care and maintenance in February 2005. In February 2007,

the Mine was sold to Wolfden Resources Inc. (Wolfden) and Lupin Mines Inc. (LMI) was created as a wholly owned subsidiary of Wolfden to receive the Mine assets. In May 2007, Wolfden was acquired by the Australian-based company Zinifex Ltd. and ownership of LMI was transferred to Zinifex Ltd. In 2008, Zinifex Ltd. and another Australian-based company, Oxiana Ltd., merged to form OZ Minerals Ltd. In 2009, the Canadian assets of OZ Minerals were sold to China Minmetals Inc. Minerals and Metals Group Ltd. was set up in Australia to receive the OZ Minerals assets. Minerals and Metals Group Ltd. is a wholly owned, privately-held subsidiary of China Minmetals Inc. In Canada, the operating company for Minerals and Metals Group Ltd. is MMG Resources Canada Inc. (MMG), which was the sole shareholder of LMI and the legal owner of the Mine until 2011. Elgin Mining Inc. (Elgin) purchased the property from MMG in July 2011. Elgin was purchased by Mandalay in 2014 and is the current the legal owner of LMI.

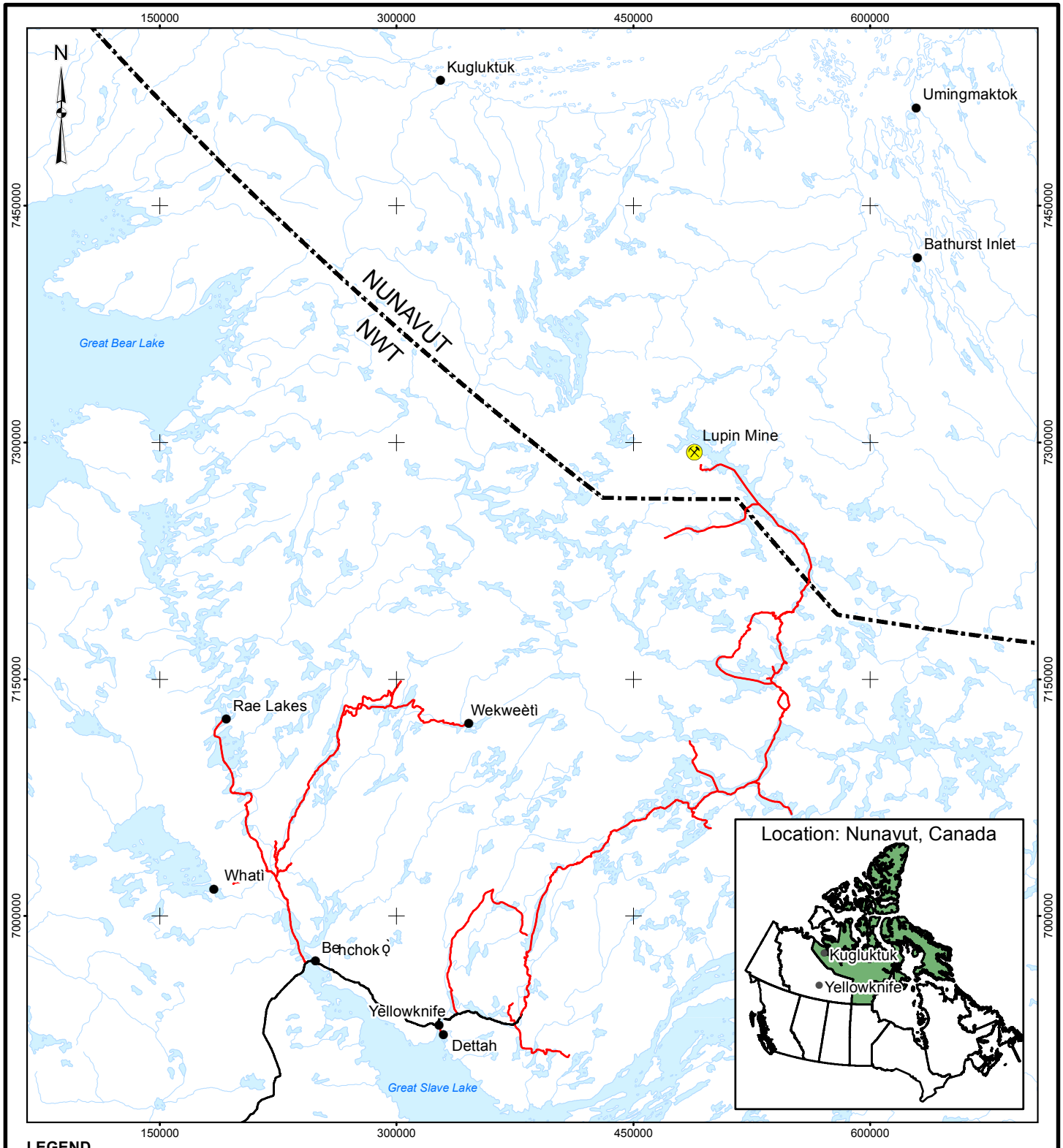
2.1.2 Location and Facilities

The Mine is located on the west shore of Contwoyto Lake, Nunavut, approximately 300 km southeast of Kugluktuk and 80 km south of the Arctic Circle at a latitude of 65°46’ N and longitude of 111°15’ W (Figure 2.2-1). The area of the land within the Mine surface lease boundary is 6,754 ha. An overview of the Mine site is depicted in Figure 2.2-2. The Mine site includes underground mining and production systems and an ore handling system. Mining and processing equipment were designed to manage up to 2,300 tonnes of ore per day (t/day). Materials and supplies are brought into the Mine site by air. The Mine is a self-contained facility with on-site power generation and sewage facilities.

The Mine site consists of two principal clusters of buildings:

- A residential complex – consisting of accommodations, a kitchen, and recreation centre.
- An industrial complex – consisting of the mine, mill, powerhouse, fuel tank farm, maintenance facility, warehouse, and offices.

Ancillary features include a sewage lagoon immediately to the south of the industrial complex, a 1.9 km airstrip, and a tailings containment area (TCA), which is located approximately 7 km south of the Mine (Figure 2.2-2).



LEGEND

- LUPIN MINE
- POPULATED PLACE
- HIGHWAY
- WINTER ROAD
- WATERCOURSE
- WATERBODY
- TERRITORIAL BOUNDARY

100 0 100

SCALE 1:3,500,000 KILOMETRES

REFERENCE

HYDROGRAPHY DATA OBTAINED FROM GEOGRATIS, © DEPARTMENT OF NATURAL RESOURCES CANADA. ALL RIGHTS RESERVED. TRANSPORTATION DATA OBTAINED FROM GEOBASE ®.
DATUM: NAD83 PROJECTION: UTM ZONE 12

PROJECT
MANDALAY RESOURCES CORPORATION

LUPIN MINE PHASE 5 ENVIRONMENTAL EFFECTS MONITORING

TITLE

LOCATION OF LUPIN MINE

Golder Associates

PROJECT	1650403 - 5000		FILE No.	
DESIGN	TL	28 Mar. 2017	SCALE AS SHOWN	REV. 1
GIS	MM/LS	10 May 2017		
CHECK	HM	29 May 2017		
REVIEW	ZK	29 May 2017		

FIGURE 2.1-1

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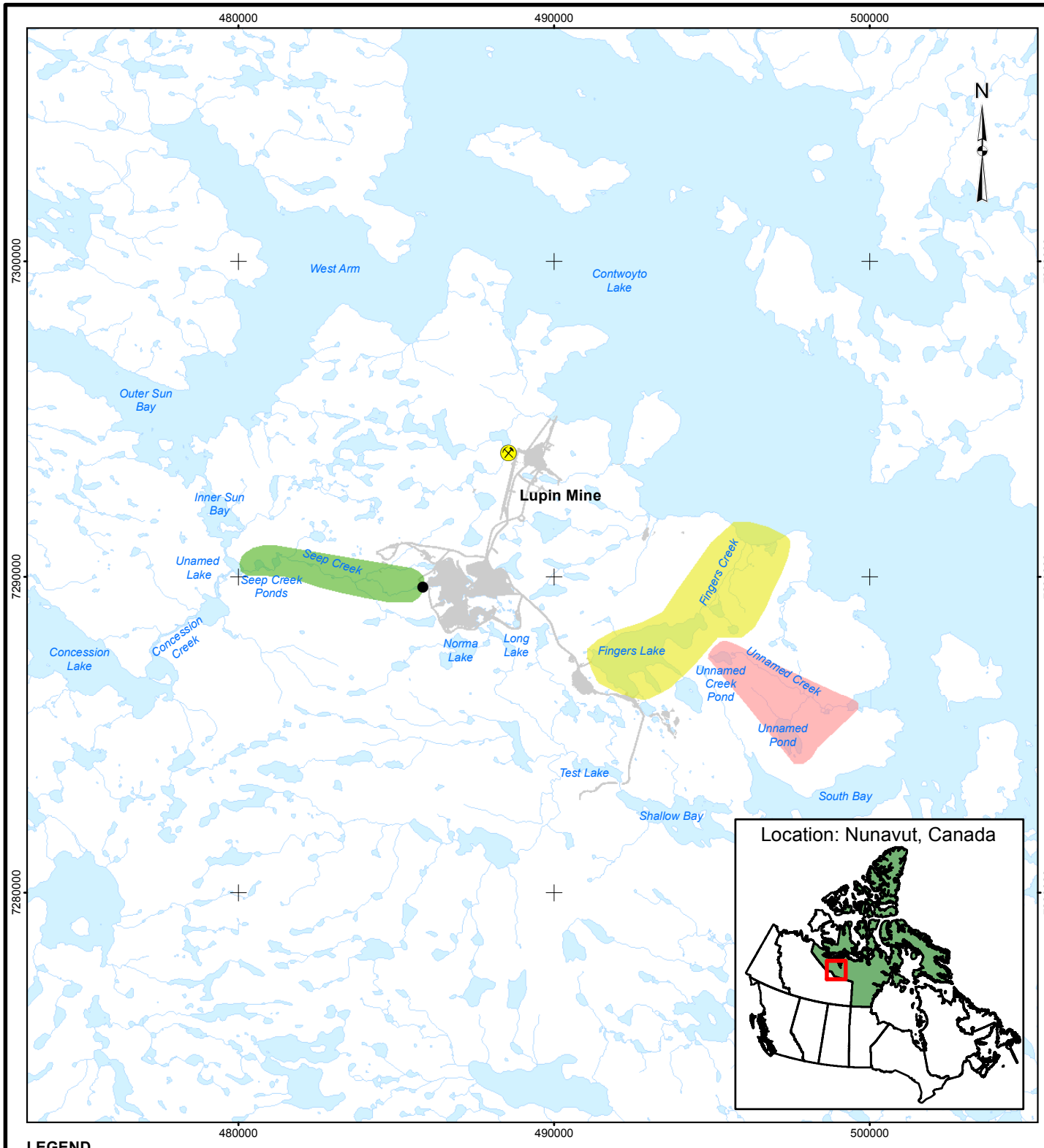

LEGEND

- FINAL DISCHARGE POINT (STATION LUP-10)
- LUPIN MINE
- WATERCOURSE
- LUPIN MINE FOOTPRINT
- WATERBODY

SAMPLING AREAS

- EXPOSURE AREA
- REFERENCE AREA 1
- REFERENCE AREA 2

5 0 5

SCALE 1:175,000 KILOMETRES

REFERENCE

HYDROGRAPHY DATA OBTAINED FROM GEOGRATIS, © DEPARTMENT OF NATURAL RESOURCES CANADA. ALL RIGHTS RESERVED.
DATUM: NAD83 PROJECTION: UTM ZONE 12

PROJECT
MANDALAY RESOURCES CORPORATION

LUPIN MINE PHASE 5 ENVIRONMENTAL EFFECTS MONITORING

TITLE

LUPIN MINE PHASE 5 EEM EXPOSURE AND REFERENCE AREAS

Golder Associates

PROJECT	1650403 - 5000		FILE No.	
DESIGN	TL	28 Mar. 2017	SCALE AS SHOWN	REV. 1
GIS	MM/LS	10 May 2017		
CHECK	HM	29 May 2017		
REVIEW	ZK	29 May 2017		

FIGURE 2.1-2

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2.1.3 Historical Operations

2.1.3.1 Description of Production Processes

The ore body at the Mine extends from the surface to about 2,000 m below the surface. Gold is found primarily within a sulphide rich iron formation. The gold is fine grained (generally less than 100 µm in diameter) and is associated mainly with pyrrhotite and arsenopyrite. The Mine used cyanidation processing to leach gold into solution. A detailed description of the historical underground mining and milling techniques used at the Mine is provided by Golder (2004) and Echo Bay Mines Ltd. (2001).

2.1.3.2 Waste Rock

While the Mine was in operation, waste rock was used as roadbed material, in dams, in the airstrip, or as backfill. During the more recent years, very little development waste has been produced in the underground operation; as a result, a surface waste stockpile no longer exists.

2.1.3.3 Management of Minewater

Relative to most underground mining operations, the Mine is a dry mine, having an average water inflow of between 45 and 95 L/min. Most of this water seeps into workings below the permafrost level (500 m below surface). The main de-watering system consists of four main sumps located at the 250, 650, 890, and 1,105 m shaft stations. The discharge line is of 15.24 cm diameter, and is located in the shaft. The mine discharge water was pumped to the TCA via the mill.

Both drill water and potable water were supplied to the mine via separate pipelines located in the shaft. A brine line services the upper levels, in permafrost. A 3% to 6% brine solution (by weight) was mixed in tanks on the surface and fed to upper level sumps when needed. Also located in the shaft is a 3.81 cm fuel line, which allowed diesel fuel to be sent to a main storage facility on the 890 m level.

2.1.3.4 Management of Tailings

Originally, all waste was to be contained within the TCA. As mine capacity increased, it became necessary to expand the TCA and discharge effluent. Effluent discharge commenced on 5 September 1985. Effluent was discharged in mid-summer, generally beginning on 15 July and continued for periods that typically extended into early September. Since the initial discharge in 1985, effluent has been discharged into Seep Creek, which empties into an unnamed lake, which drains into Contwoyto Lake at the south end of Inner Sun Bay (Figure 2.2-2).

The tailings effluent management process at the Mine during operations is as follows:

- The tailings slurry is pumped from the mill to one of five solids retention cells in the TCA, where the solids settle. The tailings impounded in cells 1 and 2 has been covered with esker material and the cells closed. The tailings impounded in cells 3 and 5 have been partially covered and Cell 4 has not been used for tailings deposition.
- Each spring, usually beginning in late June, the build-up of meltwater and tailings water is decanted from Cell 3 into the polishing pond cell (Cell 4). Water is held in this cell for a one-year period, where cyanide undergoes natural degradation due to exposure to sunlight, air, and agitation by wind.
- The following year, water is released from the polishing pond through a gated culvert, into a separate holding pond (Pond 1), where it is held for one-year before being siphoned into a second holding pond (Pond 2) where it is held for another year. If necessary, the water can be treated with ferric-sulphate during the

siphoning process to precipitate arsenic in the second holding pond. Lime is also added to adjust the pH, if necessary.

- Historically, water was retained only for a two-year period, and arsenic concentrations were elevated in the first holding pond. An arsenic treatment plant was built between the two holding ponds so that the water could be injected with ferric sulphate solution as it was being siphoned between the two ponds. Between 2000 and 2005, water was been retained for a three-year period (one year in the polishing pond, one year in the first holding pond, and one year in the second holding pond). The extra year of water retention before going into the second holding pond resulted in naturally lowering the arsenic levels, thus negating the need for arsenic treatment. Periodically, treatments of ferric sulphate (two-tonne batches) were placed into the first holding pond to control arsenic concentrations.
- Depending on the water level and water quality in the second holding pond, water is released to the environment after July 15 of any year (as per requirements of Water Licence 2AM-LUP1520). Water quality is monitored daily during discharge, so that discharge criteria are not exceeded.

2.1.3.5 Sewage Disposal

Sewage and domestic grey water are pumped to the Sewage Lake Disposal System for natural degradation. The system features two small unnamed lakes located to the south of the mine and empty via an unnamed creek into Contwoyto Lake (Echo Bay Mines Ltd. 1994). Overflow pipes in the permeable dam between the two small lakes maintain the water level in the upper pond at least 1.5 m below the crest of the dam.

2.1.4 Recent Operations

2.1.4.1 Management of Minewater

The tailings line between the mill and the tailings containment area was thoroughly flushed out with fresh water and decommissioned in 2005 when the site was put under care and maintenance.

The underground drill water and potable water systems were decommissioned in 2005 along with the fuel line and storage facility on the 890 m level.

2.1.4.2 Management of Tailings

Since the Mine entered care and maintenance in 2005, there have been four campaigns to reduce the volume of water within the TCA. These campaigns occurred in 2005, 2009, 2012, and 2015.

The tailings effluent management process at Lupin during care and maintenance is as follows:

- Each spring, the build-up of meltwater on Cell 3 is released from the retention cell into the polishing pond cell (Cell 4).
- Depending on the water level in the polishing pond (Cell 4), water is periodically released from the polishing pond through a gated culvert, into holding pond, Pond 1.
- Depending on the water level in the retention Cell 5, water is periodically siphoned to the holding pond (Pond 1).

- Four overflow pipes in the dam between Pond 1 and Pond 2 keep the water level in Pond 1 at least 0.3 m below the crest of the dam. Water is periodically siphoned from first holding pond (Pond 1) to the second holding pond (Pond 2).
- The pH of the water in the second holding pond (Pond 2) is adjusted to be within discharge criteria specified by the MMER and Water Licence prior to discharge by siphons to the receiving environment after 15 July of any year (as per Water Licence requirements). Arsenic treatment has not been required. Water quality is monitored daily during discharge, so that discharge criteria are not exceeded.

2.1.4.3 Sewage Disposal

Sewage and domestic grey water is transferred to the upper pond in the Sewage Lake Disposal System for natural degradation. The sewage and domestic grey water is either trucked from holding tanks at the accommodation complex or pumped. Typically, water is released to the environment annually by siphons following the receipt of results confirming the water quality in lower pond meets the discharge criteria (as per Water Licence requirements). Discharge through the overflow pipes in the dam between the upper and lower ponds is periodically augmented by siphoning the water across the dam.

2.2 Environmental Setting

2.2.1 Climate

Climate in this region is classified as semi-arid subarctic with severe intense winters and short warm summers. Climate normals data for this area are available for 1981 to 2010 (EC 2012b). The average daily temperature is minus 10.9°C and the average annual precipitation is 298.5 mm (EC 2012b). The heaviest precipitation in the form of rainfall occurs between June and September (averaging 25.6 to 56.9 mm) with average daily temperatures ranging between 1.8 and 11.5°C during this time (EC 2012b). Snowfall can occur in any month, but the heaviest snowfalls generally occur in October (averaging 28.2 cm) with an average annual snowfall of 138 cm (EC 2012b). The Mine is subject to frequent strong winds from the northwest (Beak Consultants Ltd. and Mary Collins Consultants Ltd. 1980; Geocon Ltd. 1980; EC 2012b).

Snowmelt is generally complete by the end of June. Ice break-up on Contwoyto Lake begins in mid-July, although sometimes the lake is not ice-free until early August. Other small lakes in the region are ice-free by early July. Ice starts to form on small lakes in late August to early September. Contwoyto Lake freeze-over occurs in October. Lakes develop between 1 and 3 m of ice in the winter. The rate of ice-melt influences summer conditions. The interaction between climate and morphology of individual lakes leads to differences among the thermal regime of lakes.

2.2.2 Geology and Topography

The bedrock in the vicinity of the Mine is archean in age, including supracrustal rocks of the Yellowknife supergroup of the Slave province of the Canadian shield. Rock types occurring in the vicinity of the Mine include ultramafic, mafic, intermediate and felsic volcanic rocks, intrusive rocks, siliciclastic rocks, and ironstones. The gold mineralization at the Mine is hosted primarily by the ironstones. This region contains intrusive igneous material such as granite (NRC 2009).

In the vicinity of the Mine, terrain is low and undulating ranging between 450 and 530 m elevation. There are numerous shallow lakes and streams throughout the area (GLL 2008a,b).

2.2.3 Vegetation

The Mine is located in the subarctic tundra vegetation zone. This zone is characterized by continuous permafrost and “barren ground” vegetation including moss, lichens, heather, and dwarf shrub communities in well-drained areas, and grasses and sedges in wet areas adjacent to watercourses and lakes. Dwarf willow shrubs, up to 1 m high, occur adjacent to some watercourses (RCPL and RL&L 1985).

2.2.4 Hydrology

Contwoyto Lake is the major waterbody in the study area with a surface area of approximately 959 km^2 and a drainage area of approximately 8,000 km^2 (Roberge et al. 1986). Contwoyto Lake has two outflows. The main outflow to the north drains to the Burnside River and ultimately to Bathurst Inlet. The smaller outflow to the south drains to the Contwoyto River and into the Back River (Rescan 2002). The main basin of Contwoyto Lake is located southeast from the Mine. To the north of the Mine, the West Arm of Contwoyto Lake extends to the west into Outer Sun Bay and terminates in Inner Sun Bay. This narrow bay is located west of the Mine and ultimately receives treated effluent from the final discharge point after it has travelled through a series of small lakes and streams (Figure 2.2-2).

Seep Creek is a small stream (approximately 6.5 km in length) that flows in a westerly direction into Inner Sun Bay of Contwoyto Lake (Figure 2.2-2). The Seep Creek watershed includes three lakes (locally known as Dam 2 Lake, Dam 1a Lake, and Unnamed Lake), three headwater streams, two ponds (Seep Creek Ponds 1 and 2), and two embayment areas (Inner and Outer Sun bays). Dam 2 Lake is a small lake (maximum depth of 7 m), bordered on the north by a gravel pit and on the east by the TCA. Dam 1a Lake is a small lake (maximum depth of less than 1 m) that is located to the south of Dam 2 Lake. This waterbody freezes to the bottom in winter. A southern branch of Seep Creek, originating from Dam 1a Lake, enters Seep Creek approximately 2 km downstream of Dam 2 Lake. A third branch of the creek arises to the south of Dam 1a Lake and joins the mainstem about 400 m downstream of the confluence of Dam 2 Lake and Dam 1a Lake branches (Figure 2.2-2).

2.3 Effluent Characterization

Effluent is discharged from the TCA by siphon into Dam Lake 1a and eventually reaches Contwoyto Lake via Seep Creek, Seep Creek Ponds, Unnamed Creek, and Inner Sun Bay of Contwoyto Lake (Figure 2.2-2). The mill at the Mine has one final effluent discharge point, LUP-10, where samples are collected by LMI for effluent characterization.

2.3.1 Effluent Volume

Since 2002, when the MMER came into force, there were five scheduled effluent releases (Table 2.3-1). As per Nunavut Water Board Licence 2AM-LUP1520, effluent release occurs during the open water period, commencing no sooner than 15 July of any calendar year. The last effluent release occurred between 23 September and 29 October 2015. The largest total volume of treated effluent was discharged in 2002, the highest average daily flow occurred during the 2005 discharge, and the longest discharge occurred during 2009 (Table 2.3-1). No effluent was released in 2016; therefore, the current Phase 5 EEM study occurred during a year without effluent release. To date, only Phase 1 EEM has coincided with the discharge of treated effluent.

Table 2.3-1: Total Annual Discharge Volumes from Lupin Mine TCA (LUP-10), 2002 to 2016

Year	Annual Total [m^3]	Discharge Period	Number of Days	Flow [m^3/d]	Flow [m^3/s]	EEM Study
2002	3,102,895	July 15 to September 7	55	56,416	0.653	-
2003	0	n/a	0	n/a	n/a	-
2004	0	n/a	0	n/a	n/a	-
2005	1,682,135	July 15 to August 11	28	60,076	0.695	Yes
2006	0	n/a	0	n/a	n/a	-
2007	0	n/a	0	n/a	n/a	-
2008	0	n/a	0	n/a	n/a	Yes
2009	2,897,461	July 25 to October 7	75	38,633	0.447	-
2010	0	n/a	0	n/a	n/a	Yes
2011	0	n/a	0	n/a	n/a	-
2012	1,066,836	September 8 to 29	21	50,802	0.588	-
2013	0	n/a	n/a	n/a	n/a	Not completed
2014	0	n/a	n/a	n/a	n/a	-
2015	2,170,746	September 23 to October 29	37	58,669	0.679	-
2016	0	n/a	n/a	n/a	n/a	Yes

m^3/d = cubic metres per day; m^3/s = cubic metres per second; EEM = Environmental Effects Monitoring; n/a = not applicable; - = not conducted.

2.3.2 Effluent Chemistry

No effluent was released in 2016; therefore, effluent chemistry from 2015 is described below. In 2015, monthly and weekly grab samples were collected in September and October during effluent discharge from Station LUP-10 (final discharge point) to satisfy the requirements of the MMER (EC 2002a). Additional daily and weekly samples were collected from Station LUP-10, as required, to satisfy requirements of the Water Licence (Golder 2016b).

Effluent chemistry data collected at Station LUP-10 (final discharge point) in 2015 are summarized in Table 2.3-2; complete data are available in the Phase 5 EEM Study Design (see Appendix A, Table A-1 in Golder 2016a;). All concentrations of deleterious substances (as per Schedule 4 in the MMER) in daily grab samples were less than the MMER limits for maximum allowable concentrations in a grab sample. All pH values were within the required range of 6.0 to 9.5.

Table 2.3-2: Water Quality Monitoring of Final Effluent (Station LUP-10) in 2015

Parameter	Unit	MMER Limit[a]	n	Min	Median	Mean	Max	SD
Conventional Parameters								
Conductivity (field)	µS/cm	-	35	308	686	663	790	106
Hardness (as $CaCO_3$)	mg/L	-	31	164	179	180	203	7.8
pH (field)	-	6.0-9.5	37	6.3	6.7	7.0	9.2	0.7
Total suspended solids	mg/L	30	37	<3	<3	3.0	3.6	0.1
Total alkalinity (as $CaCO_3$)	mg/L	-	31	<2	4.2	4.2	5.9	0.9
Nutrients								
Total ammonia (as nitrogen)	mg/L	-	7	0.07	0.09	0.09	0.11	0.02
Nitrate (as nitrogen)	mg/L	-	14	0.55	0.95	0.95	1.05	0.12
Other								
Total cyanide	mg/L	2	37	<0.002	<0.005	<0.005	<0.005	0.001
Radium-226	Bq/L	1.11	5	<0.01	<0.01	0.01	0.018	0.004
Total Metals								
Aluminum	mg/L	-	31	0.019	0.030	0.034	0.100	0.019
Arsenic	mg/L	1	37	0.004	0.007	0.007	0.009	0.001
Cadmium	mg/L	-	31	0.0002	0.0002	0.0002	0.0002	0.00001
Copper	mg/L	0.6	37	0.001	0.002	0.002	0.005	0.001
Iron	mg/L	-	31	0.082	0.113	0.116	0.206	0.025
Lead	mg/L	0.4	31	<0.00005	0.00006	0.00006	0.00009	0.00001
Mercury	mg/L	-	2	<0.000005	<0.000005	<0.000005	<0.000005	-
Molybdenum	mg/L	-	31	<0.00005	<0.00005	<0.00005	0.00016	0.00002
Nickel	mg/L	1	31	0.069	0.071	0.072	0.077	0.002
Selenium	mg/L	-	31	<0.00005	<0.00005	<0.00005	0.00006	0.000002
Zinc	mg/L	1	37	0.22	0.23	0.23	0.27	0.01

Notes: due to variable detection limits between the study years, where a result was reported as less than the detection limit, the statistic was calculated by assuming the non-detectable result was equal to the detection limit. Note that an error was noted in the Phase 5 EEM Study Design summary statistics and numbers may differ from this table.

"Total Metals" for the purpose of this document include metalloids such as arsenic and non-metals such as selenium.

(a) Maximum concentration in a grab sample (EC 2002a).

MMER = Metal Mining Effluent Regulations; n = number of samples; SD = standard deviation; $CaCO_3$ = calcium carbonate; µS/cm = microSiemens per centimetre; Bq/L = Becquerels per litre; <= less than; - = not available or not applicable.

2.3.3 Effluent Toxicity

In 2015, acute toxicity testing was completed on treated effluent (Station LUP-10) monthly in September and October, while sub-lethal toxicity testing was completed once in October (Golder 2016b).

Zero mortality was recorded in both Rainbow Trout and *Daphnia magna* toxicity tests in 100% treated effluent. Therefore, the treated effluent samples collected in 2015 were determined to be not acutely toxic as tested.

Sub-lethal toxic effects on growth and/or reproduction were observed for three of four test species (i.e., *Pseudokirchneriella subcapitata, Lemna. minor, and Ceriodaphnia. dubia*); however, no effects on survival were observed. The minimum suggested threshold of 30% effluent concentration for sub-lethal toxicity (EC 2012a) was not met for growth in *Pseudokirchneriella subcapitata* (IC_{25} of 5.5%), reproduction in *Lemna minor* (IC_{25} of <1.5%), and reproduction in *Ceriodaphnia dubia* (IC_{25} of 4.7%). There was a sub-lethal effect on growth of *Lemna minor*

(IC_{25} of 57%); however, it was above the minimum threshold for sub-lethal toxicity (EC 2012a). No toxic effects were observed for growth and survival of *Pimephales promelas* and survival of *Ceriodaphnia. dubia*.

2.3.4 Effluent Plume Delineation

The MMER requires an estimate of effluent concentration at 250 m downstream from the final discharge point and a description of effluent mixing within the environment to a concentration of 1%. A 250 m distance downstream of the final discharge point is within Dam 1a Lake. Conductivity was used as a surrogate measure to delineate the effluent plume in the receiving environment.

A mixing and dispersion model was initially developed by Golder (2004) to describe the movement and concentration of effluent from the final discharge point to Outer Sun Bay. The model was developed using 2000 and 2002 data, and predicted that the 1% effluent concentration limit would extend to 850 to 1,200 m from the mouth of the narrows in Outer Sun Bay (Golder 2004). This model was then updated with data from 2005 (Golder 2006). Runoff conditions were lower in 2005 than in 2000 and 2002. When these lower runoff conditions were included and the model was re-run, the 1% concentration limit extended to 1,630 m beyond the mouth of the narrows in Outer Sun Bay.

In 2005, field specific conductivity measured in the effluent was 847 µS/cm and the laboratory measured conductivity from the sample in Dam 1a Lake was 738 µS/cm. Base flows in Seep Creek are very low and it has been estimated that up to 90% of the flow is due to effluent discharge (RL&L and DFO 1991). Based on the field measured conductivity at 250 m downstream of the final discharge point, the water in the receiving system in 2005 consisted of 87% effluent.

In 2015, conductivity values in the MMER exposure (Station LUP-24) and reference areas (Station LUP-21) were 245 and 138 µS/cm on 24 September, and below 10 µS/cm on 27 October. Using conductivity as an effluent tracer, the estimated effluent concentration at Station LUP-24 ranged between 1% (27 October 2015) and 5% (24 September 2015). The estimated effluent concentration as well as the low conductivity values at Station LUP-24 suggest that the proportion of effluent in this area was lower in 2015 than previous model predictions.

No treated effluent was discharged in 2016. However, field conductivity at 3 km from the effluent discharge point (measured during the 2016 fish survey) was 210 µS/cm. Using the 2015 average conductivity values at LUP-10 (treated effluent; 662 µS/cm) and LUP-21 (MMER reference area, 13.6 µS/cm), the water in the receiving environment in Seep Creek at 3 km from effluent discharge was calculated as 30% effluent.

2.4 Confounding Factors

In 1992, a TCA spill occurred and potentially affected an area that had been considered for use as a reference area (Golder 2004). A considerable amount of snowmelt, mixed with tailings effluent, initially spilled into Long Lake (Figure 2.2-2). This material then flowed downstream into Test Lake eventually reaching Shallow and South Bays of Contwoyto Lake. Historically, Shallow and South Bays had been used as reference areas during various fisheries assessments (AQUAMIN 1996; EVS 1996); following the spill, the reference area was relocated to eliminate this confounding factor.

Natural habitat differences exist between the Exposure Area and Reference Areas. Overall, the Exposure Area contains more pond and creek habitat, while Reference Area 1 and 2 contain more lake habitat and less creek habitat. The ponds located in Reference Areas 1 and 2 are shallower than the Exposure Area ponds. Additionally, the presence of a large sandbar at the mouth of Fingers Creek and the close proximity of both Reference Areas

to Contwoyto Lake may influence the species assemblages and population densities of Arctic Grayling in Reference Areas 1 and 2 (AECOM 2009).

3.0 ENVIRONMENTAL EFFECTS MONITORING STUDIES AT LUPIN

Three areas locations were studied for the Lupin Mine Phase 5 EEM study biological study (Figure 2.2-2):

- Exposure Area: Seep Creek (fish survey) and Seep Creek Ponds 1 and 2 (fish and benthic invertebrate survey).
- Reference Area 1: Fingers Creek (fish survey) and Fingers Lake (benthic invertebrate survey).
- Reference Area 2: Unnamed Creek (fish survey) and Unnamed Pond (benthic invertebrate survey).

Detailed background information for the Exposure Area and Reference Area 1 was provided in the EEM Phase 1 Study Design (Golder 2004), and the EEM Phase 1, 2, and 3 Interpretative Reports (Golder 2006; AECOM 2009, 2011). Background information for Reference Area 2 was provided in AECOM (2011). A summary of information relevant to the Phase 5 EEM is provided below.

3.1 Exposure Study Area

An exposure area is defined as “all fish habitat and waters frequented by fish that are exposed to effluent” (EC 2012a). Dam 1a Lake and Seep Creek are the first waterbodies to receive effluent discharge. The three shallow lakes just below the TCA (Dam 2 Lake, Dam 1a Lake, and Unnamed Lake), Seep Creek (along with the associated Seep Creek ponds), and two embayment areas of Contwoyto Lake (Inner and Outer Sun Bay) were designated as the Exposure Area. For the EEM program, Seep Creek is the Exposure Area for the fish survey, while the Seep Creek ponds are the Exposure Area for the benthic invertebrate community survey (Figure 2.2-2).

3.1.1 Physical Habitat

The small lakes and ponds of the Exposure Area, including much of Inner Sun Bay, Seep Creek and Concession Creek, freeze to the bottom in winter. One exception is Dam2 Lake which has sufficient depth for overwintering. Based on historical data, over-wintering habitat for fish is limited primarily to Outer Sun Bay and the main body of Contwoyto Lake (RCPL and RL&L 1985).

Seep Creek is approximately 6.5 km in length, flowing from its source at Dam 2 Lake and Dam 1a Lake (via separate branches, which join approximately 2 km downstream) to Unnamed Lake (Figure 2.2-2). The channel in Seep Creek just upstream of Seep Creek Pond 1 is poorly defined, often flowing through marshy areas, between large boulders or through bedrock fractures. This section of the creek is generally less than 0.5 m in depth and average channel width is 2.0 m. The dominant substrate type of Seep Creek consisted of boulders, although localized areas of cobble and gravel are present. The channel in the lower portion of Seep Creek (i.e., the 400 m section upstream of Unnamed Lake) is characterized by a well-developed channel varying in width from 1.0 to 4.0 m, although during freshet in 1985, maximum wetted width was approximately 20 m. The dominant substrate type consisted of boulders, with localized areas of cobble and gravel (RCPL and RL&L 1985; AECOM 2011).

Two ponds (Seep Pond 1 and Seep Pond 2) are located near the downstream end of Seep Creek in the Exposure Area (Figure 2.2-2). Maximum depth of these ponds was approximately 1.5 m. The surface area of Seep Creek Pond 1 is 3 ha and the surface area of Seep Creek Pond 2 is 2 ha. Unnamed Lake, the receiving waterbody for

Seep Creek has a surface area of 16 ha, a maximum depth of approximately 1.0 m, and likely freezes to the bottom during winter (RCPL and RL&L 1985). Bottom substrate was composed of organic debris/silt and small boulders (RCPL and RL&L 1985).

3.1.2 Water Quality (1985 to 2010)

In 1985, water quality was measured at stations in Seep Creek, Inner Sun Bay, and Outer Sun Bay in August (just before treated effluent discharge) and September (during the first discharge event). Conductivity values recorded at all stations in September were elevated compared to those measured in August. In September, concentrations of all metals, with the exception of arsenic, were found to be significantly higher than the concentrations measured in August. In addition, the September concentrations for arsenic, copper, and zinc exceeded Canadian Water Quality Guidelines (CWQG) (Mudroch and Sutherland 1988; Golder 2004).

Between 2005 and 2010, a total of nine water quality samples were collected in late August-early September in the Exposure Area in support of the EEM programs (Golder 2006; AECOM 2009, 2011) (Table 3.1-1). Water quality results were compared to the CWQG for the protection of freshwater aquatic life (CCME 1999). Summary statistics were calculated on these data to describe existing conditions in the Exposure Area (Table 3.1-2). From this dataset, mean pH for the Exposure Area was below the CWGQ range minimum value of 6.5 and mean concentrations of aluminum, cadmium, copper, nickel, and zinc exceeded applicable CWQG (CCME 1999). Although mean concentrations of arsenic, iron, and lead were below applicable CWQG, concentrations of these parameters occasionally exceeded the CWQG (Golder 2006; AECOM 2009, 2011).

Table 3.1-1: Water Quality Sampling Stations in the Exposure Area for EEM Phases 1 to 3

Area	Station ID	Phase 1 (2005)	Phase 2 (2008)	Phase 3 (2010)
Exposure Area	SCD1	√	√	√
	Seep Creek Pond 1 and 2	√	-	-
	SCP1	-	√	√
	SCP5	-	√	-
	LUP-20[(a)]	-	√	√

Source: Golder (2006); AECOM (2009, 2011).

(a) LUP-20 is formerly known as SNP925-20.

√ = sample collected; - = no sample collected.

Table 3.1-2: Summary of Water Quality in the Exposure Area, 2005, 2008, and 2010

Parameter	Unit	CWQG[a]	n	Exposure Area				
				Mean	SD	Median	Min	Max
Conventional Parameters								
Temperature (field)	°C	-	7	7.1	5.3	3.5	2.2	13.8
Total alkalinity (as $CaCO_3$)	mg/L	-	9	<5	-	<5	<5	6.2
Specific conductivity	µS/cm	-	9	179.8	216.7	76.8	65.4	738.0
Hardness (as $CaCO_3$)	mg/L	-	9	53.2	52.8	29.0	22.9	186.0
pH (laboratory)	-	6.5-9.0	9	**6.0**	0.7	**5.8**	**4.9**	6.9
Total suspended solids	mg/L	-	9	<3	-	<3.0	<3	8
Nutrients								
Nitrogen-Ammonia[b]	mg/L	5.8-189	9	0.068	0.166	0.009	<0.005	0.510
Nitrogen-Nitrate	mg/L	13	9	0.707	1.877	0.016	<0.006	5.700
Other								
Total cyanide	mg/L	0.005	9	<0.002	-	<0.002	<0.002	<0.002
Radium-226	Bq/L	-	9	<0.01	-	<0.01	0.006	0.01
Total Metals and Metalloids								
Aluminum[c]	µg/L	5-100	9	**143**	**126**	**110**	**31**	**461**
Arsenic	µg/L	5	9	4.2	2.4	3.8	1.7	**8.2**
Cadmium[d]	µg/L	0.009-0.056	9	**0.13**	**0.14**	**0.07**	**<0.05**	**0.41**
Copper[d]	µg/L	2-4	9	**7.31**	**5.51**	**5.20**	**3.50**	**19.70**
Iron	µg/L	300	9	210.2	228.9	109.0	30.2	**760.0**
Lead[d]	µg/L	1.0-7.0	9	0.22	0.44	0.06	<0.05	**1.39**
Mercury	ng/L	26	8	<DL[e]	-	<DL[e]	<DL[e]	0.0113
Molybdenum	µg/L	73	9	0.11	0.06	0.10	<0.06	0.24
Nickel[d]	µg/L	31-153	9	**61.0**	53.2	**42.6**	17.5	**170.0**
Selenium	µg/L	1	8	0.18	0.15	0.10	<0.10	0.48
Zinc	µg/L	30	9	**82.4**	102.0	**40.5**	8.2	**314.0**

Notes: Due to variable detection limits between the study years, where a result was reported as less than the detection limit, the statistic was calculated by assuming the non-detectable result was equal to the detection limit. Note that an error was noted in the Phase 5 EEM Study Design summary statistics and numbers may differ from this table

Bold font identifies CWQG exceedance.

(a) CWQG for the protection of freshwater aquatic life (CCME 1999).

(b) Guideline calculated using site-specific laboratory pH and field temperature.

(c) Guideline calculated using site-specific laboratory pH value.

(d) Guideline calculated using site-specific hardness value.

(e) Detection Limit ranged between 0.01 and 20 ng/L.

CWQG = Canadian Water Quality Guidelines; n = number of samples; SD = standard deviation; $CaCO_3$ = calcium carbonate; µS/cm = microSiemens per centimetre; Bq/L = Becquerels per litre; <= less than; <DL = less than a variable detection limit; - = not available or not applicable.

3.1.3 Sediment Quality (1985 to 2010)

In 1985, sediment quality was assessed in Seep Creek, Inner Sun Bay, and Outer Sun Bay in August (prior to the first discharge of treated effluent) and September (during the first discharge event). Seven stations were sampled

in the Seep Creek Ponds in the Exposure Area. Of these stations, concentrations of copper, nickel, and zinc increased at two of the stations between August and September (Mudroch and Sutherland 1988).

A second sediment study was conducted in 1990. The results of this study did not indicate any clear trends in sediment chemistry; however, there was an indication of increased deposition of fine sediment particles at some of the deeper sample stations. Therefore, there is the potential that the deeper stations may be acting as sinks for solids and metals (Porter et al. 1992).

RL&L (1996) conducted a literature survey to compare sediment chemistry trends from 1985, 1990, and 1991. No clear spatial or temporal trends were discernible. However, mean concentrations of copper, lead, and zinc tended to be greater during 1991 than in previous years.

Sediments were sampled from the same stations in the Seep Creek Ponds as part of the Phase 1, 2, and 3 EEM programs in late August-early September (Golder 2006; AECOM 2009, 2011); results were similar in all three phases. The composition of sediments was predominantly sand and silt. Concentrations of arsenic and chromium exceeded their applicable Interim Sediment Quality Guidelines (ISQG) at the Exposure Area in all phases (CCME 2001).

3.1.4 Aquatic Resources (1982 to 2010)

3.1.4.1 Fisheries

Arctic Char (*Salvelinus alpinus*), Arctic Grayling, Burbot (*Lota lota*), Cisco (*Coregonus artedi*), Lake Trout (*Salvelinus namaycush*), Ninespine Stickleback (*Pungitius pungitius*), Round Whitefish (*Prosopium cylindraceum*), and Slimy Sculpin (*Cottus cognatus*) have been previously documented in Seep Creek (RCPL and RL&L 1985; AECOM 2011). RL&L and DFO (1991) assessed Seep Creek and encountered Arctic Grayling, Ninespine Stickleback, and Round Whitefish in the stream during spring and summer. They found that fish use the stream for spawning, feeding, and juvenile rearing in the early part of the open water season. The majority of the fish were moving downstream when captured in 1990, which was likely in response to decreasing water levels as snowmelt run-off subsided. Arctic Grayling spawning was also documented in Seep Creek in 1983 and 1984 (RCPL and RL&L 1985).

In 1983 (June 19 to July 1) and 1984 (June 9 to June 29), a box trap was installed to monitor upstream fish migration into Seep Creek. In total, 26 adult Arctic Grayling were captured in 1983 and 7 adult Arctic Grayling were captured in 1984. In 1991, sampling in Seep Creek was conducted between June 28 and July 3, and of the 76 Arctic Grayling captured during this sampling event, 66 were reported as juveniles (i.e., 75 to 190 mm; age one to age three). It was concluded that Arctic Grayling use Seep Creek extensively for rearing but that very little spawning occurs in the creek (RCPL and RL&L 1985; RL&L and DFO 1991).

From 19 August to 4 September 2005, during the Phase 1 EEM fish survey (Golder 2006), four fish species (Arctic Char, Arctic Grayling, Lake Trout, and Ninespine Stickleback) were captured in the Exposure Area, specifically in Seep Creek, Seep Creek Ponds, and Inner Sun Bay. In total, 86 juvenile Arctic Grayling and 99 Ninespine Stickleback were captured (Golder 2006). During the Phase 2 EEM survey (AECOM 2009), from 28 August to 9 September 2008, seven fish species (Arctic Char, Arctic Grayling, Burbot, Lake Trout, Ninespine Stickleback, Round Whitefish, and Slimy Sculpin) were captured in the Exposure Area. A total of 112 juvenile Arctic Grayling and 130 Ninespine Stickleback were captured (AECOM 2009). During the Phase 3 EEM survey (AECOM 2011), from 18 to 29 August 2010, three fish species (Arctic Grayling, Burbot, and Ninespine Stickleback) were captured

in the Exposure Area. A total of 28 juvenile Arctic Grayling and 193 Ninespine Stickleback were captured (AECOM 2011).

3.1.4.2 Benthic Invertebrate Community

Benthic invertebrate samples were collected from the Seep Creek watershed and Inner Sun Bay in September 1982, 1983, and 1984, prior to discharge of treated effluent. In total, 30 benthic invertebrate taxa were identified, with midges and aquatic worms being the dominant taxa. Bivalves were present at Inner Sun Bay. Shallower sites were found to vary considerably in number and diversity of organisms, likely as a result of the extreme environmental conditions experienced (i.e., freezing, thawing, and temperature fluctuations; RCPL and RL&L 1985).

Following the first treated effluent discharge in 1985, Environment Canada collected samples at Inner and Outer Sun Bay to evaluate whether the effluent discharge from the Mine was having an effect on the benthic invertebrate community. The study found that the Mine effluent did not have a measurable effect on taxonomic composition or abundance (Mudroch and Sutherland 1988). A second study was conducted in 1990 (Porter et al.1992) to evaluate the longer term effects of treated effluent on the benthic invertebrate community in the West Arm of Contwoyto Lake). This study concluded that there was a decrease in abundances of common invertebrates between the two years of studies; however, this decrease may have been due to the increased percentage of fine particles in bottom sediments during the 1990 study compared to the 1985 study (Porter et al.1992).

In 2005, during the Phase 1 EEM survey, a total of 10 benthic invertebrate families were encountered in the Seep Creek Ponds. Dipterans, primarily Chironomidae (midges), were the dominant family (mean proportion of benthic invertebrates: 93%; Golder 2006). During the Phase 2 EEM survey, Chironomidae were again the most common benthic invertebrate group in the Exposure Area, representing approximately 45% of the total benthic invertebrate fauna (AECOM 2009). A benthic invertebrate community survey was not included in the Phase 3 IOC study.

3.2 Reference Study Areas

A reference area is defined as “water frequented by fish that is not exposed to effluent and that has fish habitat that, as far as practical, is most similar to that of the exposure area” (EC 2012a). For the EEM program, Fingers Creek (fish survey) and Fingers Lake (benthic invertebrate community survey) were designated in 2005 as Reference Area 1, while an unnamed creek (fish survey) and an unnamed pond (benthic invertebrate community survey) located south of Reference Area 1 were designated in 2010 as Reference Area 2 (Figure 2.2-2). Reference Area 1 was first surveyed for the Phase 1 EEM program, and again for the Phase 2 and 3 EEM programs, while Reference Area 2 was first surveyed for the Phase 3 EEM program.

3.2.1 Physical Habitat

Reference Area 1

Reference Area 1 consists of Fingers Lake, two unnamed waterbodies (ponds), and a flowing watercourse named Fingers Creek. Fingers Creek flows for approximately 3 km between Fingers Lake and Contwoyto Lake (Figure 2.2-2). There are three distinct sections of Fingers Creek qualitatively described as upstream, mid-stream, and downstream. The upstream section of the channel (approximately 400 m in length), and upstream of the two ponds, was braided and contained substrate composed primarily of silt and boulders, with some cobble. The mid-stream section of the channel widened to approximately 2.0 to 3.0 m, and substrate consisted of cobble, gravel, and silt, with some boulders (Golder 2006; AECOM 2011). The downstream end of the creek featured a narrow

channel, approximately 1.0 m wide, with substrates dominated by cobble, fines, and boulders. The upstream section was primarily shallow flat habitat whereas the midstream and downstream sections were primarily shallow run habitat. The average depth was 0.5 m through the upstream and midstream sections, and approximately 0.3 m in the downstream section (AECOM 2011). Approximately 20% of the entire creek and 40% of the midstream section contained aquatic vegetation and periphytic algae (Golder 2006). The banks were vegetated and stable, and were composed primarily of fines (Golder 2006; AECOM 2011).

Fingers Lake has a maximum depth of 6.0 m and a surface area of 370 ha (Moore 1978). Fingers Lake is a clear-water lake with little aquatic vegetation, except in the depositional areas (Golder 2006). The lake is shallower toward the northeast end, and the substrate is primarily cobble and boulders extending from the northeast shore to approximately 400 m into the lake. A boat dock is located on the southwest end of the lake. The north, east, and west shores have steep drop-offs and boulders near shore. The south shore of the lake has a gradual slope and there are four narrow shallow bays on the southeast side of the lake, which are primarily depositional habitat (Golder 2006; AECOM 2011).

Reference Area 2

A second reference area was added for the Phase 3 EEM to aid in assessing the range of natural variability among Arctic Grayling populations. Reference Area 2 is southeast of Reference Area 1; however, the two areas are not hydrologically connected. Reference Area 2, like Reference Area 1, drains to Contwoyto Lake and has two small waterbodies (ponds) in the drainage area. The creek flows for about 3 km between the upper pond and Contwoyto Lake (AECOM 2011). The creek was approximately 1.0 to 2.0 m wide and the average water depth was 0.3 m. Several residual pool depths have been recorded, with an average depth of 0.8 m. The substrate was dominated by boulders and fines. The banks were composed of fine sediments and were vertical in shape. Instream cover was provided by abundant instream, submerged vascular vegetation and moderate amounts of boulders (AECOM 2011).

3.2.2 Water Quality (2005, 2008, and 2010)

Between 2005 and 2010, in support of the EEM studies, a total of eight water samples were collected from Fingers Lake of Reference Area 1 and three water samples were collected from the small waterbodies in Reference Area 2 (Golder 2006; AECOM 2009, 2011) (Table 3.2-1). Water quality results were compared to the CWQG for the protection of freshwater aquatic life (CCME 1999). Summary statistics were calculated for these data to describe existing conditions in each reference area (Tables 3.2-2 and 3.2-3).

Table 3.2-1: Water Quality Sampling Stations in the Reference Areas for EEM Phases 1 to 3

Area	Station ID	Phase 1	Phase 2	Phase 3
Reference Area 1	FC2	√	√	√
	Fingers Lake	√	-	-
	FL1	-	√	-
	FL5	-	√	√
Reference Area 2	R2-1	-	-	√
	R2-2	-	-	√
	R2-3	-	-	√

√ = sample collected; - = no sample collected.

In Reference Area 1, pH was below the CWQG range minimum value of 6.5 (CCME 1999) in one sample in 2005, and mean concentrations of aluminum exceeded applicable CWQG in that same sample. In Reference Area 2, copper exceeded the CWQG (CCME 1999) in one sample. No other parameters in either Reference Area exceeded applicable CWQG (CCME 1999).

Table 3.2-2: Summary of Water Quality for Reference Area 1, 2005, 2008, and 2010

Parameter	Unit	CWQG[a]	Reference Area 1						
			DL Range[b]	n	Mean	SD	Median	Min	Max
Conventional Parameters									
Temperature (field)	°C	-	-	6	5.9	3.0	4.9	3.1	10.8
Total alkalinity (as $CaCO_3$)	mg/L	-	-	8	<5	-	<5	<5	<5
Specific conductivity	µS/cm	-	-	8	13.1	1.4	13.2	11.5	15.3
Hardness (as $CaCO_3$)	mg/L	-	-	8	5.2	1.3	5.0	4.0	7.0
pH (laboratory)	-	6.5-9.0	-	8	6.6	0.2	6.6	**6.3**	6.9
Total suspended solids	mg/L	-	-	8	<3	-	<3	<3	<3
Nutrients									
Nitrogen-Ammonia[c)]	mg/L	10.0-73.5	0.005 to 0.05	8	<DL	-	<DL	<DL	<DL
Nitrogen-Nitrate	mg/L	13	0.006 to 0.1	8	<DL	-	<DL	0.007	0.100
Other									
Total cyanide	mg/L	0.005	-	8	<0.002	-	<0.002	<0.002	<0.002
Radium-226	Bq/L	-	0.005 to 0.01	8	<DL	-	<DL	<DL	0.006
Total Metals and Metalloids									
Aluminum[(d)]	µg/L	5-100	-	8	18	8	16	**10**[(f)]	37
Arsenic	µg/L	5	-	8	1.5	0.3	1.6	1.2	2.1
Cadmium[(e)]	µg/L	0.002-0.003	0.002 to 0.05	8	<DL	-	<DL	<DL	0.009
Copper[(e)]	µg/L	2	-	8	<0.6	-	<0.6	0.390	0.562
Iron	µg/L	300	-	8	39.5	23.5	32.1	18.1	94.2
Lead[(e)]	µg/L	1	-	8	<0.05	-	<0.05	0.01	0.28
Mercury	ng/L	26	0.01 to 20	8	<DL	-	<DL	<DL	<DL
Molybdenum	µg/L	73	-	8	<0.06	-	<0.06	0.008	0.017
Nickel[(e)]	µg/L	25	-	8	0.51	0.12	0.52	0.34	0.65
Selenium	µg/L	1	-	8	<0.1	-	<0.1	<0.1	<0.1
Zinc	µg/L	30	-	8	<0.8	-	<0.8	1.7	2.2

Notes: Due to variable detection limits between the study years, where a result was reported as less than the detection limit, the statistic was calculated by assuming the non-detectable result was equal to the detection limit. Note that an error was noted in the Phase 5 EEM Study Design summary statistics and numbers may differ from this table.

Bold font identifies CWQG exceedance.

(a) CWQG for the protection of freshwater aquatic life (CCME 1999).

(b) Detection Limit range when parameter was measured below variable detection limits.

(c) Guideline calculated using site-specific laboratory pH and field temperature.

(d) Guideline calculated using site-specific laboratory pH value: 0.100 mg/L at pH ≥ 6.5; and 0.005 mg/L if pH <6.5 (one sample).

(e) Guideline calculated using site-specific hardness value.

(f) Minimum aluminum was above guideline because it is associated pH was below 6.5.

CWQG = Canadian Water Quality Guidelines; n = number of samples; SD = standard deviation; $CaCO_3$ = calcium carbonate; µS/cm = microSiemens per centimetre; Bq/L = Becquerels per litre; <= less than; <DL = less than variable detection limit; - = not available or not applicable.

Table 3.2-3: Summary of Water Quality for Reference Area 2, 2010

Parameter	Units	CWQG[a]	Reference Area 2					
			n	Mean	SD	Median	Min	Max
Conventional Parameters								
Temperature (field)	°C	-	2	11.7	1.6	11.7	10.5	12.8
Total alkalinity (as $CaCO_3$)	mg/L	-	3	<5	-	<5	<5	<5
Specific conductivity	µS/cm	-	3	12.2	3.2	10.6	10.2	15.9
Hardness (as $CaCO_3$)	mg/L	-	3	4.3	1.1	4.2	3.2	5.4
pH (laboratory)	-	6.5-9.0	3	6.6	0.1	6.7	6.5	6.7
Total suspended solids	mg/L	-	3	<3	-	<3	<3	<3
Nutrients								
Nitrogen-Ammonia[b]	mg/L	13.5-25.6	3	<0.005	-	<0.005	<0.005	0.008
Nitrogen-Nitrate	mg/L	13	3	<0.006	-	<0.006	<0.006	<0.006
Other								
Total cyanide	mg/L	0.005	3	<0.002	-	<0.002	<0.002	<0.002
Radium-226	Bq/L	-	3	<0.01	-	<0.01	<0.01	<0.01
Total Metals and Metalloids								
Aluminum[c]	µg/L	100	3	20	6	17	16	27
Arsenic	µg/L	5	3	0.69	0.01	0.70	0.68	0.70
Cadmium[d]	µg/L	0.002-0.003	3	<0.05	-	<0.05	<0.05	<0.05
Copper[d]	µg/L	2	3	1.60	1.20	0.93	0.88	**3.00**
Iron	µg/L	300	3	79.6	16.8	70.9	68.9	98.9
Lead[d]	µg/L	1	3	<0.05	-	<0.05	<0.05	0.09
Mercury	ng/L	26	3	<20	-	<20	<20	<20
Molybdenum	µg/L	73	3	<0.06	-	<0.06	<0.06	<0.06
Nickel[d]	µg/L	25	3	3.64	3.10	1.93	1.77	7.23
Selenium	µg/L	1	3	<0.1	-	<0.1	<0.1	<0.1
Zinc	µg/L	30	3	<0.8	-	<0.80	<0.80	1.24

Notes: due to variable detection limits between the study years, where a result was reported as less than the detection limit, the statistic was calculated by assuming the non-detectable result was equal to the detection limit. Note that an error was noted in the Phase 5 EEM Study Design summary statistics and numbers may differ from this table.

Bold font identifies CWQG exceedance.

(a) CWQG for the protection of freshwater aquatic life (CCME 1999).

(b) Guideline calculated using site-specific laboratory pH and field temperature.

(c) Guideline calculated using site-specific laboratory pH value.

(d) Guideline calculated using site-specific hardness value.

CWQG = Canadian Water Quality Guidelines; n = number of samples; SD = standard deviation; $CaCO_3$ = calcium carbonate; µS/cm = microSiemens per centimetre; Bq/L = Becquerels per litre; <= less than; - = not available or not applicable.

3.2.3 Sediment Quality (2005, 2008, and 2010)

In 2005, during the Phase 1 EEM program (Golder 2006), sediment samples were collected at Reference Area 1 (Fingers Lake). Sediments were composed primarily of sand; arsenic exceeded the ISQG for the protection of aquatic life (CCME 2001) in one of the five samples collected (Golder 2006). In 2009, for the Phase 2 EEM program, sediment samples from Reference Area 1 were again composed primarily of sand; four of the five sediment samples collected in Reference Area 1 (Fingers Lake) exceeded the arsenic ISQG for the protection of aquatic life (AECOM 2009). In the Phase 3 EEM program, sediment samples from Reference Area 1 and Reference Area 2 were composed primarily of sand. Overall, the majority of metal concentrations were similar in both Reference Areas in the Phase 3 EEM program; however, Reference Area 2 generally had higher concentrations of metals (AECOM 2011).

3.2.4 Aquatic Resources

3.2.4.1 Fisheries

Arctic Char and Lake Trout were captured in Fingers Lake (Reference Area 1) over 30 years ago (Moore 1978). There are no fish capture data for Fingers Creek (Reference Area 1) prior to the Phase 1 EEM fish survey.

Six fish species (Arctic Grayling, Burbot, Lake Trout, Ninespine Stickleback, Round Whitefish, and Slimy Sculpin) have been consistently captured from Reference Area 1 during all three EEM phases (Golder 2006; AECOM 2009, 2011). In Phase 1, a total of 52 juvenile Arctic Grayling and one Ninespine Stickleback were captured (Golder 2006). In Phase 2, 40 juvenile Arctic Grayling and 96 Ninespine Stickleback were captured (AECOM 2009). In Phase 3, a total of 25 juvenile Arctic Grayling and 16 Ninespine Stickleback were captured (AECOM 2011).

In Reference Area 2, seven fish species have been captured. Six of these fish species (Arctic Grayling, Burbot, Lake Trout, Ninespine Stickleback, Round Whitefish, and Slimy Sculpin) were also captured in Reference Area 1 during an EEM survey; with the exception of the fish captured over 30 years ago (Moore 1978), Arctic Char were only captured in Reference Area 2. During the Phase 3 EEM fish survey, a total of 33 juvenile Arctic Grayling and 158 Ninespine Stickleback were captured (AECOM 2011).

3.2.4.2 Benthic Invertebrate Community

In 2005, during the Phase 1 EEM survey, a total of 11 benthic invertebrate families were encountered in Reference Area 1, which was dominated by dipterans, primarily Chironomidae (mean proportion of benthic invertebrates: 91%) (Golder 2006). During the Phase 2 EEM survey, Chironomidae were again the most common benthic invertebrate group in Reference Area 1, representing 45 to >90% of the total benthic invertebrates (AECOM 2009).

A benthic invertebrate community survey was not included in the Phase 3 IOC study; therefore, no benthic invertebrate data is available for Reference Area 2.

3.3 Summary of Results from Phase 1 to Phase 4 EEMs

3.4 Phase 1 (2005)

Phase 1 was conducted as a Periodic Monitoring – Surveillance study (Golder 2004 and 2006). The biological studies for the Phase 1 EEM program were completed during late summer/early fall between 19 August and 4 September 2005. The Phase 1 EEM program consisted of the following components:

- water quality monitoring
- sediment quality monitoring
- lethal fish survey with juvenile Arctic Grayling as the sentinel fish species
- benthic invertebrate community survey
- effluent sub-lethal toxicity sampling

Mercury concentrations in treated Mine effluent were below 0.1 µg/L; therefore, fish tissue chemistry analysis was not required (EC 2002a).

3.4.1 Sampling Areas

Sampling for the Phase 1 EEM program was conducted in one Exposure Area and one Reference Area (Figure 2.2-2; Golder 2006, Figure 2.1):

- Exposure Area – Seep Creek (fish survey) and Seep Creek Ponds (fish and benthic invertebrate community surveys).
- Reference Area 1 – Fingers Creek (fish survey) and Fingers Lake (fish and benthic invertebrate community surveys).

3.4.2 Water Quality

Water quality was evaluated during the field survey to aid with the interpretation of the biological data. Conductivity, nitrate, ammonia, and most metal concentrations were greater in the Exposure Area than in Reference Area 1. In addition, aluminum, arsenic, cadmium, copper, lead, nickel, and zinc were above applicable CWQG in the Exposure Area for the protection of freshwater aquatic life (CCME 1999). Measurements of pH were below minimum CWQG range in the Exposure Area and in one sample from Reference Area 1. Aluminum exceeded CWQG in one sample from Reference Area 1.

3.4.3 Sediment Quality

Sediments collected within the Exposure Area had elevated concentrations of arsenic, barium, cobalt, chromium, copper, nickel, vanadium, and zinc relative to Reference Area 1. Sediment arsenic concentrations in all samples collected in the Exposure Area, and one sample from Reference Area 1, exceeded the ISQG for the protection of freshwater aquatic life (CCME 2001). Chromium exceeded the ISQG in two Exposure Area sediment samples. Exposure Area sediments were composed of sand and silt, whereas Reference Area sediments consisted of mainly sand, with little silt or clay.

3.4.4 Fish Survey

A lethal Arctic Grayling survey was conducted to investigate the effects of treated Mine effluent on fish in the Exposure Area by exploring differences in population characteristics between fish inhabiting the Exposure Area and Reference Area 1. This was accomplished by assessing size and energy storage characteristics in juvenile Arctic Grayling.

Fishing methods involved fyke nets, hoop nets, gill nets, and backpack electrofishing. In total, 42 Arctic Grayling from the Exposure Area and 36 Arctic Grayling from Reference Area 1 were captured and sacrificed. Overall, the state of general health (i.e., occurrence of abnormalities and parasites) of Arctic Grayling was similar in the Exposure and Reference Areas. Age one Arctic Grayling were 31% heavier and 7% longer, on average, in Reference Area 1 than in the Exposure Area. Condition was 8% greater in the Reference Area 1 fish than in Exposure Area fish. Liver weight-at-carcass weight was not statistically different between Reference and Exposure Area fish.

3.4.5 Benthic Invertebrate Community Survey

Depositional habitat was sampled in each pond, and habitat features (i.e., depth and substrate) were standardized to the extent possible. Five replicate stations were sampled in each area. At each replicate station, five subsamples were collected and pooled into a single composite sample. All samples were collected in water depths between 0.3 and 1.3 m.

Although benthic invertebrate density was greater in the Reference than in the Exposure Area, differences were not statistically significant. Family richness was similar in both areas, and was not significantly different. The Bray-Curtis Index (BCI) was more variable in Reference Area 1, but was not significantly different from the Exposure Area. Simpson's Diversity Index (SDI) and evenness were significantly lower in the Exposure Area than Reference Area 1. The differences in SDI and evenness may be linked to elevated arsenic and chromium concentrations in the Exposure Area sediments, as well as elevated metal concentrations in the water column of the Exposure Area.

3.4.6 Sub-lethal Toxicity Testing

Test organisms used for sub-lethal toxicity testing were Fathead Minnow (*Pimephales promelas*), a water flea (*Ceriodaphnia dubia),* an aquatic plant (*Lemna minor*), and an alga (*Pseudokirchneriella subcapitata*). Endpoints were 25% inhibition concentration (IC_{25}) values for all organisms and median lethal concentration (LC_{50}) values for Fathead Minnow and *C. dubia*.

Effluent was non-lethal to Fathead Minnow and *C. dubia*, but was sub-lethally toxic to all organisms except for Fathead Minnow. *P. subcapitata* growth was inhibited in effluent concentrations greater than 17%. *C. dubia* reproduction was inhibited in a 12% effluent concentration on August 30, 2005, but no sub-lethal effects were observed for this test organism in effluent from July 27, 2005. *L. minor* growth and reproduction were inhibited at concentrations greater than 10% and 6.1%, respectively.

3.4.7 Overall Assessment

The Phase 1 EEM program concluded that benthic invertebrates and fish showed effects related to Mine effluent discharge. While the general health of Arctic Grayling was similar in Reference and Exposure Areas, Arctic Grayling were heavier, longer, and in better condition in Reference Area 1. Differences in the benthic invertebrate communities may be related to elevated metal concentrations in water and sediment within the Exposure Area.

3.5 Phase 2 (2008)

The Phase 2 EEM program for the Mine occurred from 28 August to 9 September 2008 (AECOM 2009). The main objective of the Phase 2 EEM program was to confirm the findings from the Phase 1 EEM program by evaluating the effects, if any, of treated mine effluent on the Ninespine Stickleback population, juvenile Arctic Grayling population, and benthic invertebrates in the Exposure Area. As such, Phase 2 was conducted as a Periodic Monitoring – Confirmation study that involved the following components:

- water quality monitoring
- sediment quality monitoring
- lethal fish survey with juvenile Arctic Grayling and Ninespine Stickleback as the sentinel fish species
- benthic invertebrate community survey

Mercury concentrations in treated mine effluent were again below 0.1 µg/L; therefore, fish tissue chemistry analysis was not required (EC 2002a). However, at the request of Environment Canada juvenile Arctic Grayling tissues were analyzed for copper.

In 2008, no treated effluent was discharged; therefore, no sub-lethal toxicity testing was conducted.

3.5.1 Sampling Areas

Sampling for the Phase 2 EEM was conducted within the same sampling areas as the Phase 1 EEM program (Figure 2.2-2; AECOM 2009, Figure 1):

- Exposure Area – Seep Creek (fish survey) and Seep Creek Ponds (fish and benthic invertebrate community surveys).
- Reference Area 1 – Fingers Creek (fish survey) and Fingers Lake (fish and benthic invertebrate community surveys).

3.5.2 Water Quality

Concentrations of routine water chemistry parameters (i.e., hardness, TOC, and DOC), major ions (i.e., manganese, magnesium, and sodium) and total metals (i.e., aluminum, boron, cadmium, cobalt, copper, iron manganese, and zinc) were elevated in the Exposure Area relative to Reference Area 1. In addition, pH was lower in the Exposure Area compared to Reference Area 1. None of the water samples from the Reference Area exceeded applicable CWQG for the protection of freshwater aquatic life (CCME 1999). In the Exposure Area, pH was below the minimum CWQG range value of 6.5 for protection of freshwater aquatic life (CCME 1999), and aluminum, cadmium, copper, nickel, and zinc were above the CWQG for the protection of freshwater aquatic life (CCME 1999). These findings were similar to those of Phase 1.

3.5.3 Sediment Quality

Exposure Area sediments were composed of sand, silt, and clay while Reference Area 1 sediments were composed mainly of sand with little silt or clay. Sediment samples collected in the Exposure Area had elevated concentrations of arsenic, cobalt, copper, nickel, and zinc relative to sediments from Reference Area 1. Sediment arsenic concentrations exceeded the ISQG for the protection of freshwater aquatic life (CCME 2001) in the Exposure Area and Reference Area 1. Chromium concentration exceeded the ISQG for the protection of freshwater aquatic life (CCME 2001) in the Exposure Area.

3.5.4 Fish Survey

A lethal Ninespine Stickleback and juvenile Arctic Grayling survey were conducted to investigate the effects of treated Mine effluent discharge on fish in the Exposure Area.

Fishing methods involved backpack electrofishing, gill nets, and minnow traps. In total, 109 Arctic Grayling from the Exposure Area and 39 Arctic Grayling from Reference Area 1 were captured, while 104 and 95 Ninespine Stickleback were captured from the Exposure Area and Reference Area 1, respectively.

Since the majority of Arctic Grayling were young-of-the-year (YOY), statistical analyses were restricted to assessing energy use and storage in YOY Arctic Grayling. Overall, the state of general health (i.e., occurrence of abnormalities and parasites) of YOY Arctic Grayling was similar between the Exposure Area and Reference Area 1. YOY Arctic Grayling were 44% heavier and 16% longer, on average, in Reference Area 1 than in the Exposure Area. Condition was 18% greater in the Reference Area 1 fish than in Exposure Area fish. Liver weight relative to length was 4% greater in the Reference Area 1 fish. There was no significant difference in copper concentration in Arctic Grayling muscle tissues between the Exposure and Reference Areas.

All of the captured Ninespine Stickleback were classified as immature fish. Ninespine Stickleback in the Exposure Area weighed 138% more and were 40% longer than Ninespine Stickleback captured from Reference Area 1. Conversely, condition was 15% higher in Reference Area 1 than in the Exposure Area. Liver weight relative to carcass weight was not significantly different in fish between the Reference and Exposure Areas. Liver weight relative to fork length was 9% higher in fish in the Exposure Area than those in Reference Area 1.

3.5.5 Benthic Invertebrate Community Survey

Depositional habitat was sampled at stations with similar habitat features (i.e., depth and substrate). Five replicate stations were sampled at each area. At each replicate station, five subsamples were collected and pooled into a single composite sample. All samples were collected in water depths between 0.5 and 1.2 m.

Density of benthic invertebrates and BCI were higher in the Exposure Area compared to Reference Area 1. Species diversity and evenness were higher in Reference Area 1 compared to the Exposure Area. As the Phase 2 study was conducted three years after the last effluent discharge, these results suggest that density and richness are variable by area and may not be influenced by historical effluent discharge.

3.5.6 Overall Assessment

The Phase 2 EEM field program was conducted more than three years after the last treated Mine effluent discharge period in 2005. Results of the Phase 2 EEM program indicated that benthic invertebrates and fish may be influenced by historical mine contamination. Effects observed in the YOY Arctic Grayling population in the Exposure Area consisted of decreased growth (based on length and weight). Effects observed on the Ninespine Stickleback population in the Exposure Area consisted of increased growth (based on length and weight). The effects observed in the YOY Arctic Grayling confirmed the Phase 1 results and, as such, triggered an IOC study for Phase 3. Although differences were detected in the benthic invertebrate community, they did not confirm the Phase 1 results and benthic invertebrates were excluded from the Phase 3 IOC study.

3.6 Phase 3 (2010)

Results of the Phase 1 and 2 fish surveys indicated that juvenile Arctic Grayling were consistently shorter and lighter in the Exposure Area as compared to Reference Area 1 (Golder 2006; AECOM 2009). As such, an IOC Study was initiated for the Phase 3 EEM program to determine the cause of growth differences in juvenile Arctic Grayling (AECOM 2011). It was hypothesized that Fingers Lake, in the Reference Area, provided thermal warming, facilitating YOY Arctic Grayling growth. The Exposure Area temperatures were expected to be colder due to lack of significant lake or ponded water. Due to the potential that the previously used Reference Area may not be the

most appropriate for comparison to the Exposure Area due to differences in ponded water, an Alternative Reference Area was selected based on the absence of ponded water and the presence of YOY Arctic Grayling. The Phase 3 fish survey was conducted in late summer between 18 and 29 August 2010.

The following components were included in the Phase 3 EEM program:

- water quality monitoring
- sediment quality monitoring
- non-lethal fish survey with juvenile Arctic Grayling

Mercury concentrations in treated mine effluent continued to be below 0.1 µg/L; therefore, fish tissue chemistry sampling was not required (EC 2002a). However, metal concentrations in whole-bodied Arctic Grayling were analyzed to determine whether there was a link between tissue metal concentrations and decreased growth.

A benthic invertebrate community survey was not included in the Phase 3 IOC study because effects were not confirmed between Phase 1 and Phase 2. In 2010, no treated effluent was discharged; therefore, no sub-lethal toxicity testing was conducted.

3.6.1 Sampling Areas

Sampling for the Phase 3 EEM program was conducted at the same sampling areas as the previous EEM programs. In addition, a second reference area, Reference Area 2, was added for Phase 3 (Figure 2.2-2; AECOM 2011, Figure 1-1):

- Exposure Area – Seep Creek and Seep Creek Ponds
- Reference Area 1 – Fingers Creek and Fingers Pond
- Reference Area 2 – Unnamed Creek and Unnamed Ponds

3.6.2 Water Quality

Conductivity was higher in the Exposure Area as compared to both Reference Areas. Nutrients were generally lower in the Reference Areas compared to the Exposure Area. None of the water samples from either Reference Area 1 or Reference Area 2 exceeded the CWQG for the protection of freshwater aquatic life (CCME 1999); this was consistent with Reference Area 1 results from both the Phase 1 and 2 EEM studies. Samples from the Exposure Area exceeded the CWQG (CCME 1999) for cadmium, copper, iron, nickel, and zinc.

3.6.3 Sediment Quality

Sediments from the Exposure Area were composed of sand, silt, and clay. In contrast, sediments from the Reference Areas were composed mainly of sand, with little silt or clay. Sediments within the Exposure Area had elevated concentrations of arsenic, cobalt, copper, molybdenum, nickel, strontium, and zinc relative to both Reference Areas.

3.6.4 Fish Survey

In 2010, a non-lethal juvenile Arctic Grayling survey was conducted on fish captured in the Exposure Area and Reference Areas 1 and 2 (Figure 2.2-2; AECOM 2011, Figure 1-1). Supporting environmental information (stream

temperature and habitat information) was also collected in 2010 to help determine the cause(s) of the size differences in juvenile Arctic Grayling observed during Phases 1 and 2.

Arctic Grayling were captured by backpack electrofishing. In total, 33 Arctic Grayling from the Exposure Area, 25 Arctic Grayling from Reference Area 1, and 33 Arctic Grayling from Reference Area 2 were captured. Overall, the state of general health (i.e., occurrence of external abnormalities and parasites) of Arctic Grayling was similar in all three areas. Juvenile Arctic Grayling were heavier and longer, on average, in both Reference Areas as compared to the Exposure Area. These findings were consistent with the findings of Phases 1 and 2. Arsenic was significantly elevated in juvenile Arctic Grayling collected from the Exposure Area in comparison with the two Reference Areas.

To investigate the impacts of potential differences between stream temperature on Arctic grayling growth and survival, six temperature data loggers were installed in early from 7 July to 27 August 2010. Each logger was set to record water temperature every 30 minutes. Differences in stream temperatures were determined by comparing the temperature profiles recorded by each data logger (n=2441) using an ANOVA with repeated measures. Stream temperatures were significantly greater in the Exposure Area compared to both Reference Area 1 and Reference Area 2. This pattern was inconsistent with the hypothesis that Reference Area 1 was warmer than the Exposure Area.Within the Exposure Area, the downstream location was significantly warmer than the upstream location. Between the reference areas, the upstream location in Reference Area 2 had significantly greater temperatures than both of the locations within Reference Area 1. No other statistically significant comparisons were identified.

3.6.5 Overall Assessment

The Phase 3 IOC Study evaluated whether differences in temperature between the Exposure and two Reference Areas caused decreased growth of juvenile Arctic Grayling in the in Exposure Area. A literature review was conducted to investigate the potential effects of metal concentrations on growth of Arctic Grayling. Overall, temperature was found to be warmer in the Exposure Area by 1.0 to 1.5°C during the summer months, refuting the hypothesis that decreased growth in the Exposure Area was the result of lower temperature than in the reference areas. Additionally, as described in the Phase 3 EEM report, metal concentrations observed in the Exposure Area water samples were not sufficiently elevated to have resulted in decreased growth in Arctic Grayling.

3.7 Phase 4

A Phase 4 Periodic Monitoring – Surveillance program EEM Study Design was submitted in February 2013. A reconnaissance visit was conducted in July 2013. Temperature loggers were installed at the Exposure Area (Seep Creek and Seep Creek Pond 2) and Reference Areas (R2-5, Fingers Creek, Fingers Creek Pond), and habitat information was collected. The biological investigation was not completed as all activities on the mine were suspended in August 2013.

3.8 Phase 5 EEM Study Design Synopsis

The proposed biological monitoring Study Design for Phase 5 included a fish survey, a benthic invertebrate community survey, and the collection of supporting environmental data (Golder 2016a). Fish tissue analysis was not required because mercury concentrations in the effluent have been consistently below 0.1 µg/L (AECOM 2011; EC 2002a). The program was designed as a Periodic Monitoring – Surveillance Program as per Schedule 5,

Section 19 of the MMER (EC 2002a). The Phase 5 EEM Study Design was submitted to the Regional Director in February 2016, six months in advance of the biological monitoring study being conducted.

The Phase 5 EEM fish survey Study Design was based on a single exposure/multiple reference design, with one exposure area and two reference areas for each fish species (Figure 2.2-2). Multiple locations within each area were proposed for sampling to assist in determining the extent of potential effluent-related effects:

- Exposure Area – Seep Creek (fish survey) and Seep Creek Ponds 1 and 2 (fish and benthic invertebrate survey).
- Reference Area 1 – Fingers Creek, which includes two small drainage ponds, herein referred to as Fingers Creek Ponds (fish survey), and Fingers Lake (benthic invertebrate survey).
- Reference Area 2 – Unnamed Creek, which includes a small drainage pond (fish survey) and two unnamed ponds, herein referred to as Unnamed Pond and Unnamed Creek Pond (benthic invertebrate survey).

A summary of the proposed Phase 5 EEM program is provided in Table 3.8-1.

The Phase 5 EEM fish survey Study Design proposed documenting supporting environmental information (e.g., habitat characteristics, weather conditions; field water quality variables, sediment characteristics) at fish and benthic invertebrate community Exposure and Reference sampling areas. In addition, water chemistry and sediment quality samples were proposed to be collected in each of the Exposure and Reference areas. To assess seasonal differences in water temperature, it was proposed to deploy two temperature data loggers in the each of the Exposure and Reference Areas.

The two sentinel fish species proposed for the Phase 5 EEM fish survey were Ninespine Stickleback and Arctic Grayling. A lethal survey was proposed for Ninespine Stickleback and a non-lethal survey was proposed for YOY and juvenile Arctic Grayling. The proposed target sample sizes were 30 adult males, 30 adult females, and 30 juveniles Ninespine Stickleback, and a minimum of 100 YOY, and a maximum of 400 YOY Arctic Grayling from each of the Exposure and Reference Areas. Backpack electrofishing and seine nets were proposed as methods of capture for fish.

The proposed benthic invertebrate community sampling coincided with water and sediment quality sampling. Ekman grabs (five subsamples per station, five stations from each of the Exposure and Reference Areas) were proposed as method of sampling for benthic invertebrates.

Table 3.8-1: Outline of the Lupin Mine Proposed Phase 5 Environmental Effects Monitoring Field Study Components, 2016

Survey Component	Rationale	Study Area	Waterbody	Timing	Sample Size		Parameters Measured
Plume Delineation							
Distribution of treated effluent	In the absence of treated effluent discharge in 2016, determine whether water flowing from Dam 1a Lake can be detected at 250 m from the discharge point in the Exposure Area.	Exposure Area	Dam 1a Lake	Late August	10 measurements		Field conductivity will be measured every 25 m from the discharge point (i.e., LUP-10) to 250 m downstream. Field measured conductivity data will be used to estimate the percent effluent at 250 m from the discharge location.
Ninespine Stickleback Fish Survey							
Monitoring of adult and juvenile fish (lethal)	As required in the TGD (EC 2012a).	Exposure Area	Seep Creek and Seep CreekPonds	Late August	30 adult males	Total = 90	Fish will be examined for fork length, weight, external condition (wounds, tumours, parasites, fin fraying, gill parasites, lesions), internal condition (presence/absence of parasites, proportion of mesenteric fat, abnormalities such as tumours on liver, spleen, gall bladder, kidney and gonads), maturity, sex, gonad weight, liver weight, carcass weight, stomach fullness. Sagittal otoliths will be collected for ageing analyses. All gonads will be preserved in 10% formalin and archived.
					30 adult females		
					30 juveniles		
		Reference Area 1	Fingers Creek		30 adult males	Total = 90	
					30 adult females		
					30 juveniles		
		Reference Area 2	Unnamed creek		30 adult males	Total = 90	
					30 adult females		
					30 juveniles		
Arctic Grayling Fish Survey							
Monitoring of YOY (non-lethal)	As required in the TGD (EC 2012a).	Exposure Area	Seep Creek and Seep Creek Ponds	Late August	100-400 young-of-year		Fish will be examined for total and fork length, weight and external condition (wounds, tumours, parasites, fin fraying, gill parasites, lesions). Scales will be collected from all captured fish for ageing analyses. Sagittal otoliths will be removed from a sub-set of each size class of fish for confirmation of scale age determination. Following the external examination, Arctic Grayling will be released with the exception of the sub-set lethally sampled for otoliths.
		Reference Area 1	Fingers Creek		100-400 young-of-year		
		Reference Area 2	Unnamed creek		100-400 young-of-year		
Benthic Invertebrate Community Survey							
Invertebrate community	As required in the TGD (EC 2012a).	Exposure Area	Seep Creek Ponds	Late August	5 stations per area each with five sub-samples per station combined into a single composite sample per station		Density[b], richness[a,b], Simpson's Evenness Index[a,b], Bray-Curtis Index[a,b], community composition, EPT density, diptera density
		Reference Area 1	Fingers Lake				
		Reference Area 2	Unnamed pond				
Supporting Environmental Data							
Water quality	As required in the TGD (EC 2012a).	Exposure Area	Seep Creek and Seep Creek Ponds	Late August	3 surface water samples		Full characterization as outlined in the TGD (EC 2012a), including one duplicate, one field blank and one travel blank.
		Reference Area 1	Fingers Creek and Fingers Lake		3 surface water samples		
		Reference Area 2	Unnamed creek and unnamed pond		3 surface water samples		
Sediment quality	As required in the TGD (EC 2012a).	Exposure Area	Seep Creek Ponds	Late August	5 stations per area each witha single composite sample per station consisting of 5 Ekman grabs (top 5 cm)		Full characterization as outlined in the TGD (EC 2012a) including appropriate field duplicates.
		Reference Area 1	Fingers Lake				
		Reference Area 2	Unnamed pond				
Seasonal water temperature	Water temperatures in the Exposure and Reference Areas were documented in previous phases and will be measured in Phase 5.	Exposure Area	Seep Creek and Seep Creek Ponds	Early-June to late-August	2 loggers in each fish Exposure and Reference Areas		Water temperature recorded hourly from spring to fall.
		Reference Area 1	Fingers Creek				
		Reference Area 2	Unnamed creek				

(a) Assessed at the family level and lowest taxonomic level.
(b) Endpoint will be analyzed statistically.
TGD = Metal Mining Technical Guidance for Environmental Effects Monitoring; YOY = young-of-year; EPT = Ephemeroptera, Plecoptera, Trichoptera

3.8.1 Deviations from the Phase 5 EEM Study Design

The Phase 5 EEM program followed the approved study design with the following exceptions:

- Effluent Characterization – Plume Delineation:
  - As part of the supporting field data, field conductivity was to be measured every 25 m from the discharge point (i.e., LUP-10) to 250 m downstream (Golder 2016c), and the field measured conductivity was to be used to estimate the percent effluent at 250 m from the discharge location. In 2016, due to field logistics, field conductivity was not collected at 250 m from the effluent discharge, therefore, percent effluent at 250 m from discharge could not be calculated. Percent effluent was calculated at 3000 m from discharge (Section 2.3.4).
- Supporting Environmental Data:
  - Habitat characterization was completed by the fish team in all areas where fish sampling was conducted. As Seep Creek Pond 2 and the portion of Seep Creek downstream of Seep Creek Pond 2 were not visited by the fish crew, habitat characterization was not completed in that portion of the creek.
  - An Onset HOBO Data Loggers Tidbit V2 Water Temperature Data Logger – UTBI-001 was used to collect seasonal water temperature instead of an Onset HOBO Water Temp Pro.
  - Turbidity measurements were only collected in three sampling stations at each of the Exposure and Reference Areas.
  - Hardness, calcium, magnesium, potassium, sodium were not analyzed in the Exposure and Reference Area water samples because of an oversight by the laboratory.
  - Sediment particle size criteria in the study design differed from the criteria used by ALS (Section 4.2.2). As per the study design, size criteria was: gravel (>2 mm), sand (2 to 0.063 mm), silt (0.063 to 0.004 mm), and clay (<0.004 mm); particle size categories used for the Phase 5 EEM were gravel (>2 mm), sand (2 to 0.05 mm), silt (0.05 to 0.002 mm), and clay (<0.002 mm).
- Fish:
  - Despite greater than seven days of fishing effort, the proposed sample size of lethally sampled Ninespine Stickleback (i.e., 30 adult male, 30 adult female and 30 juveniles per area) was not met for the following areas:
    - Exposure Area: adult female (28 fish lethally-sampled) and adult male (28 fish lethally-sampled)
    - Reference Area 1: adult male (20 fish lethally sampled)
    - Reference Area 2: adult female (29 fish lethally-sampled) and juveniles (28 fish lethally-sampled)
    - It is noted that sample sizes did meet the TGD (EC 2012a) requirement (i.e., at least 20 adult male, 20 adult female)
  - The proposed minimum sample size for non-lethally sampled YOY Arctic Grayling (i.e., 100 fish per area) was not met at Reference Area 2:only 12 juvenile fish were captured. It is likely that the YOY Arctic Grayling may have already moved out of the unnamed creek at the time of sampling. After 5 days of fishing effort using minnow traps, electrofishing, and seine netting, a permit amendment to allow use of fyke nets, in addition to continuing the seining effort, minnow trapping and backpack electrofishing was

obtained. Additional YOY Arctic Grayling were still not captured despite 7 days of fishing effort and new gear type Reference Area 2.

- In addition to seine nets and a backpack electrofisher, minnow traps were also used to capture Ninespine Stickleback in the Exposure and Reference Areas, and as noted above, fyke nets were used in Reference Area 2.
- Ten percent of sacrificed fish were to be preserved for species verification. No samples were archived for fish species verification, as confidence in field observations was sufficient.
- Due to logistical constraints, fish sampling was conducted from early to mid-day (i.e., 9:00 to 14:00).

4.0 SUPPORTING ENVIRONMENTAL DATA – PHASE 5

4.1 Introduction and Objectives

Collection of key supporting environmental information is required during the biological monitoring of fish and benthic invertebrates (EC 2012a). The supporting environmental variables component provides information that allows for comparison of aquatic habitats between the Exposure and Reference Areas, and assists with the interpretation of biological results for fish and benthic invertebrates. Of particular importance is the effect of temperature on fish spawning, growth, and other aspects of energy utilization. Detailed information related to the quantity and quality of treated effluent is presented in Section 2.3; relevant information is summarized herein.

4.2 Methods

4.2.1 Field Program

The following supporting environmental variables were documented in the Exposure and Reference Areas in the Phase 5 EEM biological program:

- detailed description of the habitat
- seasonal water temperatures
- weather conditions (i.e., air temperature, wind direction, precipitation type, and percent cloud cover)
- in situ field water quality variables (i.e., water temperature, dissolved oxygen (DO), specific conductivity, pH, and turbidity)
- surface water quality
- macrophyte cover
- sediment characteristics (visual description of texture, colour, odour, organic content)
- sediment quality

4.2.1.1 Habitat

Habitat in the Exposure and Reference Areas was documented during the fish survey. This included visual assessments of the following:

- substrate type (e.g., boulder, cobble, gravel)

- water depth (m)
- type and proportion of vegetative or substrate cover
- proportion of overhanging vegetation
- habitat type (e.g., run, riffle, pool, eddy)

In addition, water velocities were measured intermittently throughout the program at each Exposure and Reference Areas using a Marsh-McBirney velocity meter. Habitat characterization was completed by the fish team in all areas where fish sampling was conducted. Habitat was not characterized in Seep Creek Pond 2 and the smaller pond in Reference Area 1 because fish were not collected in those areas.

During the benthic survey, the type and relative coverage of vegetation and macrophytes in the ponds, and the proportion of benthic algae was recorded for each Exposure and Reference Areas.

4.2.1.2 Seasonal Water Temperature

Temperature data loggers (Onset HOBO Data Loggers Tidbit V2 Water Temperature Data Logger – UTBI-001) were deployed in the Exposure and Reference Areas to assess seasonal differences in water temperature. A total of six temperature loggers were deployed: one in the creek section and one in a pond section in each Exposure and Reference Areas (Table 4.2-1; Figures 4.2-1 and 4.2-2). Temperature loggers were set to record temperature on an hourly basis (i.e., 24 readings per day). Deployment occurred following ice break up on 10 June 2016, and were removed after completion of the fish survey in early September; this is the length of time encompassing the principal period of growth for fish and as well as the spawning period of Arctic Grayling. The temperature loggers were installed within a protective casing, anchored on a rock which was attached to the shore with sideline, and placed in the water at depths ranging from 0.2 to 0.6 m (Table 4.2-1; Appendix A, Photographs 1 and 2).

Table 4.2-1: Temperature Logger Deployment Details, 2016

Sampling Area	Location	Easting (m)[a]	Northing (m)[a]	Installation Date	Retrieval Date	Water Depth (m)	Substrate Type
Exposure Area	Seep Creek	482172	7290467	10 June	6 September	0.3	boulder/cobble
	Seep Creek Pond 2	480778	7290414	10 June	6 September	0.2	fines
Reference Area 1	Fingers Creek	495109	7289461	10 June	5 September	0.5	gravel/cobble/ boulder
	Fingers Creek Pond	495291	7288979	10 June	4 September	0.5	boulder
Reference Area 2	Unnamed Creek	499174	7285902	10 June	2 September	0.4	boulder
	Unnamed Creek Pond (R2-5)	495515	7287279	10 June	5 September	0.6	angular boulder and cobble

(a) Zone 12 W.

m = metres.

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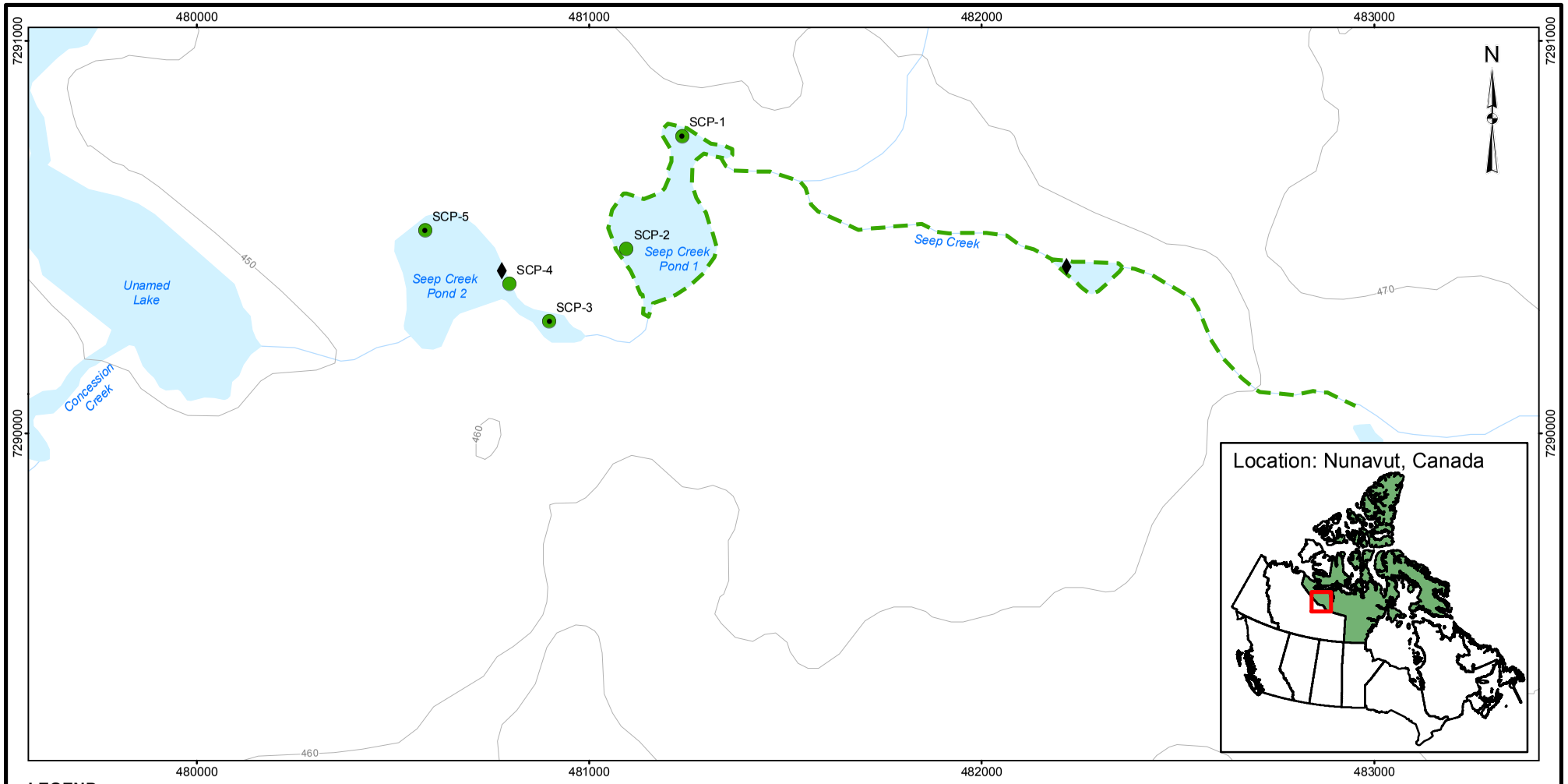

LEGEND

- BENTHIC INVERTEBRATE AND SEDIMENT SAMPLING STATION – EXPOSURE AREA
- TEMPERATURE LOGGER LOCATION
- WATER QUALITY SAMPLING STATION
- CONTOUR (10 m)
- FISH SAMPLING LOCATION - EXPOSURE AREA
- WATERCOURSE
- WATERBODY

500 0 500

SCALE 1:15,000 METRES

REFERENCE

DATUM: NAD83 PROJECTION: UTM ZONE 12

PROJECT
MANDALAY RESOURCES CORPORATION

LUPIN MINE PHASE 5 ENVIRONMENTAL EFFECTS MONITORING

TITLE

PHASE 5 EEM EXPOSURE AREA SAMPLING LOCATIONS

Golder Associates

PROJECT	1650403 - 5000		FILE No.	
DESIGN	TL	28 Mar. 2017	SCALE AS SHOWN	REV. 1
GIS	MM/LS	29 May 2017		
CHECK	HM	29 May 2017	**FIGURE 4.2-1**	
REVIEW	ZK	29 May 2017		

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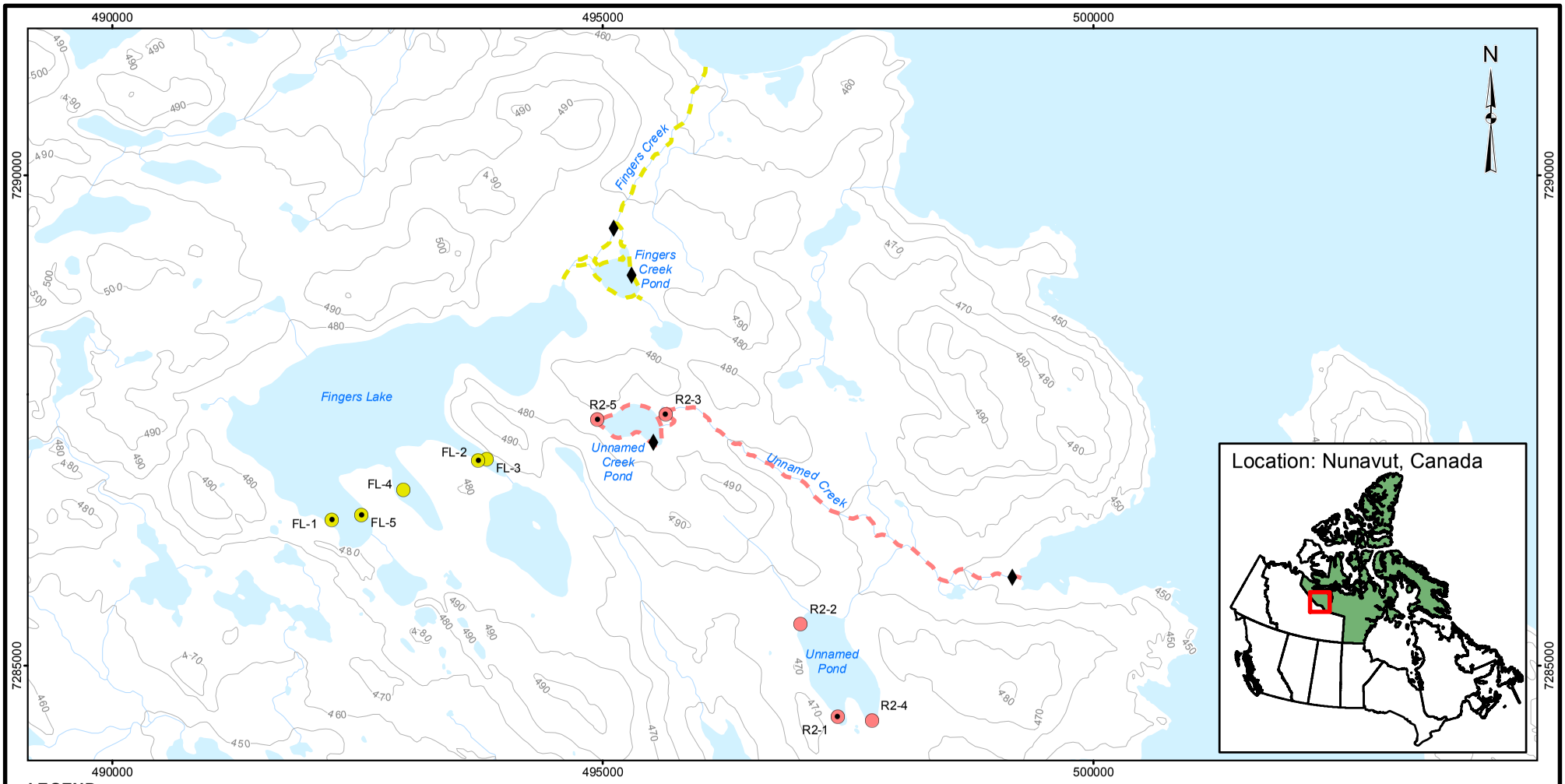


LEGEND

- BENTHIC INVERTEBRATE AND SEDIMENT SAMPLING STATION - REFERENCE AREA 1
- BENTHIC INVERTEBRATE AND SEDIMENT SAMPLING STATION - REFERENCE AREA 2
- TEMPERATURE LOGGER LOCATION
- WATER QUALITY SAMPLING STATION
- CONTOUR (10 m)
- FISH SAMPLING LOCATION - REFERENCE AREA 1
- FISH SAMPLING LOCATION - REFERENCE AREA 2
- WATERCOURSE
- WATERBODY

2 0 2

SCALE 1:60,000 KILOMETRES

REFERENCE

HYDROGRAPHY AND CONTOUR DATA OBTAINED FROM GEOGRATIS, © DEPARTMENT OF NATURAL RESOURCES CANADA. ALL RIGHTS RESERVED.
DATUM: NAD83 PROJECTION: UTM ZONE 12

PROJECT
MANDALAY RESOURCES CORPORATION

LUPIN MINE PHASE 5 ENVIRONMENTAL EFFECTS MONITORING

TITLE

PHASE 5 EEM REFERENCE AREA SAMPLING LOCATIONS

Golder Associates

PROJECT	1650403 - 5000		FILE No.	
DESIGN	TL	28 Mar. 2017	SCALE AS SHOWN	REV. 1
GIS	MM/LS	29 May 2017		
CHECK	HM	29 May 2017		
REVIEW	ZK	29 May 2017		

FIGURE 4.2-2

4.2.1.3 Water Quality

Water quality grab samples were collected at three benthic invertebrate community stations (SCP-1, SCP-3, SCP-5, FL-1, FL-3, FL-5, R2-1, R2-3, and R2-5) in each of the Exposure and Reference Areas in late-August and Early-September (Figure 4.2-1 and 4.2-2).

Field measurements of the following parameters were recorded once a day at each fish survey location and each benthic invertebrate station:

- water depth (m)
- water temperature (°C)
- DO (mg/L) and percent saturation
- pH
- specific conductivity (µS/cm)

In addition, measurements of turbidity (nephelometric turbidity units [NTU]) were recorded at the water quality sampling stations.

A YSI 556 MPS water quality meter was used for measurements of temperature, pH, DO, and specific conductivity. Field turbidity measurements were obtained using a LaMotte turbidity meter; turbidity measurements were an average of three readings taken of the same sample.

Water quality samples and field measurements were collected from an approximate depth of 0.2 m below the water surface in accordance with the methods outlined in the TGD (EC 2012a) and Golder TP 8.3-1 Surface Water Sampling Methods (Golder, unpublished). Water quality samples were collected in plastic and glass bottles, depending on the parameter or parameter groups being sampled, and were filtered and/or preserved in the field, when necessary, according to laboratory instructions. Samples were placed in coolers containing ice-packs, stored in a refrigerator, and shipped to the analytical laboratory ALS Canada Ltd. (ALS) in Yellowknife, NT, who in turn shipped the samples to ALS in Edmonton, Alberta (AB) for analysis.

4.2.1.4 Sediment Quality

Sediment samples were collected at five stations at each of the Exposure and Reference Areas in conjunction with collection of benthic invertebrate community samples (Figures 4.2-1 and 4.2-2). Sediment samples were collected using a standard Ekman grab sampler (15.24 centimetre [cm] × 15.24 cm) with a sampling area of 0.0232 square metres (m^2) according to procedures in the TGD (EC 2012a) and the Phase 5 EEM Study Design (Golder 2016a).

Five Ekman grabs (top 5 cm) were combined into a composite sample at each station. After each grab sample was collected, the overlying water was siphoned off and the sediment was sub-sampled using a plastic spoon. The upper 5 cm layer of sediment, from the inner portion of the Ekman (i.e., avoiding the sides of the Ekman) were removed from each of the five replicate grabs, and combined in a pre-cleaned plastic bowl. The composite samples were thoroughly mixed by stirring until the colour and texture of the mixture were homogenous. Samples were placed in coolers containing ice-packs, stored in a refrigerator, and shipped to the analytical laboratory ALS in Yellowknife, NT, who in turn shipped the samples to ALS in Edmonton, AB for analysis. The Ekman sampler was cleaned thoroughly at each sampling station following sample collection, and was rinsed with water from the next sampling station prior to sampling there.

4.2.2 Laboratory

4.2.2.1 Water Quality

Water samples were analyzed by ALS for the following parameters:

- conventional parameters: specific conductivity, hardness, pH, alkalinity, TDS, turbidity, and total suspended solids (TSS)
- major ions: bicarbonate, carbonate, calcium, magnesium, potassium, sodium, chloride, fluoride, hydroxide, sulphide, and sulphate
- nutrients: ammonia-nitrogen (N), nitrate-N, nitrite-N, total Kjeldahl nitrogen, total phosphorus, and total dissolved phosphorus
- radium-226
- total organic carbon (TOC) and dissolved organic carbon
- total metals: aluminum, antimony, arsenic, barium, beryllium, bismuth, boron, cadmium, chromium, cobalt, copper, iron, lead, lithium, manganese, molybdenum, nickel, selenium, silicon, silver, strontium, sulphur, thallium, tin, titanium, uranium, vanadium, zinc, and zirconium
- total cyanide

Because of an oversight by the laboratory, hardness, calcium, magnesium, potassium, and sodium were not analyzed in samples from the Exposure and Reference Area 1.

4.2.2.2 Sediment Quality

Sediment samples were analyzed by ALS for the following parameters:

- particle size
- % moisture
- TOC
- total metals: aluminum, antimony, arsenic, barium, beryllium, bismuth, boron, cadmium, chromium, cobalt, copper, iron, lead, lithium, manganese, mercury, molybdenum, nickel, selenium, silver, strontium, thallium, tin, titanium, uranium, vanadium, and zinc

Particle size was analyzed according to the following classification:

- gravel (>2 mm)
- sand (2 to 0.05 mm)
- silt (0.05 to 0.002 mm)
- clay (<0.002 mm)

4.2.3 Data Analysis

Daily water temperature averages were calculated for each individual temperature logger and then a daily average within each sampling area was calculated combining the two loggers. Data for the dates of logger deployment and retrieval were not included because they included times when the loggers were not yet installed. Statistical comparisons of temperature were not performed; the loggers were installed at different depths due to the different habitat types present and as such statistical comparisons are inappropriate.

Data from the water and sediment quality samples collected in 2016 from each of the Exposure and Reference Areas were obtained from laboratory files submitted by ALS. Data were imported into a database; an export file by station was created.

Water quality parameters in the Exposure Area were compared to the Reference Areas; concentrations that differed by more than a factor of two were identified as outlined in the TGD (EC 2012a). In addition, concentrations of parameters in water quality sampling areas were compared to applicable CWQG (CCME 1999). Because hardness and some major ions (i.e., calcium, magnesium, potassium, sodium) concentrations were not available for the Exposure Area and Reference Area 1, and as some guidelines are hardness dependent, average 2016 hardness from Reference Area 2 was used as an estimate of hardness for Reference Area 1, and average 2005 to 2010 hardness from the Exposure Area was used as an estimate of hardness for the Exposure Area in 2016.

Sediment quality data were compared to the Canadian ISQG guidelines for the protection of aquatic life and Probable Effect Levels (PELs) (CCME 2001).

4.2.4 Quality Assurance and Quality Control

Quality Assurance and Quality Control (QA/QC) procedures and requirements are an important aspect of any field or laboratory testing program. The objective of having good QA/QC practices is to standardize methods and to allow field sampling, data entry, data analysis, and report preparation to produce technically sound and scientifically defensible results. Further details on QA/QC procedures pertaining to the water and sediment quality data are provided in Appendix B.

As part of routine practices for field operations for this program, the following QA procedures were undertaken:

- Detailed specific work instructions outlining each field task were provided to the field personnel prior to the field program.
- Samples were collected by experienced personnel and were labelled, filtered, preserved, and shipped according to Golder's Technical Procedures and the TDG (EC 2012a).
- Field equipment, such as water quality meters, was calibrated according to manufacturer recommendations.
- Detailed field notes were recorded in pencil in waterproof field notebooks and on pre-printed waterproof field data sheets.
- Field data were checked at the end of the day for completeness and accuracy.
- Chain-of-custody forms were used to track sample shipments from the field to the analytical laboratory (ALS in Edmonton, AB).

As part of the QA/QC process for the field program, a series of QC samples were incorporated into the water and sediment program, and analyzed for the same suite of water and sediment quality parameters. Specifically, these included:

- Field blank (water quality only), which consists of samples of deionized (DI) water poured into laboratory-provided sample bottles in the field, and analyzed for the same parameters as the field samples, to detect potential sample contamination during sample collection, handling, shipping, and analysis.
- Trip blank (water quality only), which consists of unopened sample bottles of DI (same analysis as the field samples) water which were transported to the field and back to detect potential sample contamination due to ambient conditions, or that may have occurred during shipping and laboratory analysis.
- Duplicate water and sediment samples, which consist of additional samples collected at the same time and location as sediment and water samples collected during the field program (i.e., Stations R2-5 [water] and SCP-2 and FL-3 [sediment]), using the same procedures. Duplicate samples were collected in the field to assess within-site variation, and the precision of field sampling methods and laboratory analysis.

In addition, internal laboratory QC data (e.g., use of laboratory replicate samples) were analyzed to assess variability resulting from the analytical methods used for the water and sediment quality of the samples.

Field data entered into the electronic format (including the EC EEM database) underwent a 100% transcription and validity check by a second person not involved in the initial data entry process. Calculated values, tables, and summary figures generated from the dataset underwent an additional QA/QC verification by a second person not involved in the initial calculations.

Temperature recorded by the temperature loggers at the time of installation was compared to the temperature measured with the YSI multi-meter.

4.3 Results and Discussion

4.3.1 Quality Assurance and Quality Control

Detailed results and discussion of the QA/QC analysis are provided in Appendix B. Due to an oversight by the laboratory, water hardness and dissolved calcium, magnesium, potassium, and sodium were not analyzed in the samples from the Exposure and Reference Area 1. Sediment sample hold time qualifiers were reported by ALS for mercury in five samples (FL-4, FL-5, R2-1, R2-2, and R2-4). Mercury hold time was exceeded because the sediment samples were not shipped to Edmonton in a timely manner by ALS in Yellowknife, and therefore ALS in Edmonton did not receive the samples until 26 September (sample collection occurred between 1 and 3 September). These results are considered reliable because hold time was only exceeded by a day and mercury in the affected samples were similar to those measured in the unaffected samples collected in Reference Area 1.

Due to the remoteness of the site, and the frequency of the flights, the hold times for pH, turbidity, nitrate, and nitrite were exceeded for all samples. Hold time qualifiers were also reported by ALS for DOC and TOC from all Exposure and Reference Area 1 samples, and for conductivity, total alkalinity, TDS, TSS, sulphide and total cyanide from the Reference Area 2 samples. Hold time exceedances for turbidity, pH, conductivity, total alkalinity, TDS, TSS, sulphide and total cyanide were very large in the Reference Area 2 samples because the samples were not shipped by ALS Yellowknife in a timely manner and ALS Edmonton did not receive samples until 26 September (sample collection occurred on 3 September). Even with the hold time exceedance, parameter

concentrations in the Reference 2 samples were similar to those measured in samples from Reference Area 1 where hold time was not exceeded. Results from Reference Area 2 with hold time exceedances are flagged in Appendix C.

The overall quality of the water and sediment quality data was determined to be acceptable; therefore, the results reported herein are adequate for the objectives of the Phase 5 EEM.

4.3.2 Habitat

A summary of the in situ water quality data collected during the Phase 5 EEM fish program is shown in Table 4.3-1. Supporting environmental variables collected at each benthic invertebrate sampling station are shown in Table 4.3-2.

4.3.2.1 Exposure Area

The creek portion in the Exposure Area was predominantly composed of large cobbles and boulders, with gravel as the subdominant substrate (Appendix A, Photograph 3). The majority of the creek length surveyed was of a run-riffle morphology, with occasional pools dispersed throughout (Appendix A, Photograph 4). The substrate in these pools differed from that in the run-riffle sections, and was dominated by fines, with cobble being the subdominant substrate. These pools also contained approximately 75% aquatic vegetation coverage (Appendix A, Photograph 5), while the run-riffle sections of the watercourse contained no aquatic vegetation (Appendix A, Photograph 4). The water depths in the creek portion of the Exposure Area ranged from 0.2 m to 0.6 m with an average of 0.4 m, and average water velocity was 0.11 m/s.

The Seep Creek Ponds in the Exposure Area were dominated by fine substrates (Appendix A, Photographs 6 and 7), with boulders and bedrock being the subdominant substrate. Approximately 15% of the ponds contained aquatic vegetation. Overhanging vegetation and woody debris were not observed. The water depths in Seep Creek Ponds ranged from 0.3 m to 0.4 m with an average of 0.4 m. Undercut banks and boulders were observed throughout the length of the Exposure Area where fishing occurred.

4.3.2.2 Reference Area 1

Fingers Creek in Reference Area 1 was similar to the creek in the Exposure Area and was predominantly composed of large cobbles and boulders, with gravel being the subdominant substrate (Appendix A, Photograph 8). The majority of the length studied was a run-riffle morphology, with occasional pools dispersed throughout (Appendix A, Photograph 9). The substrate in these pools differed from that in the run-riffle sections, and was dominated by fines, with cobble and gravel being the subdominant substrates. These pools also contained approximately 75% aquatic vegetation coverage (Appendix A, Photograph 10), while the run-riffle sections of the watercourse contained approximately 5% aquatic vegetation.

The larger pond in Reference Area 1, as well as the outflow from Fingers Lake in Reference Area 1 were dominated by fines, with boulders and bedrock being the subdominant substrate (Appendix A, Photograph 11). The portion of Fingers Creek that extended 200 m downstream of Fingers Creek Pond was dominated by fines (Appendix A, Photograph 12). Downstream of this section, towards Contwoyto Lake, the stream returned to that previously described. Overhanging vegetation and woody debris was not observed. Undercut banks and boulders were observed throughout the length of the Reference Area 1 creek. The average water depth in the creek ranged from 0.2 m to 0.6 m with an average of 0.4 m, and an average velocity of 0.04 m/s.

Habitat at Fingers Lake was composed of boulder, cobble and sand with no macrophytes or vegetation and low amounts of visible benthic algae (Appendix A, Photograph 13). Water depth at the benthic sampling stations in Fingers Lake varied from 0.6 to 2.0 m.

4.3.2.3 Reference Area 2

The Reference Area 2 creek was comprised of a run-flat-pool morphology for the majority of its length, with two sections of run-riffle morphology within 200 m of the confluence with Contwoyto Lake and within 200 m of the confluence with the upper pond (Appendix A, Photographs 14 and 15). In the riffle-run sections, the channel became braided and the dominant substrate was cobbles, with the subdominant being boulders. In the run-flat-pool sections, the dominant substrate was fines, with the subdominant being cobbles that were frequently embedded (Appendix A, Photograph 14). Aquatic vegetation was more common in Reference Area 2 in comparison to the other two areas (Appendix A, Photograph 16), and covered approximately 50% of the flowing watercourse. Overhanging vegetation and woody debris were not observed. Undercut banks were observed throughout the watercourse and boulders were observed in the ponds of Reference Area 2. The average water depth ranged from 0.2 m to 0.6 m with an average of 0.3 m, and average velocity was 0.35 m/s. The two ponds in Reference Area 2 were similar to those found in Exposure Area and Reference Area 1, and were dominated by fines, with boulders and bedrock being the subdominant substrate (Appendix A, Photograph 17).

Table 4.3-1: Supporting Environmental Variables for the Fish Survey, 2016

Area	UTM Coordinates[a]		Sampling Date	Average Depth (m)	Depth of Sample (m)	pH[b]	Water Temperature (°C)	Conductivity (µS/cm)	DO[c] (mg/L)	DO (% Sat)	Comment
	Easting (m)	Northing (m)									
Exposure	481579	7290598	25-Aug-16	0.5	0.25	**5.3**	7.4	120	10.9	90.3	Creek
	482946	7290070	27-Aug-16	0.4	0.3	**5.3**	7.5	210	11.4	95.1	Creek
	481636	7290523	28-Aug-16	0.4	0.3	**5.3**	7.5	73	11.4	95.1	Creek
	482172	7290467	01-Sep-16	0.3	0.3	**4.7**	7.0	215	11.2	92.5	Creek
	480778	7290414	06-Sep-15	0.4	0.3	**4.7**	7.9	147	11.0	92.3	Pond
Reference Area 1	494804	7289166	29-Aug-15	0.3	0.3	**5.7**	9.1	21	11.6	100.1	Creek
	494776	7289163	04-Sep-16	0.4	0.4	**5.7**	6.8	16	**9.3**	76.6	Creek
	496041	7291102	05-Sep-16	0.4	0.3	**5.7**	6.8	16	**9.3**	76.6	Creek
	494587	7288879	08-Sep-16	0.4	0.3	**6.0**	5.3	16	**9.3**	80.1	Creek
	494600	7288941	09-Sep-17	0.3	0.3	**5.7**	6.8	16	**9.4**	77.2	Creek
Reference Area 2	499243	7285883	02-Sep-16	0.3	0.3	**4.2**	4.1	54	12.6	96.5	Creek
	499151	728900	02-Sep-16	0.3	0.3	8.8	9.0	33	10.4	90.1	Creek
	494959	7287526	04-Sep-16	0.4	0.3	**8.8**	9.1	33	10.4	90.1	Creek
	494941	7287525	03-Sep-16	0.3	0.2	**5.9**	8.3	36	10.8	91.6	Pond
	495041	7287544	09-Sep-16	0.4	0.3	8.8	7.0	33	10.4	90.1	Creek

Bolded values are outside of Canadian Water Quality Guidelines (CWQG) range.

(a) All UTM coordinates are in Zone 12, NAD 83.

(b) CWQG for pH = 6.5 to 9.0 (CCME 1999).

(c) CWQG for dissolved oxygen (DO) for cold water biota = 9.5 mg/L for early life stages and 6.5 for other life stages (CCME 1999).

UTM = Universal Transverse Mercator coordinate system; NAD = North American Datum; µS/cm = microSiemens per centimetre; Sat = saturation.

Table 4.3-2: Supporting Environmental Variables for the Benthic Invertebrate Community Survey, 2016

Area	Waterbody	Station	Sampling Date	UTM Coordinates[a]		Water Depth (m)	Field Water Quality Data					Habitat Data				Sediment Description	Sediment Quality Data			
				Easting (m)	Northing (m)		pH[b]	Water Temperature (°C)	DO[c] (mg/L)	Specific Conductivity (µS/cm[c])	Turbidity[d] (NTU)	Benthic Algae (N/L/M/H)	Vegetation	Macrophytes (species, % cover)	Habitat Type		Total Organic Carbon (%)	Sediment Particle Size		
																		Sand (%)	Silt (%)	Clay (%)
Exposure Area	Seep Creek Pond 1	SCP-1	25-Aug-16	481236	7290758	0.5	**5.3**	7.4	10.91	117	0.4	H	None	0	-	Red/brown. Sulfur smell. Hard substrate, only 30% pull. Organic substrate with sand, silt, clay.	2.91	67.0	31.8	1.2
		SCP-2	27-Aug-16	481095	7290470	0.4	**6.0**	8.9	10.57	-	-	L	Very few green stems growing from sediment	-	-	Brown/gray. Manually pushed Ekman into sediment, and manually pushed jaws closed. Block of sediment often stayed in the Ekman.	0.40	89.5	10.3	0.2
	Seep Creek Pond 2	SCP-3	31-Aug-16	480898	7290286	0.4	**5.7**	7.9	11.34	128	0.5	M	-	-	-	Sulfur smell. Rusty brown/red at surface (organic). Dark grey organic underneath.	1.69	92.0	7.5	0.5
		SCP-4	31-Aug-16	480794	7290381	0.3	**6.3**	8.9	11.24	130	-	L	Very few small shoots in sample	0	clay bottom, shallow	Light brown/grey/black. Sulfur smell.	1.43	49.4	49.6	1.0
		SCP-5	27-Aug-16	480582	7290517	0.4	**5.9**	7.4	11.41	124	0.5	M	-	20	-	Clay, earthy smell.	2.44	0.2	97.1	2.8
Reference Area 1	Fingers Lake	FL-1	1-Sep-16	492233	7286488	2.0	6.5	10.0	10.45	14	0.4	L	None	0	-	Rusty/brown on top. Clay underneath. Light sulfur smell.	0.59	93.1	6.9	<1
		FL-2	26-Aug-16	493811	7287107	1.0	**5.6**	11.6	10.04	14	-	L	None	0	boulder/cobble near shoreline	Needed to step on Ekman to sink into sediment and step on jawn in order to close them. Little organic matter on sediment surface.	0.24	71.0	28.6	0.5
		FL-3	26-Aug-16	493728	7287075	0.6	6.5	12.3	10.01	15	0.3	L	-	0	-	Hard clay bottom. Little organic matter on sediment surface. Dark grey/brown. Metallic smell.	0.65	82.7	17.1	0.2
		FL-4	1-Sep-16	492966	7286793	2.0	**6.0**	8.7	10.59	15	-	L	None	-	-	Soft, soupy, mostly organic. Slight sulfur smell. Green-grey.	5.00	64.0	35.3	<1
		FL-5	1-Sep-16	492536	7286538	0.9	**6.3**	9.8	10.47	14	0.3	N	None	0	boulder with sand	Coarse sand with little or no organic material on top.	0.30	96.8	3.1	<1
Reference Area 2	Unnamed Pond	R2-1	2-Sep-16	497392	7284481	0.2	6.5	10.8	11.16	14	0.5	N	None	none	shallow, sandy bay	Sand, no odour.	0.26	99.6	<1	<1
		R2-2	2-Sep-16	497015	7285423	0.4	**5.5**	7.2	11.98	18	-	M	Thin vegetation mat over sediment	-	-	Sand, no odour.	1.21	82.7	17.2	<1
		R2-4	2-Sep-16	497741	7284442	0.4	**6.3**	10.2	10.37	16	-	L	-	-	sand and rock shoreline	Rusty brown, grey clay, sulfur smell.	0.34	99.4	<1	<1
	Unnamed Creek Pond	R2-3	3-Sep-16	495638	7287564	0.4	**6.0**	9.4	10.23	37	1.9	H	-	algal mat	-	Sand, no odour.	0.59	74.9	24.6	<1
		R2-5	3-Sep-16	494945	7287510	0.4	**5.9**	8.3	10.79	36	0.9	H	-	-	-	Surface of sediment cover with organics/algal mat with 1-2 cm thick rusty brown/orange on top, and deep green on underside over clay. Sulfur smell.	0.65	97.3	2.6	<1

Bolded values are outside of Canadian Water Quality Guidelines (CWQG) range.

(a) All UTM coordinates are in Zone 12, NAD 83.

(b) CWQG for pH = 6.5 to 9.0 (CCME 1999).

(c) CWQG for dissolved oxygen for cold water biota = 9.5 mg/L for early life stages and 6.5 for other life stages (CCME 1999).

(d) Turbidity is an average of three readings.

UTM = Universal Transverse Mercator coordinate system; NAD = North American Datum; DO = dissolved oxygen; µS/cm[c] = microSiemens per centimetre, corrected to 25 degrees Celsius; NTU = nephelometric turbidity units; N = none; L = low; M = moderate; H = high; - not applicable or data no available; < = less than.

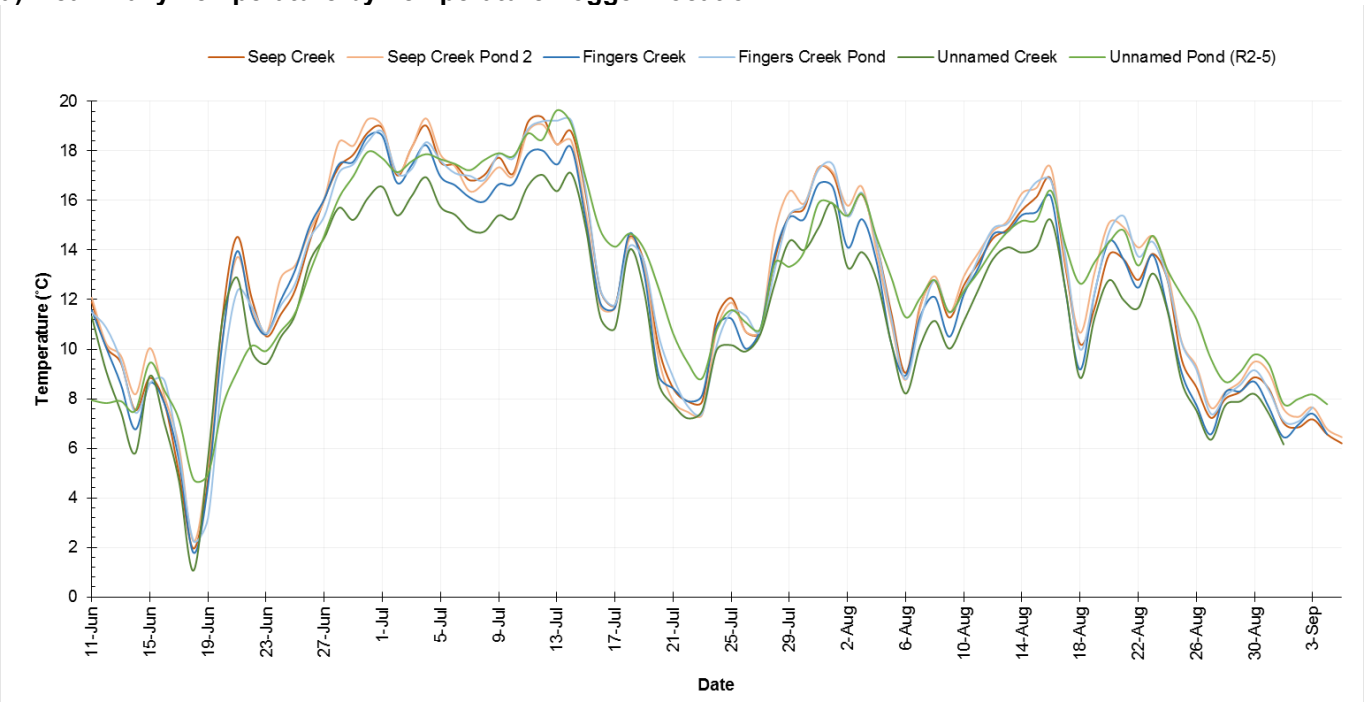
4.3.3 Seasonal Water Temperature

Water temperatures in 2016 followed similar trends through the late spring and summer at both the Exposure and Reference Areas (Figure 4.3.1). In all locations, water temperatures dropped from the time of installation (10 June 2016) to 18 June 2016, then climbed steadily until early-July, and reached a peak of 19.4°C on 13 July 2016 at Reference Area 2 unnamed creek pond. Water temperature fluctuated at each area, but generally decreased, until temperature logger retrieval in September 2016. The lowest temperature recorded was 1.1°C on 18 June 2016 at Reference Area 2 unnamed creek.

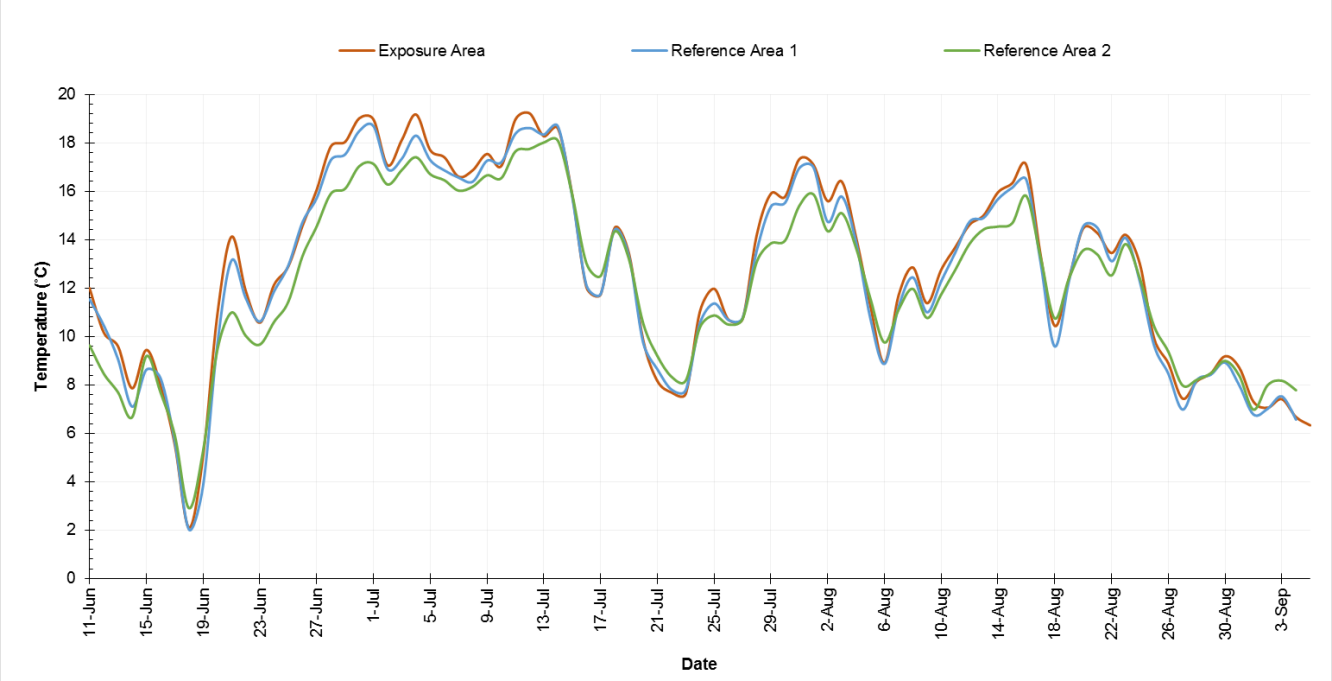
In general, water was warmer in the ponds than in the creeks. The Exposure Area was warmer than Reference Area 1 and Reference Area 2 by an average of 0.3°C and 3°C respectively, and the differences of temperatures were more notable in the creeks than in the ponds. These difference may be explained by faster water velocity in Reference Area 2 and shallower depths in the Exposure Area than the Reference Areas. No consistent bias was observed in the temperatures from the deeper sites (i.e., R2-5 did not consistently have colder temperatures). Despite the habitat differences (velocity, depth), water temperatures can be compared across the Exposure and Reference Areas.

Figure 4.3-1: Exposure and Reference Areas Seasonal Water Temperature, Lupin Mine, 2016

a) Mean Daily Temperature by Temperature Logger Location



b) Mean Daily Temperature by Area



4.3.4 Water Quality

Water quality was compared throughout the receiving environment between the Exposure and Reference Areas to determine whether any patterns were evident. There is evidence of presence of historical treated effluent in the Exposure Area (Section 2.3.4), with decreasing concentrations of some parameters from upstream to downstream (e.g., total beryllium, barium, boron, cadmium, cobalt, copper, manganese, nickel, silicon, uranium). Concentrations of lead and chloride appeared to be increasing from upstream to downstream. Detailed water quality tables are provided in Appendix C, Table C-1. Relevant plots of metals concentrations are shown below in Figure 4.3-2.

Concentrations of parameters in the Exposure Area were compared to the average Reference Areas concentrations (Appendix C, Table C-1). Concentrations that differed by more than a factor of two in the Exposure Area (as per EC 2012a) include: specific conductivity, TDS, chloride, sulphate, most nutrients (i.e., total ammonia, total Kjeldahl nitrogen, total phosphorous), and most metals (i.e., aluminum, arsenic, cadmium, cobalt, copper, iron, lead, lithium, manganese, nickel, silicon, strontium, uranium, zinc).

Parameters that exceeded CWQGs (CCME 1999) are summarized in Table 4.3-3. In general, pH was below minimum CWQG range in every area in 2016. Copper, nickel, zinc in the Exposure Area exceeded CWQGs in 2005, 2008, 2010 and 2016 (Table 4.3-3; Appendix C, Table C-1). Cadmium at the Exposure Area exceeded CWQG in 2005, 2008 and 2010 but not in 2016. In 2016, aluminum exceeded the CWQG at five out of six Reference Area stations, and copper exceeded the CWQG at one Reference Area 2 station. Since 2005, there has been a general decreasing trend in concentrations at the Exposure Area (Figure 4.3-2).

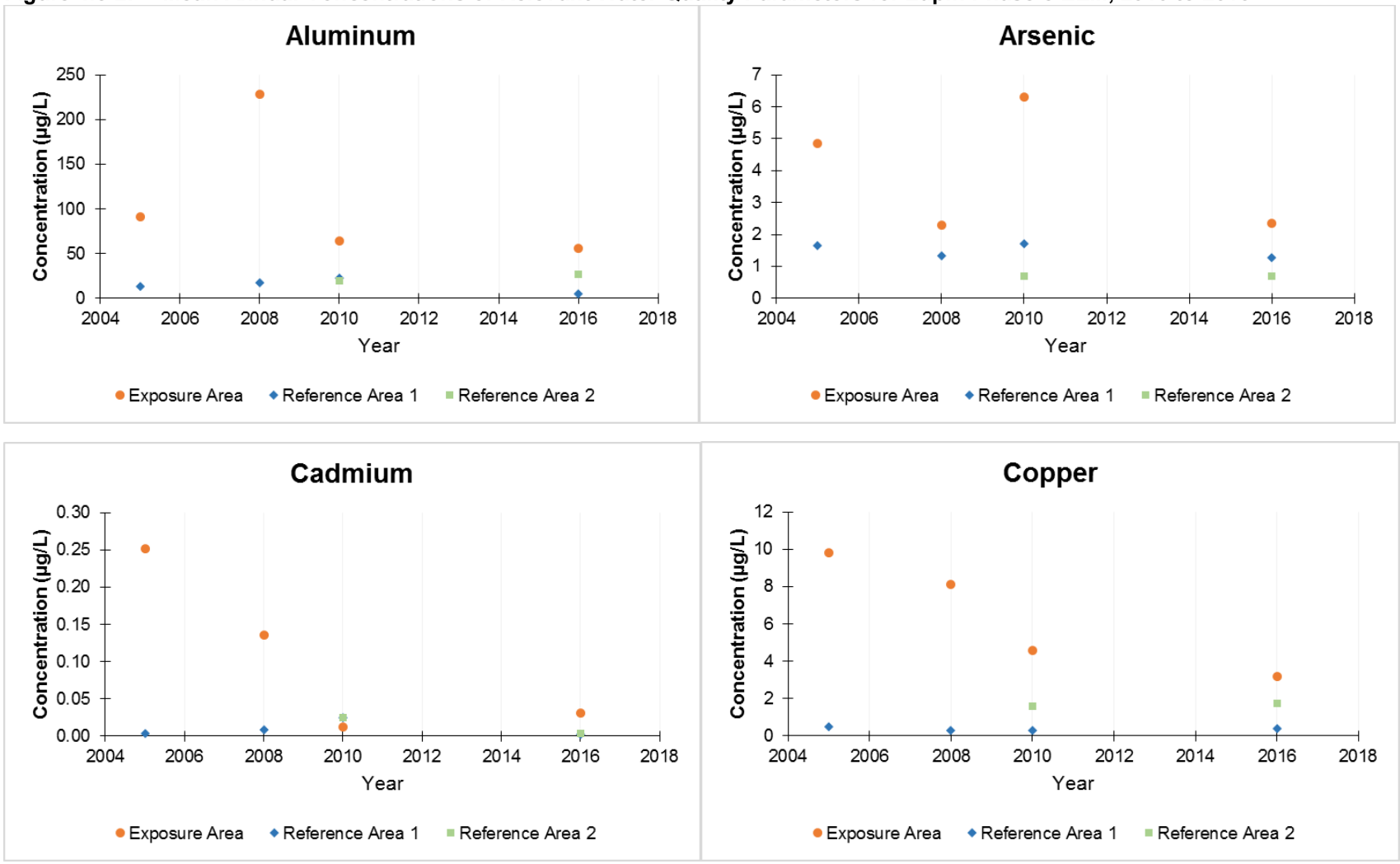
Table 4.3-3: Summary of Exceedances of Applicable Canadian Water Quality Guidelines in the Exposure and Reference Areas, 2005 to 2016

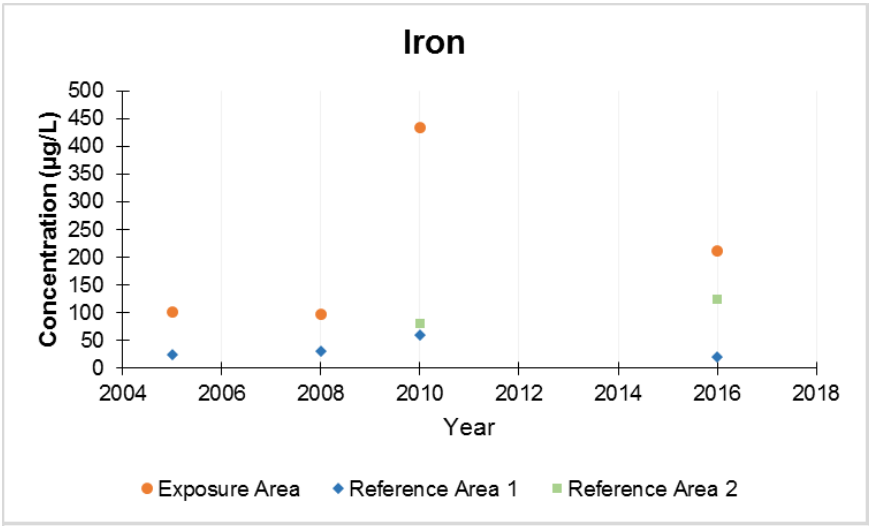
Parameter	Exposure Area				Reference Area 1				Reference Area 2	
	2005	2008	2010	2016	2005	2008	2010	2016	2010	2016
pH (laboratory)	X	X	X	X	X	-	-	X	-	X
pH (field)	-	-	-	X	-	-	-	X	-	X
Aluminum	X	X	-	X	X	-	-	X	-	X
Arsenic	X	-	X	-	-	-	-	-	-	-
Cadmium	X	X	X		-	-	-	-	-	-
Copper	X	X	X	X	-	-	-	-	-	X
Iron	-	-	X	-	-	-	-	-	-	-
Lead	X	-	-	-	-	-	-	-	-	-
Nickel	X	X	X	X	-	-	-	-	-	-
Zinc	X	X	X	X	-	-	-	-	-	-

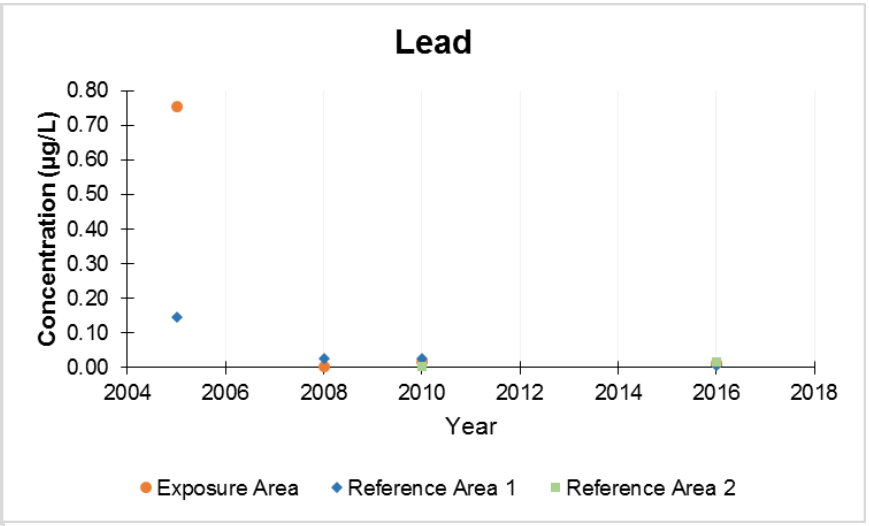
Source: CCME (1999).

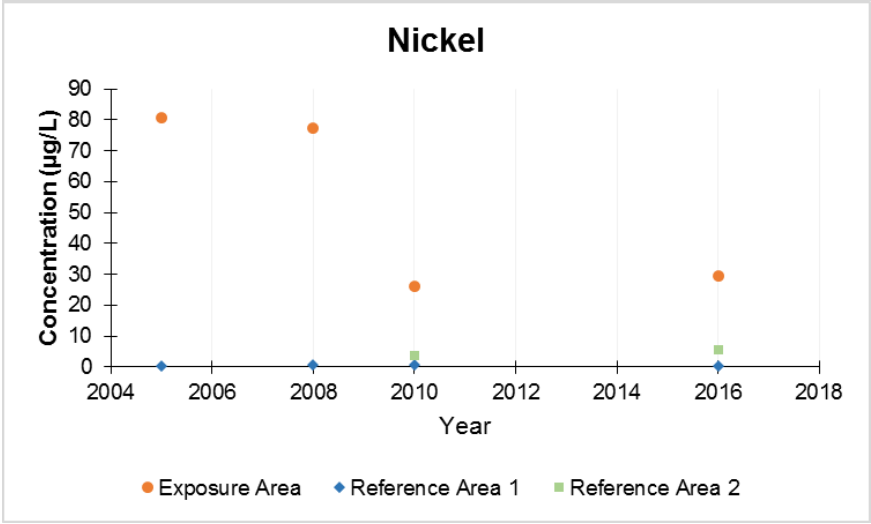
X = indicates at least one sample exceeded parameter guideline; - = indicates no guideline exceedance.

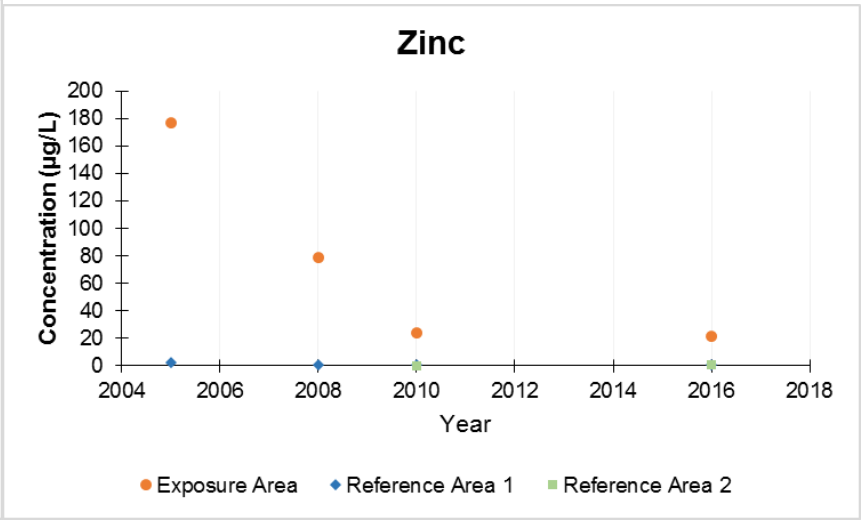
Figure 4.3-2: Mean Annual Concentrations of Relevant Water Quality Parameters for Lupin Phase 5 EEM, 2010 to 2016




Iron
Concentration (µg/L)
500
450
400
350
300
250
200
150
100
50
0
2004
2006
2008
2010
2012
2014
2016
2018
Year
Exposure Area
Reference Area 1
Reference Area 2


Lead
Concentration (µg/L)
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
2004
2006
2008
2010
2012
2014
2016
2018
Year
Exposure Area
Reference Area 1
Reference Area 2


Nickel
Concentration (µg/L)
90
80
70
60
50
40
30
20
10
0
2004
2006
2008
2010
2012
2014
2016
2018
Year
Exposure Area
Reference Area 1
Reference Area 2


Zinc
Concentration (µg/L)
200
180
160
140
120
100
80
60
40
20
0
2004
2006
2008
2010
2012
2014
2016
2018
Year
Exposure Area
Reference Area 1
Reference Area 2

4.3.5 Sediment Quality

Sediment composition was similar among sampling areas and consisted predominately of sand (average of 77%) and silt (average of 22%) (Table 4.3-2; Appendix A, Photographs 18 to 22; Appendix C, Table C-2). An exception occurred at SCP-5 (Exposure Area; Appendix A, Photograph 19) where silt was predominant (97%) and sand content was very low (0.2%). The proportion of clay was relatively consistent among areas, with most stations having less than 1% clay. TOC was the highest at Station FL-4 (5%), but on average was higher in the Exposure Area as compared to the Reference Areas.

All parameters were at similar concentrations in Reference Area 1 and Reference Area 2, with the exception of a number of metals measured at FL-4 (Reference Area 1) which had comparable, or sometimes higher concentrations than in the Exposure Area (e.g., arsenic, boron, copper, lead, manganese, mercury, molybdenum, uranium, vanadium). Higher concentrations at FL-4 are likely due to different composition of sediment at this station compared to the other four stations in Fingers Lake. At FL-4, the sediment was described as soupy, mostly organic, and containing a lower percentage of sand (Appendix A, Photograph 21); sediment at the other Fingers Lake stations was described as having little organic matter (Table 4.3-2).

Concentrations of arsenic, beryllium, cadmium, cobalt, copper, manganese, mercury, molybdenum, nickel, sulphur and zinc were elevated in the Exposure Area sediments compared to the Reference Areas sediments, with the exception of parameters at SCP-2 (Exposure Area) which were similar to the concentrations measured in the Reference Areas. Lower concentrations at SCP-2 are likely due to the sediment being described as hard and compacted, and having lower percentage of TOC (Table 4.3-2).

Arsenic concentrations were above the PEL at all sampling stations except one (SCP-2) in the Exposure Area. In contrast, arsenic was below the PEL at all sampling stations except one (FL-4) in Reference Area 1, and below the ISQG at all sampling stations but FL-4 and one station (R2-2) in Reference Area 2 (Appendix C, Table C-2). Copper concentrations were below the PEL at all sampling stations and below the ISQG at all but one station (SCP-5) in the Exposure Area.

A clear spatial pattern was not evident for all sediment parameters; however, there was a general decrease in cadmium and sulphur concentrations within the Exposure Area from upstream to downstream. In addition, parameter concentrations, such as aluminum, barium, beryllium, titanium, and uranium, were higher at the farthest downstream station in each pond (i.e., SCP-5, R2-2 and R2-5), as compared to the upstream station, within each Exposure Area (excluding SCP-2) and Reference Area 2 ponds (Appendix C, Table C-2).

4.4 Summary

Overall, the habitat found in Exposure Area and Reference Area 1 was similar, with frequent cobble and gravel bars available for Arctic Grayling spawning habitat, as well as cover provided by the substrate and aquatic vegetation that would provide rearing habitat for both Arctic Grayling and Ninespine Stickleback. The habitat found in Reference Area 2 differed from Reference Area 1 and the Exposure Area in that little to no gravel was found to provide spawning habitat for Arctic Grayling, average water depth was lower, and average velocity was higher. Significant cover was available for rearing of both Arctic Grayling and Ninespine Stickleback.

Seasonal water temperature was in general warmest in the Exposure Area and coolest in Reference Area 2. Velocity in the creek portion of Reference Area 2 was higher than at the Exposure Area and Reference Area 1, which may have contributed to the colder water. In general, water was warmer in the ponds than in the creeks.

Water temperature is also influential on fish development and growth (Jobling 1995; Moyle and Cech 2004). Warmer waters can often lead to larger, faster growing fish. Temperature data recorded during the growing season indicated that Ninespine Stickleback and Arctic Grayling in the Exposure Area and Reference Area 1 were likely exposed to warmer water earlier in the season than the Reference Area 2 which could result in increased fish size.

Concentrations of water and sediment quality parameters were generally higher in the Exposure Area than in the Reference Areas. Cadmium, copper, nickel, zinc in water in the Exposure Area exceeded CWQGs in 2005, 2008, 2010 and 2016. Aluminum concentrations in water exceeded CWQG at most of the Reference Area stations, and copper exceeded CWQG at one Reference Area 2 station. Concentrations of parameters in the Exposure Area have been decreasing since 2005.

Arsenic concentrations were above the PEL at all sampling stations but one within the Exposure Area and at one sampling station in Reference Area 1; arsenic was above the ISQG at one station within Reference Area 2. Copper concentrations were above ISQG but below PEL at one station within the Exposure Area; concentrations were below the ISQG at all other sampling stations.

A clear spatial pattern was not evident for sediment parameters; however, there was a decrease in sediment cadmium and sulphur concentrations within the Exposure Area from upstream to downstream.

5.0 BENTHIC INVERTEBRATE COMMUNITY SURVEY – PHASE 5

5.1 Introduction and Objectives

The main objectives of the Phase 5 EEM benthic invertebrate community survey were to:

- confirm the Phase 2 benthic invertebrate community assessment results
- determine whether residual effluent from the Mine is affecting the benthic invertebrate community in Seep Creek Ponds 1 and 2 (Exposure Area) relative to Fingers Lake, Unnamed Pond, and Unnamed Creek Pond (Reference Areas)

The Phase 5 EEM benthic invertebrate community survey was conducted based on a multiple control-impact (MC-I) design, with one Exposure Area and two Reference Areas. The MC-I design was consistent with previous EEM benthic invertebrate surveys (Golder 2004, 2006; AECOM 2009). The design also considered the Environment Canada Metal Mining Technical Guidance Document (TGD) (EC 2012a), and comments from the TAP review of the Phase 5 EEM Study Design (Golder 2016a, 2016c).

5.2 Methods

5.2.1 Study Areas and Sampling Locations

5.2.1.1 Exposure Area

The effluent receiving environment, or Exposure Area (EXP), is the Seep Creek system composed of small ponds and tributaries eventually discharging to Outer Sun Bay in the West Arm area of Contwoyto Lake. Seep Creek flows for approximately 2.5 km before it enters two ponds (Seep Creek Pond 2 and Seep Creek Pond 1). The Exposure Area for the benthic invertebrate community survey was restricted to Seep Creek Ponds 1 and 2, which are shallow (less than 1.5 m) waterbodies located approximately 4 km west of the Mine (Figure 2.1-2).

Two stations were sampled in Seep Creek Pond 1 and three stations in Seep Creek Pond 2, for a total of five stations in the Exposure Area (Figure 4.2-1). Coordinates for each station are provided in Table 5.2-1. The Phase 5 EEM stations were established as close to previous EEM station locations as possible.

5.2.1.2 Reference Areas

Two Reference Areas were defined for the Phase 5 EEM benthic invertebrate community survey. One of the Reference Areas (Fingers Lake) was also sampled during the Phase 2 EEM survey.

Reference Area 1 (REF1) is the Fingers Creek system, also composed of small ponds and tributaries. Reference Area 1 is located southeast of the tailings area and drains into the central region of Contwoyto Lake (Figure 2.1-2). For the benthic invertebrate community survey, Fingers Lake was designated as Reference Area 1. Similar to the Seep Creek watershed, the Fingers Lake watershed also has a connection to Contwoyto Lake. Fingers Creek flows for approximately 3 km between Fingers Lake and Contwoyto Lake. Fingers Lake has a maximum depth of 6 m and a surface area of 3.7 km^2.

Reference Area 2 (REF2) is an unnamed system located southeast of the tailings area and drains into the northern part of South Bay on Contwoyto Lake (Figure 2.1-2). Reference Area 2 is located southeast of Reference Area 1, but the two areas are not hydrologically connected. Reference Area 2, also drains to Contwoyto Lake, but unlike Reference Area 1, it contains two small ponds, as opposed to one large pond. The two ponds are referred to as Unnamed Pond and Unnamed Creek Pond. Unnamed Creek flows for about 3 km between the upper pond (Unnamed Creek Pond) and Contwoyto Lake.

Five stations were sampled in each Reference Area for a total of 10 stations (Figure 4.2-2). Coordinates for each station are provided in Table 5.2-1. The Phase 5 EEM reference stations were established as close to previous EEM station locations as possible.

Table 5.2-1: Location of Stations and Sampling Dates for the Benthic Invertebrate Community Survey, 2016

Area	Waterbody	Station	Sampling Date	UTM Coordinates[a]		Latitude	Longitude
				Easting (m)	Northing (m)		
Exposure Area (EXP)	Seep Creek Pond 1	SCP-1	25-Aug-16	481236	7290758	65°44'16.5" N	111°24'33.2" W
		SCP-2	27-Aug-16	481095	7290470	65°44'07.1" N	111°24'44.2" W
	Seep Creek Pond 2	SCP-3	31-Aug-16	480898	7290286	65°44'01.1" N	111°24'59.5" W
		SCP-4	31-Aug-16	480794	7290381	65°44'04.2" N	111°25'07.7" W
		SCP-5	27-Aug-16	480582	7290517	65°44'08.5" N	111°25'24.5" W
Reference Area 1 (REF1)	Fingers Lake	FL-1	1-Sep-16	492233	7286488	65°42'00.2" N	111°10'08.9" W
		FL-2	26-Aug-16	493811	7287107	65°42'20.3" N	111°08'05.3" W
		FL-3	26-Aug-16	493728	7287075	65°42'19.3" N	111°08'11.8" W
		FL-4	1-Sep-16	492966	7286793	65°42'10.1" N	111°09'11.5" W
		FL-5	1-Sep-16	492536	7286538	65°42'01.8" N	111°09'45.2" W
Reference Area 2 (REF2)	Unnamed Pond	R2-1	2-Sep-16	497392	7284481	65°40'55.7" N	111°03'24.3" W
		R2-2	2-Sep-16	497015	7285423	65°41'26.1" N	111°03'53.9" W
		R2-4	2-Sep-16	497741	7284442	65°40'54.4" N	111°02'57.0" W
	Unnamed Creek Pond	R2-3	3-Sep-16	495638	7287564	65°42'35.2" N	111°05'42.1" W
		R2-5	3-Sep-16	494945	7287510	65°42'33.4" N	111°06'36.4" W

(a) UTM coordinates are in Zone 12, NAD 83.

UTM = Universal Transverse Mercator coordinate system; NAD = North American Datum; ° = degree; ' = minute; " = second; N = north; W = west.

5.2.2 Study Timing

The benthic invertebrate community survey was completed on the following dates (Table 5.2-1):

- Exposure Area (Seep Creek Ponds 1 and 2) – August 25 to 31, 2016
- Reference Area 1 (Fingers Lake) – August 26 to September 1, 2016
- Reference Area 2 (Unnamed Pond and Unnamed Creek Pond) – September 2 to 3, 2016

The timing of the Phase 5 EEM benthic invertebrate community survey was consistent with the timing of surveys in Phases 1 and 2. Sampling typically occurred from mid-morning to mid-afternoon (i.e., 09:50 to 16:15).

5.2.3 Field Methods

5.2.3.1 Supporting Environmental Data

As recommended in the TGD (EC 2012a), key supporting environmental data were collected during the Phase 5 EEM benthic invertebrate community survey (Section 4). Supporting environmental data were used to assist in the interpretation of results from the benthic invertebrate community survey, as well as to assess similarity in habitat among study areas. Supporting environmental data relevant to the benthic invertebrate component included physical habitat characteristics, as well as water and sediment quality data.

5.2.3.2 Invertebrate Sample Collection

Two different types of samples were collected as part of the benthic invertebrate community survey: discrete and composite samples. Discrete samples were collected to assess within-station natural variability. At one station in each sampling area (i.e., Station 5), the five subsamples/grabs were kept as *discrete* samples (i.e., each subsample placed into an individual sample bottle) for taxonomic analysis. At four of the stations in each sampling area (i.e., Stations 1 to 4), individual subsamples/grabs were combined into a single *composite* sample (i.e., five subsamples placed into a single sample bottle) for taxonomic analysis. A total of 15 discrete and 12 composite samples were collected.

Composite Sampling

Composite benthic invertebrate samples were collected according to the TGD (EC 2012a) and Golder's *Technical Procedure 8.6-1: Benthic Invertebrate Sampling* (Golder internal document). Benthic invertebrate samples were collected using a standard Ekman grab (15.24 cm × 15.24 cm) with a bottom sampling area of 0.0232 m^2 (Appendix A, Photographs 18 to 28), with the exception of Station FL-5 that as sampled using a Ponar sampler. At each station, five field subsamples were collected and combined to form a single composite sample. Samples were sieved in the field using a 500 micrometre (µm) mesh Nitex sieve bucket or mesh bag, until the fine sediments have passed through the mesh. The material retained in the mesh was transferred to a pre-labelled one litre sample bottle and preserved with 10% buffered formalin.

Discrete Sampling

Five Ekman grab samples were collected at Station 5 in each of the Exposure and Reference Areas using the methods outlined above for composite sampling. Each sample was sieved through a 500 µm mesh sieve bucket or mesh bag. The material retained in the mesh was placed into a separate sample bottle and preserved with 10% buffered formalin, thus creating five discrete samples for each station.

5.2.4 Laboratory Methods (Sample Sorting and Taxonomic Identification)

Benthic invertebrate samples were shipped to a qualified taxonomist (J. Zloty, PhD in Summerland, BC) for enumeration and taxonomic identification. Each sample was processed separately and was divided into coarse and fine fractions. The coarse fractions were sorted completely and, if necessary due to large amounts of detritus or animals, the fine fractions were subsampled independently using the subsampling method approved for EEM (EC 2012a).

Invertebrates were identified to the lowest practical taxonomic level using current literature and nomenclature:

- phylum – Nematoda, and Ostracoda
- order – Microturbellaria (several combined orders)
- superfamily or higher group – Hydracarina
- family/sub-family – Oligochaeta
- genus – Bivalvia, Hydracarina, Trichoptera, Diptera (aside from those taxa identified to the species level)

Organisms that could not be identified to the desired taxonomic level (e.g., immature or damaged specimens) were reported as a separate category at the lowest level of taxonomic resolution possible. This was typically the family level, which is the level recommended in the TGD (EC 2012a). The most common taxa were distinguishable based on gross morphology and required only a few slide mounts (five to ten) for verification. Organisms that required detailed microscopic examination for identification (i.e., Chironomidae and Oligochaeta) were mounted on microscope slides using an appropriate medium (i.e., CMC-9AF). All rare or less commonly occurring taxa were mounted on slides for identification. A reference collection was prepared, consisting of representative specimens from each taxon. The reference collection was archived by the taxonomist for possible comparison with benthic invertebrate community data from future studies and for quality control of future taxonomic identifications.

Raw invertebrate abundance data were received from the taxonomist in electronic format. Data were entered into the latest version of Environment Canada's EEM Metal Mining Data Entry Software, as required for EEM programs. Data used in the benthic invertebrate community assessment are maintained in Excel spreadsheets.

5.2.5 Data Analyses

5.2.5.1 Supporting Environmental Data

Habitat information was summarized in tabular format and compared among areas. *In situ* water and sediment quality variables were summarized in tabular format and compared to applicable CWQGs (CCME 1999) and CSQGs, specifically ISQGs and PELs (CCME 2001) as well as among areas. Data analysis methods for the water and sediment chemistry data are provided in Section 4.2-3.

5.2.5.2 Data Screening

Raw invertebrate abundance data were screened during the preparation of data for analysis and the following organisms were removed:

- Nematoda – removed because samples were sieved through a 500 µm mesh sieve, which results in unreliable estimates of nematode numbers (EC 2012a)

- Copepoda and Cladocera – removed because they are planktonic organisms
- Chironomidae pupae – removed because they are not a benthic life stage
- terrestrial organisms – removed because they are not aquatic organisms

The data were also screened visually for anomalous values and potential data entry errors, using both box-and-whisker plots and scatter-plots for each variable. Statistical outliers were identified during statistical testing as values having studentized residuals (SR) greater than or equal to an absolute value of 3. Outliers were checked and their validity confirmed. Unless otherwise warranted, statistical analyses were run both with and without outliers.

5.2.5.3 Variables Analyzed and Descriptive Statistics

Descriptive statistics for the following effect endpoints were calculated for each station:

- total benthic invertebrate density (density)
- family level richness
- family level Simpson's Evenness Index (SEI)
- family level Bray-Curtis Index (BCI), using both the standard EEM method using Reference Area medians (EC 2012a) and the pairwise comparison method (Huebert et al. 2011).

Descriptive statistics for the following supporting endpoints were calculated for each station:

- lowest taxonomic level richness
- family level Simpson's Diversity Index (SDI)
- family level density
- family level proportion (i.e., relative density)
- family and lowest taxonomic level presence/absence.

Density was calculated as the total number of organisms per square metre (org/m^2) for each station. These calculations were based on the bottom area of the Ekman grab (0.0232 m^2). Density was used to indicate an effect, as recommended in the TGD (EC 2012a).

Family level richness is the total number of different taxonomic groups per station (taxa/station) at the level of family. Lowest taxonomic level richness is the total number of different taxa (typically at the genus/species level) per station at the lowest level of taxonomy achieved by the taxonomist. Richness provides an indication of the diversity of invertebrates in an area. A higher richness value typically indicates a more healthy and balanced community. Family and lowest taxonomic level richness were calculated for each station; however, only family level richness was used to indicate an effect, as recommended in the TGD (EC 2012a).

SDI measures the proportional distribution of organisms in the community, taking into account the density patterns and taxonomic richness of the community. SDI values range from 0 and 1; higher values indicate a community consisting of more taxa among which density is more equitably distributed. Lower values indicate a community

dominated by few taxonomic groups, which may reflect natural or anthropogenic stresses. Family level SDI was used as a supporting endpoint, as recommended in the TGD (EC 2012a).

SEI is a measure of the relative densities of the different taxa contributing to richness in an area. SEI compares the observed community to a hypothetical community, which consists of the same number of taxa that are equally abundant. A community dominated by one or two species is considered to have lower evenness than one in which several different species have similar densities. SEI values range from 0 and 1; higher values indicate a balanced community consisting of more taxa evenly distributed among taxonomic groups. Lower values indicate a community dominated by few taxa. Family level SEI was used to indicate an effect, as recommended in the TGD (EC 2012a).

The above indices are measures of total density and taxonomic richness, but they do not take into account any quantitative information on the types of organisms present among sampling areas. Therefore, the BCI, which is a dissimilarity index, was calculated to compare entire invertebrate communities among sampling areas. The BCI summarizes the overall difference in community structure between the reference and exposure areas. BCI values range from 0 and 1; lower values indicate that the community in the Exposure Area is more similar to the reference community. Family level BCI was used to indicate an effect, as recommended in the TGD (EC 2012a). The BCI was calculated using two different methods:

1) the standard EEM method using Reference Area medians (EC 2012a); and

2) the pairwise comparison method (Huebert et al. 2011).

Family level density is the total number of organisms of each family expressed per unit area (org/m^2). Family level proportion is the relative density for each taxon at the family level composing the invertebrate community, expressed as a percentage (%). Presence/absence was quantified through a presence/absence matrix at the family level and lowest taxonomic level for each sampling area. These biotic measures were used as additional descriptors and were not used to indicate an effect, as recommended in the TGD (EC 2012a).

In addition to the standard community descriptors listed above, the spatial trend in the density of Chironomidae (the dominant invertebrate group in the study area) was examined to further investigate potential differences between the Reference and Exposure Areas.

Mean total invertebrate density and invertebrate endpoints were plotted for both Exposure and Reference Areas for comparison purposes. The relative density of invertebrates (at the family and sub-family levels) and the density of Chironomidae were also plotted. The Chironomidae (midges) family was split into the dominant sub-families and tribes for the sub-family level relative density plot, because they differ in their feeding preferences. Scatter-plots were created in SigmaPlot v.13 (SYSTAT 2014).

5.2.5.4 Habitat Relationships

Relationships between selected habitat variables and invertebrate community variables were evaluated by calculating Spearman rank correlation coefficients (r_s) and examining scatter-plots. Correlations were evaluated between the biological variables identified above and water depth, total organic carbon (TOC), and the percentage of fine sediments, which consists of the combined silt and clay particle size fractions.

Spearman's r_s values were compared to two-tailed critical values at the appropriate sample size. A critical value of 0.521 was used to determine the significance of the correlations, based on a *P-value* of 0.05 and a sample size

of 15 (Zar 1999). Spearman correlations were performed using SYSTAT v.13.1 (SYSTAT 2009). Habitat variables with significant correlations that were consistent in direction among sampling areas were considered for addition into statistical comparisons as covariates.

5.2.5.5 Statistical Analysis

5.2.5.5.1 Approach

The objective of the statistical analyses was to evaluate whether there were significant differences in the invertebrate endpoints among the Exposure and Reference Areas. Statistical testing was conducted by analysis of variance (ANOVA). All statistical analyses were performed using SYSTAT version 13.1 (SYSTAT 2009).

5.2.5.5.2 Testing Assumptions for Analysis of Variance

Parametric tests such as ANOVA assume that the data fit the normal distribution, because the residuals (or error terms of the variates) are assumed to fit the normal distribution. If a measurement variable is not normally distributed, there is an increased chance of a false positive result (Type I error). ANOVA is not sensitive to moderate deviations from normality, because when a large number of random samples are taken from a population, the means of those samples are approximately normally distributed even when the population is not normal (Sokal and Rohlf 1995).

The goodness-of-fit of the data to the normal distribution were tested with the Kolmogorov-Smirnov test with the Lillieffors correction. Many datasets that are significantly non-normal are still appropriate for an ANOVA; therefore, issues with non-normality were addressed with a *P* value less than 0.01. No violations of the normality assumption were found in the untransformed 2016 benthic invertebrate data used in the statistical analysis.

Another important assumption in ANOVA is that group variances are equal (i.e., homogeneity of variances). When variances differ markedly, various data transformations will typically remedy the problem. As with normality, the consequences of moderate deviations from the assumption of equal variances do not compromise the overall test of significance by ANOVA. Homogeneity of variances was tested using the Levene's test ($P<0.01$). For the variables selected for statistical analysis, variances were found to be homogeneous based on untransformed data.

5.2.5.5.3 Analysis of Variance

The mean values of the three sampling areas (i.e., EXP, REF1, and REF2) were compared to one another in an overall ANOVA. If the overall ANOVA model was significant, the Exposure Area mean was compared to the mean of the two Reference Areas using a linear orthogonal contrast (Hoke et al. 1990). Statistical tests were considered significant at a *P*-value <0.10, as recommended by the TGD (EC 2012a).

5.2.5.5.4 Detecting Effects

The magnitude of differences in effect endpoints between the Exposure and Reference Areas were calculated by expressing the difference as a percentage of the mean of the Reference Areas according to the following equation:

*Magnitude = [(exposure area mean – mean of reference area means)/mean of reference area means)]*100*
[Equation 5.1]

The critical effect size (CES) was calculated as plus or minus two standard deviations (±2 SD), expressed as a percentage of the overall Reference Area mean. Magnitudes of differences between Reference and Exposure Areas were considered biologically significant if they exceeded the CES.

5.2.5.5.5 Power Analysis

The probability of a Type I error (α) was set to the same level as a Type II error (β), because the probability of missing important effects (Type II error) is deemed to be as important as the probability of finding an effect when none exists (Type I error). For a study design with five stations per area (i.e., five replicates), the TGD (EC 2012a) recommends that α and β be set equally at 0.10 to allow a CES equal to ±2 SD from the Reference Area mean (see Table 8-7 of the TGD [EC 2012a]). Using this design, power (1-β) was set *a priori* at 0.90. For non-significant results, *post hoc* power analyses were calculated to determine the actual power to detect a biologically meaningful effect (i.e., with a magnitude equal to the CES). The power of the statistical tests would be 0.9 by default if the effect size is calculated as 2SD using the pooled SD for all areas combined. To account for the possibility that the Exposure Area data were more variable than the Reference Area data, power was recalculated for non-significant tests using effect sizes based on the variation observed in the two Reference Areas (i.e., 2 times the pooled Reference Area SD).

5.2.6 Quality Assurance and Quality Control

QA/QC procedures and requirements are an important aspect of any field or laboratory testing program. Good QA/QC practices result in field sampling, data entry, data analyses, and report preparation that produce technically sound and scientifically defensible results.

Detailed specific work instructions outlining each task were provided to the field personnel prior to the field program. Samples were collected by experienced personnel and were labelled, preserved, and shipped according to Golder's *Technical Procedure 8.6-1: Benthic Invertebrate Sampling* (Golder internal document). Applicable guidance from the TGD (EC 2012a) was incorporated into sampling protocols. Field equipment, such as water quality meters, was regularly calibrated according to the manufacturer's recommendations. Benthic invertebrate community samples were accompanied by a chain-of-custody form.

Invertebrate sample sorting efficiency was verified by the taxonomist by performing spot-checks on left-over debris. Ten percent of the samples were randomly selected and re-sorted by an individual not involved in initial sample sorting. The data quality objective was a minimum recovery of 90% of the total organisms. If more than 10% of the total number of organisms removed from the sample were found in the debris, then all samples were re-sorted by a different individual. In addition, if an entire taxonomic group was omitted by the sorter, then all samples were re-sorted, again by a different individual.

Subsampling precision and accuracy of the fine fractions were verified by the taxonomist by analyzing all subsamples in one sample. Realized precision and accuracy of subsampling were verified according to the methods recommended by Environment Canada (2002b, 2012a).

Raw benthic invertebrate abundance data were visually screened to identify extreme values and to assess whether the identified taxa were appropriate for the waterbody sampled. If questionable data were identified, the taxonomist was contacted for clarification and the necessary corrections were made.

Field data entered into electronic format (including the Environment Canada EEM database) underwent a 100% transcription and validity check by a second person not involved in the initial data entry process. Calculated values, tables, statistical test results and figures generated from the dataset were reviewed by a senior biologist of appropriate qualifications.

5.3 Results and Discussion

Detailed taxonomic results and abundance data are provided in Appendix D, Table D-1.

5.3.1 Quality Assurance and Quality Control

All data collected during the Phase 5 EEM benthic invertebrate community survey were deemed valid. Higher than expected among-station variability in invertebrate abundance values was identified. However, the data were verified by the taxonomist.

Low sampler fullness was achieved at several of the stations across all the three sampling areas, reflecting the compact nature of bottom sediments in the study area. Mean sampler fullness was plotted against total invertebrate density to examine the relationship between these two variables. Results revealed that higher percent sampler fullness generally resulted in higher invertebrate densities (Appendix D, Figure D-1). However, since sampler fullness reflected the amount of non-compacted sediment, and thus available habitat "depth" at the stations sampled, this variation was considered representative of site conditions and was not considered further during data analysis.

Three samples (FL-1, R2-2, and SCP-5-3) were randomly selected and re-sorted to measure the invertebrate sample sorting efficiency. The sorting efficiency ranged from 97% to 100%, with an average of 98% (Appendix D, Table D-2). Because all three of the samples met the minimum data quality objective of 90%, data quality was considered acceptable.

Subsampling was done on a total of six samples (SCP-1, SCP-3, SCP-4, FL-4, R2-4, and R2-3) in the benthic invertebrate dataset. Subsampling factors are presented in Appendix D, Table D-3. Subsampling precision and accuracy of the fine fractions were verified by the taxonomist by analyzing all subsamples in one sample collected from Station FL-4, representing 10% of subsampled samples. Precision and accuracy results are presented in Appendix D, Table D-4. Overall, accuracy ranged from 0.2 to 3.5% (mean 1.9%) and precision ranged from 0.8 to 5.6%. Both subsampling accuracy and precision estimates were below the QC criterion of 20% outlined by Environment Canada (2002b, 2012a). A comparison of the actual and predicted abundances for the subsampled sample are provided in Appendix D, Table D-5.

5.3.2 Supporting Environmental Data

Detailed results for the supporting environmental data are provided in Section 4. Supporting environmental data relevant to the benthic invertebrate component included physical habitat characteristics, as well as water and sediment quality data.

5.3.3 Benthic Invertebrate Community Analysis

5.3.3.1 Data Screening

Two anomalous values or statistical outliers (i.e., values with SR values ≥3) were identified in the dataset; these were the total density (SR = 4.8) and Chironomidae density (SR = 6.7) values for Station SCP-3. Statistical analyses were run with and without these values. Since statistical tests results were not affected by removal of the outliers, results are presented for the full data set, with outliers included.

5.3.3.2 Within-Station Variation

Within-station variability was evaluated for the three stations (SCP-5, FL-5, and R2-5) where discrete samples were collected. The number of subsamples required to achieve a precision of 20% (D= 0.20) was estimated for total abundance and richness. Results revealed that to achieve the desired level of precision for total abundance, a range of 2 to 30 subsamples would be required, depending on station (Table 5.3-1). Excluding the outlier of 46 organisms/sample from the FL-5 dataset, the range would decrease to 2 to 12 subsamples, which illustrates the influence of a single unusual subsample on the estimate. For richness, the desired level of precision can be reached based on 1 to 7 subsamples. For both variables, the number of subsamples collected in 2016 (5 samples) is within the estimated range for the appropriate level of precision.

Table 5.3-1: Estimation of the Number of Subsamples Required for a Desired Level of Precision Based on Variation among the Discrete Benthic Invertebrate Samples, 2016

Station	Subsample					Mean	Minimum	Maximum	SD	SE	No. of Subsamples Required[(a)]
	1	2	3	4	5						
Total Abundance (no./sample)											
SCP-5	31	15	29	26	28	26	15	31	6.3	2.8	2
FL-5	6	5	46	13	9	16	5	46	17.2	7.7	30
R2-5	24	21	50	14	7	23	7	50	16.4	7.3	12
Richness (taxa/station)											
SCP-5	7	7	7	7	8	7	7	8	0.4	0.2	1
FL-5	4	3	8	6	5	5	3	8	1.9	0.9	3
R2-5	7	10	17	9	4	9	4	17	4.8	2.2	7

(a) The number of subsamples required to achieve a precision of 20% (D = 0.20) is calculated using the formula, as recommended by EC (2012a).

SD = standard deviation; SE = standard error; No. = number; no./sample = number of organisms per sample; SCP = Seep Creek Pond; FL = Fingers Lake; R2 = Reference Area 2.

5.3.3.3 Effects of Habitat Variation

In the field, habitat was standardized among stations to the extent possible; however, the following natural factors may have influenced the assemblages present in the depositional sediments sampled during this study:

- water depth, which typically has a strong influence on invertebrate abundance and distribution in lakes, with deeper stations frequently having lower abundances in deep lakes
- proportion of fine sediments (i.e., percentage of silt and clay), which can influence the abundance and type of invertebrates present, through physical means (e.g., amount of interstitial space, compaction of sediment)
- TOC, which is a measure of how much organic material is in the sediment, can affect dissolved oxygen (DO) concentrations and food availability for invertebrates, as well as complex with metals and modify their bioavailability

Habitat variability was observed among sampling stations and areas (Table 5.3-2). Mean water depths were similar in the Exposure Area and Reference Area 2 (0.4 m in both), and lower than in Reference Area 2 (1.3 m), where three stations were ≥1 m deep (Appendix D, Table D-6). The mean percentage of fine sediments was higher in

the Exposure Area (40%) compared to the Reference Areas (REF1: 19%; REF2: 10%), but this was largely due to one exposure station (SCP-5) having predominantly fine sediments. Consistent with this observation, the benthic invertebrate community of Station SCP-5 was co-dominated by aquatic worms (Lumbriculidae), which was unique in the study area. Excluding Station SCP-5, the mean percentage of fine sediments in the Exposure Area was 25%, which is only slightly higher than the mean value (19%) for Reference Area 1. The mean percentage of TOC in sediments was highest in the Exposure Area (1.8%), followed by Reference Area 1 (1.4%), and Reference Area 2 (0.6%). However, overall, TOC varied over a narrow range (0.24% to 5%) in the study area. Although the habitat variation observed in 2016 is expected to account some of the among-station variation in benthic community structure, it is unlikely to be a major influence accounting for the variation in the benthic invertebrate community among sampling areas.

Table 5.3-2: Summary of Habitat Characteristics in Benthic Invertebrate Sampling Areas, 2016

Area	Waterbody	Mean (and Range) of Habitat Variable		
		Water Depth (m)	Fine Sediments (%)	TOC (%)
Exposure Area (EXP)	Seep Creek Ponds 1 and 2	0.4 (0.3 to 0.5)	40 (8 to 100)	1.8 (0.4 to 2.9)
Reference Area 1 (REF1)	Fingers Lake	1.3 (0.6 to 2.0)	19 (4 to 36)	1.4 (0.2 to 5.0)
Reference Area 2 (REF2)	Unnamed Pond and Unnamed Creek Pond	0.4 (0.2 to 0.4)	10 (1 to 25)	0.6 (0.3 to 1.2)

TOC = total organic carbon.

Consistent with the expectation of a minor influence of habitat variation on the biological dataset, there were no significant correlations between habitat variables and biological variables (Table 5.3-3). Two habitat variables (% fines and%TOC) were significantly correlated, which is the expected relationship between these variables. This had no effect on the statistical analysis or the interpretation of results.

Table 5.3-3: Spearman Rank Correlation Coefficients for Benthic Invertebrate Community Variables and Selected Habitat Variables, 2016

Taxonomic Level	Variable	Spearman Rank Correlations (r_s)		
		Fine Sediments	TOC	Water Depth
Correlations Among Habitat Variables				
-	% Fines	1	-	-
-	% TOC	**0.673**	1	-
-	Water depth	0.029	0.024	1
Correlations Between Habitat Variables and Benthic Community Variables				
n/a	Total density	0.248	0.436	0.123
Family Level	Richness	0.160	0.413	-0.057
	Simpson's Diversity Index	0.338	0.329	0.002
	Simpson's Evenness Index	0.179	0.083	-0.091
	Chironomidae density	0.042	0.260	0.089

Table 5.3-3: Spearman Rank Correlation Coefficients for Benthic Invertebrate Community Variables and Selected Habitat Variables, 2016

Taxonomic Level	Variable	Spearman Rank Correlations (r_s)		
		Fine Sediments	TOC	Water Depth
Lowest Taxonomic Level	Richness	0.116	0.374	0.380

Notes: Significant correlations (P<0.05) are shown in **bold**. The two-tailed critical value for data correlations was 0.521 for all three habitat variables (n = 15).

TOC = total organic carbon; - = not applicable; P = probability value; <= less than; n = number of samples.

5.3.3.4 Evaluation of Effects on the Benthic Invertebrate Community

The total density of invertebrates ranged from 439 to 19,435 org/m^2 in the Exposure Area, from 534 to 8,938 org/m^2 in Reference Area 1, and from 362 to 7,319 org/m^2 in Reference Area 2 (Table 5.3-4; Figure 5.3-1). Total density was higher (111%) in the Exposure Area compared to the Reference Areas. However, the magnitude of the difference did not exceed the CES (164%; Table 5.3-7) and no significant difference was detected between the Exposure Area and Reference Areas. These results suggest that this difference in density was not biologically significant. For total density, the direction of difference between the Exposure and Reference Areas was inconsistent with an adverse effect (i.e., lower in the Exposure Area) on the benthic invertebrate community.

Mean densities of Chironomidae were variable, but generally low among the sampling areas (Figure 5.3-1). Mean Chironomidae density was highest in the Exposure Area (5,327 org/m^2), followed by Reference Area 2 (2,335 org/m^2), and Reference Area 1 (1,417 org/m^2) (Table 5.3-4). Chironomidae density was higher (184%) in the Exposure Area compared to the Reference Areas; however, the magnitude of the difference did not exceed the CES (214%; Table 5.3-7). No significant difference was detected in Chironomidae density between the Exposure Area and Reference Areas. These results suggest that the difference in Chironomidae density was not biologically significant.

Richness was generally low at both the family and lowest taxonomic levels, reflecting the habitat type sampled. Richness varied moderately among stations and areas (Table 5.3-4; Figures 5.3-1 and 5.3-2). Family level richness ranged from 5 to 11 taxa/station in the Exposure Area, from 3 to 9 taxa/station in Reference Area 1, and from 4 to 6 taxa/station in Reference Area 2. Lowest level taxonomic richness ranged from 13 to 24 taxa/station in the Exposure Area, from 12 to 24 taxa/station in Reference Area 1, and from 9 to 21 taxa/station in Reference Area 2. No significant difference was detected in either the family or lowest taxonomic level richness among areas (Table 5.3-7). Both family and lowest taxonomic level richness were higher (30% and 7%, respectively) in the Exposure Area compared to the Reference Areas, which is inconsistent with an adverse effect. The magnitude of the differences did not exceed the CES (46% and 40%, respectively; Table 5.3-7). These results suggest that the differences in family and lowest taxonomic level richness were not biologically significant.

Family level SDI values were low to moderate and in similar ranges in the Exposure Area (0.15 to 0.60), Reference Area 1 (0.18 to 0.61), and Reference Area 2 (0.08 to 0.46; Table 5.3-4; Figure 5.3-1). Family level SDI values were not significantly different between the Exposure Area and Reference Areas (Table 5.3-7). Family level SDI was lower (7%) in the Exposure Area compared to the Reference Areas; however, this difference did not exceed the CES (68%), suggesting that the observed difference was not biologically significant.

Family level SEI values were in similar ranges in all sampling areas (Exposure Area: 0.11 to 0.42; Reference Area 1: 0.26 to 0.41; Reference Area 2: 0.22 to 0.39) (Table 5.3-4; Figure 5.3-1). No significant differences were detected in SEI among areas (Table 5.3-7). The magnitude of difference in the Exposure Area (-19%) did not exceed the CES (28%) (Table 5.3-7). These results also suggest that the differences among areas were not biologically significant.

Dissimilarity index (BCI) values calculated using the EEM method were lower for comparisons between Reference Areas and their own medians compared to differences between the Exposure Area and Reference Area medians (Tables 5.3-5 and 5.3-6; Figure 5.3-3). Based on the pairwise comparison method, BCI values were relatively similar among the three comparisons. Significant differences in BCI were not detected among areas at the family level for either calculation method (Tables 5.3-5 and 5.3-6). Based on the standard EEM method (EC 2012a), family level BCI was 47% greater in the Exposure Area compared to the Reference Areas, but the increases were only 10% when the pairwise comparison method (Huebert et al. 2011) was used (Table 5.3-7). The magnitude of differences in family level BCI did not exceed the CES (98% and 28%, respectively) for the EEM or pairwise comparison methods. These results suggest that the differences were not biologically significant.

A basic control/impact study design requires five samples per area to attain a power of 0.90 to detect an effect size of ±2 SD (EC 2012a). The benthic invertebrate survey was designed to achieve this level of power. *Post hoc* power analysis indicated that using Reference Area data to estimate effect size, there was insufficient power for total density and Chironomidae density, but sufficient or nearly sufficient power for all other variables (Appendix D, Table D-7). Re-running the power analysis without the outlier density data for SCP-3 in the Exposure Area resulted in sufficient power to detect increased total density and Chironomidae density (i.e., the observed direction of difference), but not for decreases in density. Given that magnitudes of differences were lower that the CES for all variables, these results do not affect the conclusion of the study regarding the presence/absence of effects.

The total number of families varied among areas, ranging from nine families in Reference Area 1 to 15 families in the Exposure Area (Table 5.3-8). Three families of Acari-Hydracarina (Aturidae, Arrenuridae, and Pionidae), one family of Trichoptera (Phryganeidae), and one family of Diptera (Tipulidae) were exclusive to the Exposure Area. Two families (Microturbellaria and Pisidiidae) were absent from the Exposure Area, but present in at least one of the Reference Areas. The total number of taxa was similar among areas, with the Reference Areas having the lowest (36 taxa) and the Exposure Area having the highest (37 taxa) total number of taxa (Table 5.3-9).

Family level invertebrate density and proportion (i.e., relative density) for each station and area are summarized in Appendix D, Table D-8.

Table 5.3-4: Benthic Invertebrate Community Endpoints, 2016

Area	Waterbody	Station	Total Invertebrate Density (org/m^2)	Family Level			Lowest Taxonomic Level
				Richness (taxa/station)	SDI	SEI	Richness (taxa/station)
Exposure Area	Seep Creek Pond 1	SCP-1	5,830	5	0.15	0.24	19
		SCP-2	439	7	0.28	0.20	19
	Seep Creek Pond 2	SCP-3	19,435	11	0.15	0.11	24
		SCP-4	3,634	6	0.46	0.31	19
		SCP-5	1,042	6	0.60	0.42	13
Mean			6,076	7	0.33	0.25	19
Median			3,634	6	0.28	0.24	19
Minimum			439	5	0.15	0.11	13
Maximum			19,435	11	0.60	0.42	24
Standard Deviation			7,772	2	0.20	0.12	4
Standard Error			3,476	1	0.09	0.05	2
Reference Area 1	Fingers Lake	FL-1	3,427	9	0.61	0.28	24
		FL-2	1,154	5	0.22	0.26	19
		FL-3	534	3	0.18	0.41	15
		FL-4	8,938	6	0.50	0.33	22
		FL-5	629	4	0.33	0.37	12
Mean			2,936	5	0.37	0.33	18
Median			1,154	5	0.33	0.33	19
Minimum			534	3	0.18	0.26	12
Maximum			8,938	9	0.61	0.41	24
Standard Deviation			3,555	2	0.18	0.06	5
Standard Error			1,590	1	0.08	0.03	2
Reference Area 2	Unnamed Pond	R2-1	766	4	0.36	0.39	9
		R2-2	362	6	0.46	0.31	15
		R2-4	7,319	6	0.37	0.26	21
	Unnamed Creek Pond	R2-3	4,822	5	0.08	0.22	18
		R2-5	844	6	0.44	0.30	21
Mean			2,823	5	0.34	0.30	17
Median			844	6	0.37	0.30	18

Table 5.3-4: Benthic Invertebrate Community Endpoints, 2016

Area	Waterbody	Station	Total Invertebrate Density (org/m^2)	Family Level			Lowest Taxonomic Level
				Richness (taxa/station)	SDI	SEI	Richness (taxa/station)
Minimum			362	4	0.08	0.22	9
Maximum			7,319	6	0.46	0.39	21
Standard Deviation			3,099	1	0.16	0.06	5
Standard Error			1,386	0	0.07	0.03	2

org/m^2 = organisms per square metre; taxa/station = number of taxa per station; SDI = Simpson's Diversity Index; SEI = Simpson's Evenness Index.

Table 5.3-5: Bray-Curtis Index Values Calculated using Standard EEM Method, 2016

Areas Compared	Stations Compared	BCI[(a)]
EXP vs REF1 MEDIAN	SCP-1 vs REF1 MEDIAN	0.70
	SCP-2 vs REF1 MEDIAN	0.47
	SCP-3 vs REF1 MEDIAN	0.90
	SCP-4 vs REF1 MEDIAN	0.56
	SPC-5 vs REF1 MEDIAN	0.54
Mean		0.63
Median		0.56
Minimum		0.47
Maximum		0.90
Standard Deviation		0.17
Standard Error		0.08
EXP vs REF2 MEDIAN	SCP-1 vs REF2 MEDIAN	0.80
	SCP-2 vs REF2 MEDIAN	0.29
	SCP-3 vs REF2 MEDIAN	0.93
	SCP-4 vs REF2 MEDIAN	0.69
	SPC-5 vs REF2 MEDIAN	0.41
Mean		0.63
Median		0.69
Minimum		0.29
Maximum		0.93
Standard Deviation		0.27
Standard Error		0.12
REF1 vs REF1 MEDIAN	FL-1 vs REF1 MEDIAN	0.50
	FL-2 vs REF1 MEDIAN	0.04
	FL-3 vs REF1 MEDIAN	0.36
	FL-4 vs REF1 MEDIAN	0.78
	FL-5 vs REF1 MEDIAN	0.34
Mean		0.41
Median		0.36
Minimum		0.04
Maximum		0.78
Standard Deviation		0.27
Standard Error		0.12
REF2 vs REF2 MEDIAN	R2-1 vs REF2 MEDIAN	0.13
	R2-2 vs REF2 MEDIAN	0.37
	R2-3 vs REF2 MEDIAN	0.82
	R2-4 vs REF2 MEDIAN	0.76
	R2-5 vs REF2 MEDIAN	0.17
Mean		0.45
Median		0.37
Minimum		0.13
Maximum		0.82
Standard Deviation		0.32
Standard Error		0.14

(a) Bray-Curtis Index (BCI) calculated using the standard EEM method using Reference Area medians (EC 2012a).
EXP = Exposure Area; REF1 = Reference Area 1; REF2 = Reference Area 2.

Table 5.3-6: Bray-Curtis Index Values Calculated using Pairwise Comparison Method, 2016

Areas Compared	Stations Compared	BCI[(a)]
REF1 vs REF2	FL-1 vs REF2	0.67
	FL-2 vs REF2	0.52
	FL-3 vs REF2	0.50
	FL-4 vs REF2	0.75
	FL-5 vs REF2	0.49
Mean		0.58
Median		0.52
Minimum		0.49
Maximum		0.75
Standard Deviation		0.12
Standard Error		0.05
EXP vs REF1	SCP-1 vs REF1	0.70
	SCP-2 vs REF1	0.53
	SCP-3 vs REF1	0.87
	SCP-4 vs REF1	0.63
	SPC-5 vs REF1	0.58
Mean		0.66
Median		0.63
Minimum		0.53
Maximum		0.87
Standard Deviation		0.13
Standard Error		0.06
EXP vs REF2	SCP-1 vs REF2	0.55
	SCP-2 vs REF2	0.54
	SCP-3 vs REF2	0.79
	SCP-4 vs REF2	0.61
	SPC-5 vs REF2	0.64
Mean		0.63
Median		0.61
Minimum		0.54
Maximum		0.79
Standard Deviation		0.10
Standard Error		0.04

(a) Bray-Curtis Index (BCI) calculated using the pairwise comparison method (Huebert et al. 2011).

EXP = Exposure Area; REF1 = Reference Area 1; REF2 = Reference Area 2.

Table 5.3-7: Summary of Benthic Invertebrate Community Effects Analyses, 2016

Effect or Supporting Endpoint	ANOVA (*P*-value)	Direction	Magnitude (%)	CES (%)	Magnitude >CES?
Total Density	0.555	n/a	111	232	N
Chironomidae Density	0.391	n/a	184	214	N
Family Level					
Richness	0.363	n/a	30	65	N
SDI	0.943	n/a	-7	96	N
SEI	0.388	n/a	-19	40	N
BCI[(a)]	0.423	n/a	47	139	N
BCI[(b)]	0.596	n/a	10	40	N
Presence/Absence	Number of families was highest in the Exposure Area (15 families), followed by Reference Area 2 (12 families), and then Reference Area 1 (9 families).				
Community Composition[(c)]	All areas were dominated by Chironomidae (Chironominae, Orthocladiinae, and Tanypodinae) and to a lesser extent, Ostracoda. Diptera (Empididae) made up a noticeable proportion of the community composition in the Reference Areas, but not in the Exposure Area.				
Lowest Taxonomic Level					
Richness	0.776	n/a	7	57	N
Presence/Absence	Total number of taxa was similar among the Exposure and References Areas (36 to 37 taxa).				

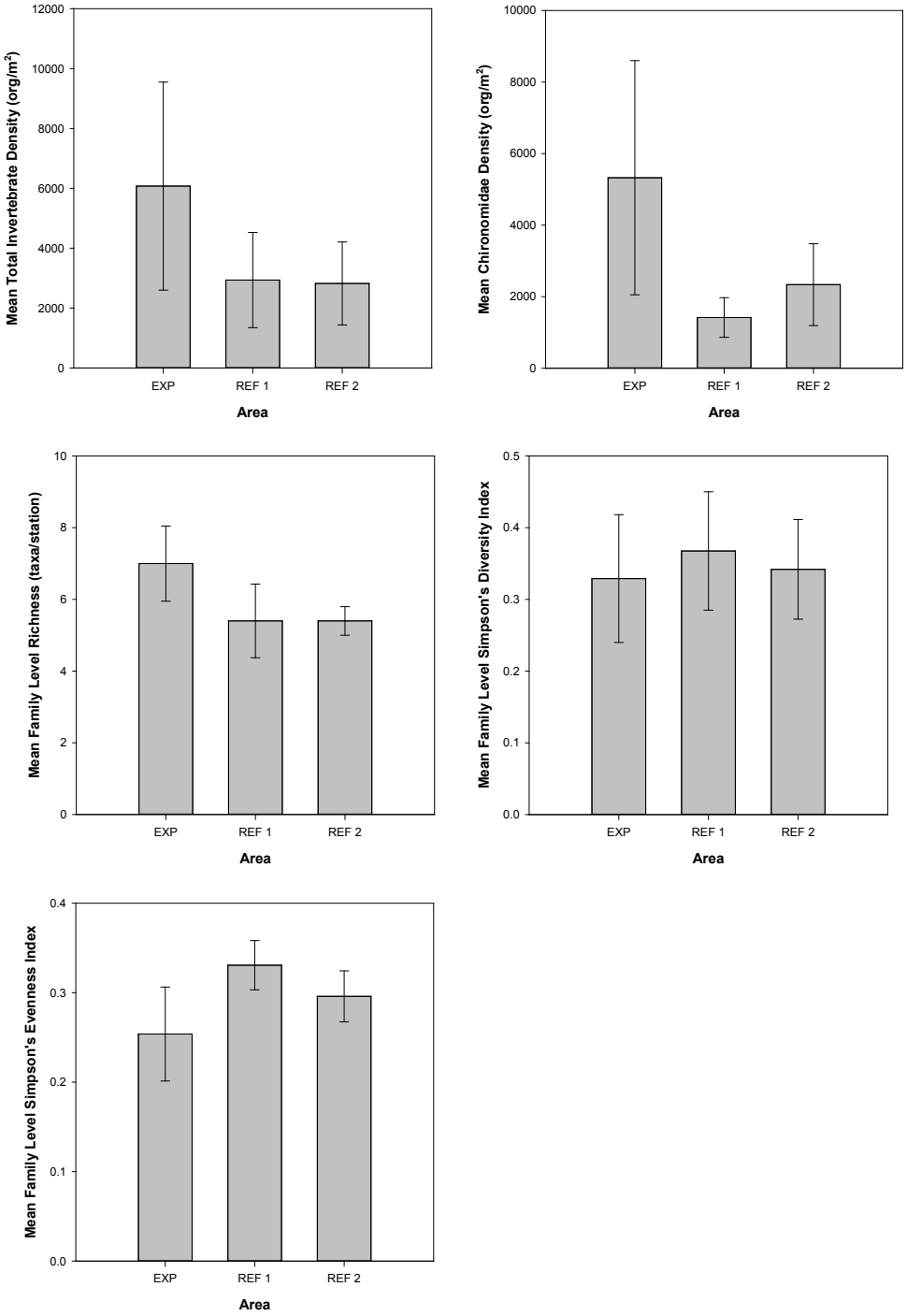
(a) Bray-Curtis Index calculated using the standard EEM method using Reference Area medians (EC 2012a).

(b) Bray-Curtis Index calculated using the pairwise comparison method (Huebert et al. 2011).

(c) Community composition based on percentages of major invertebrate groups.

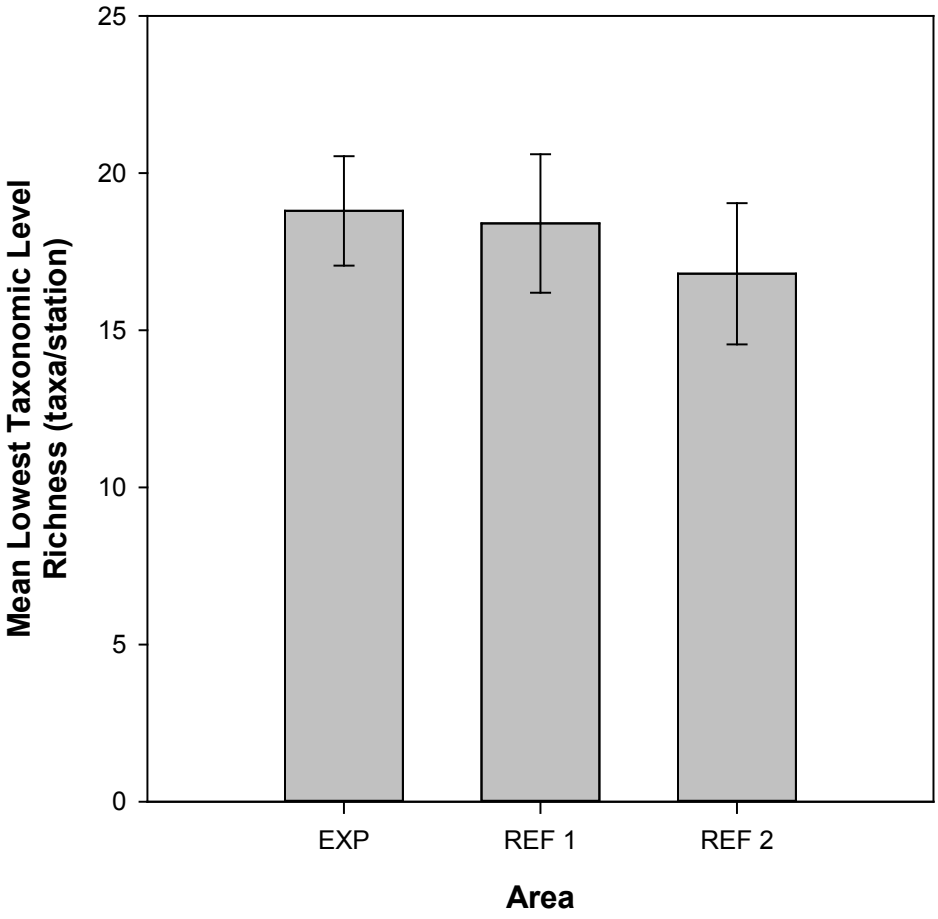
ANOVA = analysis of variance; *P*-value = probability value; CES = critical effect size; n/a = not applicable.

Figure 5.3-1: Mean Total Invertebrate Density and Family Level Invertebrate Endpoints, 2016



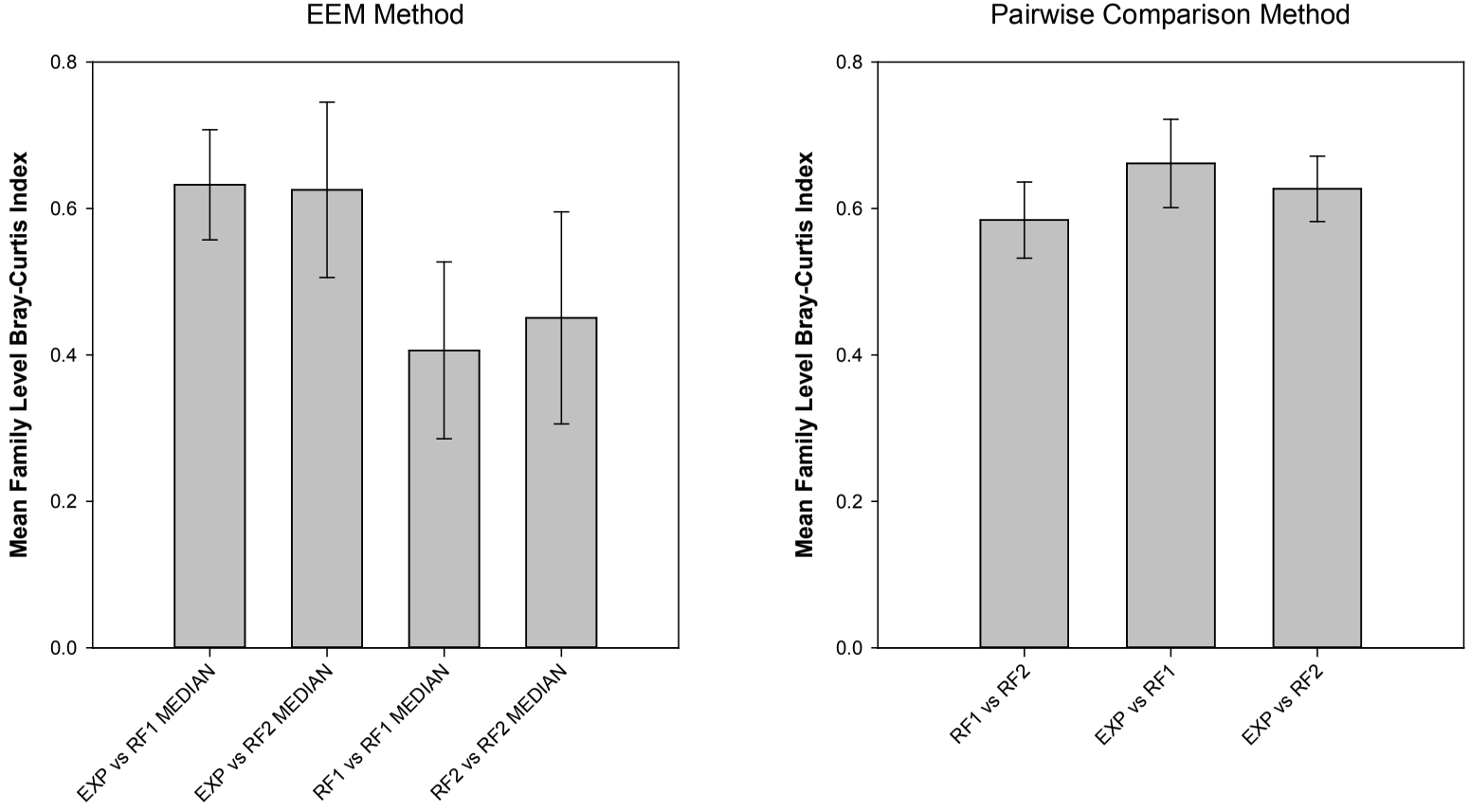
Note: Error bars represent ±1 standard error of the mean.
org/m^2 = organisms per square metre; taxa/area = number of taxa per area.

Figure 5.3-2: Mean Lowest Taxonomic Level Invertebrate Endpoints, 2016



Note: Error bars represent ±1 standard error of the mean.
taxa/area = number of taxa per area.

Figure 5.3-3: Mean Family Level Bray-Curtis Index Calculated Using the EEM and Pairwise Comparison Methods, 2016



Notes: Error bars represent ±1 standard error of the mean.

Bray-Curtis Index was calculated using the standard EEM method using Reference Area medians (EC 2012a) and pairwise comparison method (Huebert et al. 2011).

Table 5.3-8: Benthic Invertebrate Families Documented in Samples Collected from the Exposure and Reference Areas, 2016

Major Taxonomic Group	Family	Exposure Area – Seep Creek Ponds	Reference Area 1 – Fingers Lake	Reference Area 2 – Unnamed Ponds
Microturbellaria (i/d)	-	-	-	X
Oligochaeta	Enchytraeidae	X	-	X
Oligochaeta	Lumbriculidae	X	X	X
Oligochaeta	Naididae	X	X	X
Bivalvia	Pisidiidae	-	X	X
Acari - Hydracarina	Aturidae	X	-	-
Acari - Hydracarina	Arrenuridae	X	-	-
Acari - Hydracarina	Lebertiidae	X	X	X
Acari - Hydracarina	Pionidae	X	-	-
Ostracoda	-	X	X	X
Trichoptera	Leptoceridae	X	X	X
Trichoptera	Phryganeidae	X	-	-
Diptera	Chironomidae	X	X	X
Diptera	Ceratopogonidae	X	X	X
Diptera	Empididae	X	X	X
Diptera	Muscidae	X	-	X
Diptera	Tipulidae	X	-	-
	Total Number of Families	**15**	**9**	**12**

i/d = immature or damaged specimen identified to the lowest level possible; X = taxon was present; - = taxon was absent, or not applicable.

Table 5.3-9: Benthic Invertebrate Taxa (at the Lowest Level) Documented in Samples Collected from the Exposure and Reference Areas, 2016

Major Taxonomic Group	Family	Subfamily	Tribe	Genus/Species	Exposure Area – Seep Creek Ponds	Reference Area 1 – Fingers Lake	Reference Area 2 – Unnamed Ponds
Microturbellaria (i/d)	-	-	-	-	-	-	X
Oligochaeta	Enchytraeidae	-	-	-	X	-	X
	Lumbriculidae	-	-	-	X	X	X
	Naididae	Naidinae	-	-	-	-	X
	Naididae	Tubificinae	-	-	X	X	-
Bivalvia	Pisidiidae	-	-	*Sphaerium*	-	X	X
				Pisidium	-	X	-
Acari - Hydracarina	Aturidae	-	-	*Aturus*	X	-	-
	Arrenuridae	-	-	*Arrenurus*	X	-	-
	Lebertiidae	-	-	*Lebertia*	X	X	X
	Pionidae	-	-	*Piona*	X	-	-
Ostracoda	-	-	-	-	X	X	X
Trichoptera	Leptoceridae	-	-	*Oecetis*	X	X	X
				Mystacides	-	X	X
	Phryganeidae	-	-	*Agrypnia*	X	-	-
Diptera	Chironomidae	Tanypodinae	Pentaneurini	*Ablabesmyia*	X	X	-
				Thienemannimyia group	-	X	X
			Procladiini	*Procladius*	X	X	X
		Diamesinae	Diamesini	*Potthastia longimanus* group	-	X	-
		Prodiamesinae	-	*Monodiamesa*	-	X	X
		Orthocladiinae	Orthocladiini	*Cricotopus / Orthocladius*	X	X	X
				Heterotrissocladius	X	X	X
				Parakiefferiella	X	X	X
				Psectrocladius	X	X	X
				Zalutschia	X	X	X
		Chironominae	Chironomini	*Chironomus*	X	X	-
				Cladopelma	X	-	X
				Cryptochironomus	X	X	X
				Cryptotendipes	-	-	X

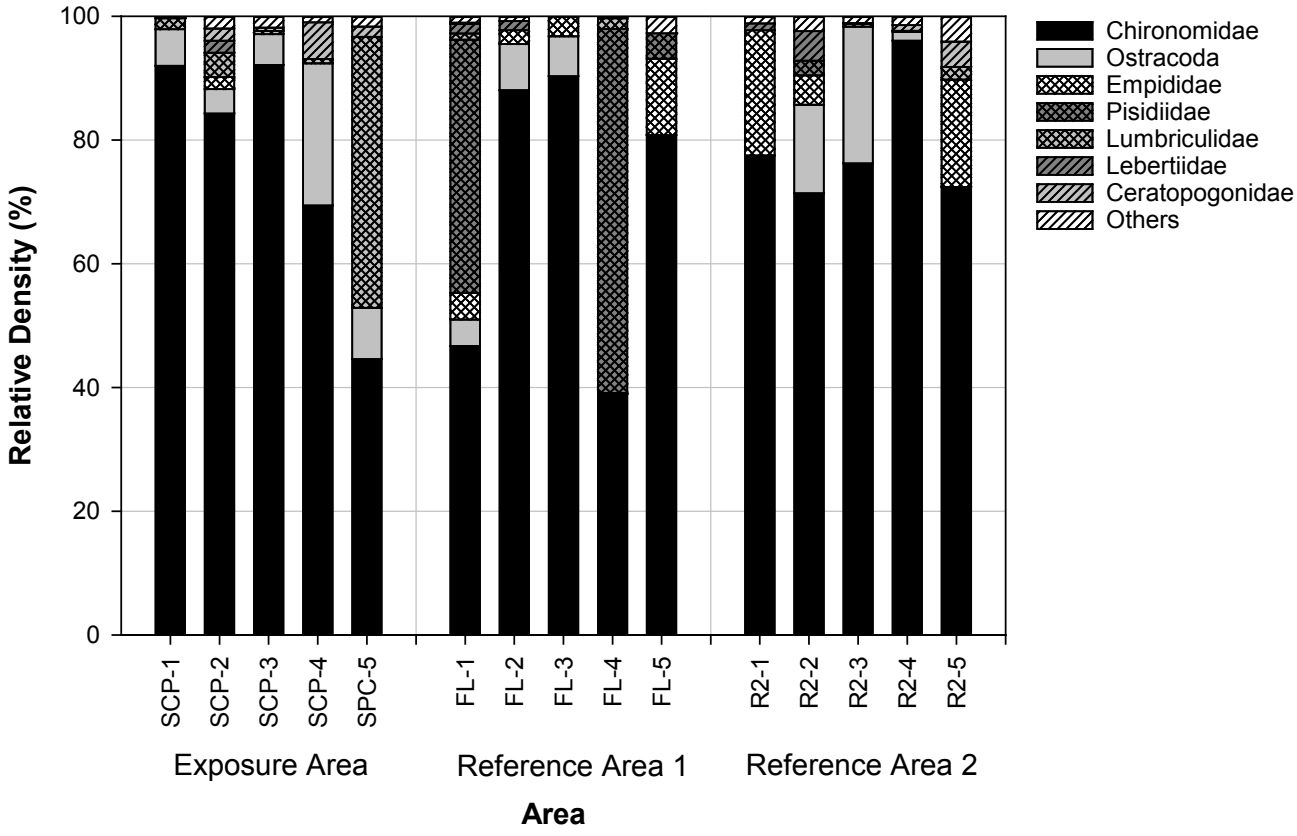
Table 5.3-9: Benthic Invertebrate Taxa (at the Lowest Level) Documented in Samples Collected from the Exposure and Reference Areas, 2016

Major Taxonomic Group	Family	Subfamily	Tribe	Genus/Species	Exposure Area – Seep Creek Ponds	Reference Area 1 – Fingers Lake	Reference Area 2 – Unnamed Ponds
				Demicryptochironomus	X	X	X
				Dicrotendipes	X	X	X
				Glyptotendipes	-	-	X
				Microtendipes	X	X	X
				Pagastiella	-	X	-
				Paracladopelma	-	X	-
				Paratendipes	X	-	-
				Polypedilum	X	X	X
				Sergentia	-	X	-
				Stictochironomus	X	X	X
			Pseudochironomini	*Pseudochironomus*	X	X	X
			Tanytarsini	*Cladotanytarsus*	X	-	X
				Constempellina	X	X	-
				Corynocera	-	X	-
				Micropsectra	-	X	X
				Paratanytarsus	X	X	X
				Tanytarsus	X	X	X
	Ceratopogonidae	Ceratopogoninae	-	*Bezzia*	X	X	X
				Ceratopogon	X	-	-
				Probezzia	X	-	X
		Dasyheleinae	-	*Dasyhelea*	-	-	X
	Empididae	-	-	*Chelifera / Metachela*	X	X	X
	Muscidae	-	-	*Limnophora*	X	-	X
	Tipulidae	-	-	*Tipula*	X	-	-
				Total Number of Taxa	**37**	**36**	**36**

i/d = immature or damaged specimen identified to the lowest level possible; X = taxon was present; - = taxon was absent, or not applicable.

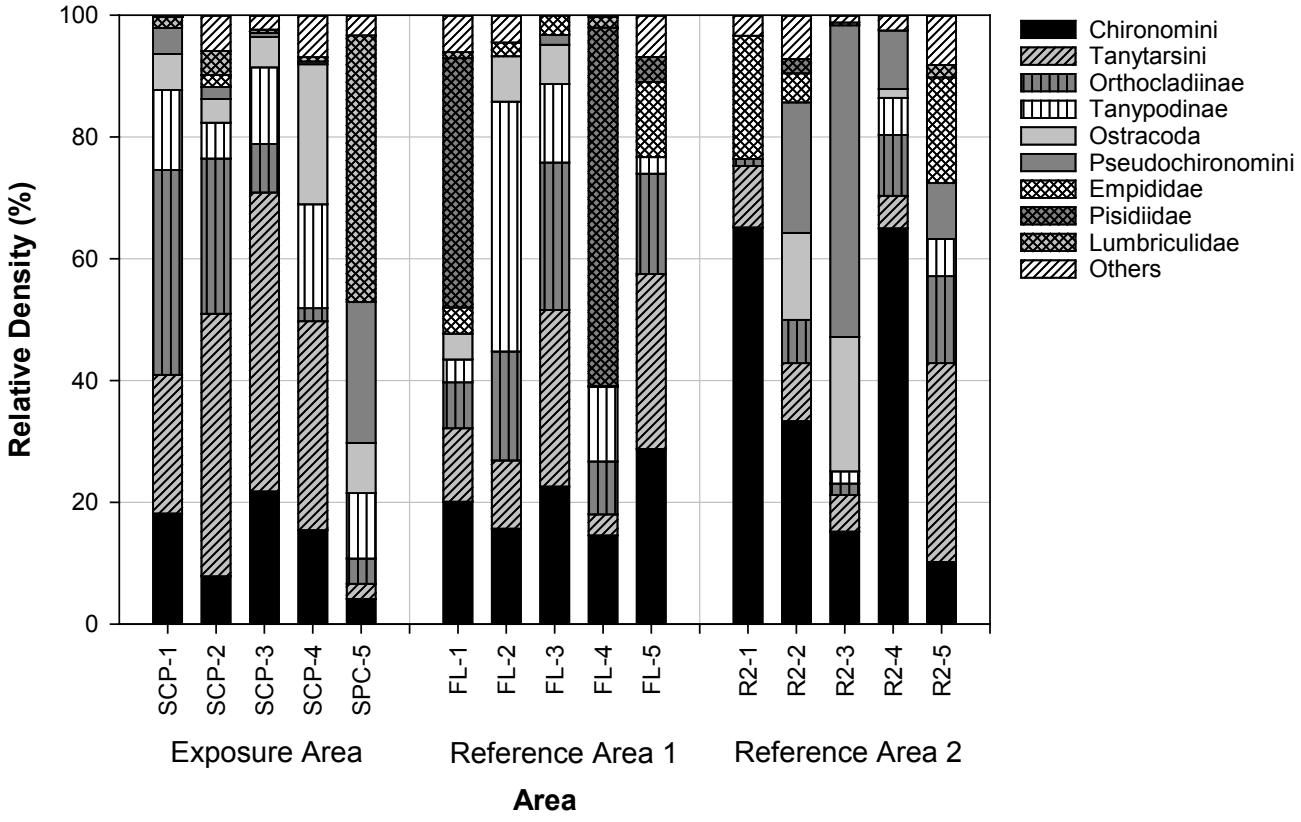
The relative densities of major taxonomic groups at the family level were similar in the Exposure and Reference Areas (Figure 5.3-4). The benthic invertebrate community in all three sampling areas consisted predominately of Chironomidae (midges), which accounted for 69% to 96% of the total density among stations, with the exception of three stations (SCP-5, FL-1, and FL-4), which were co-dominated by Chironomidae and a second major taxonomic group. Station SCP-5 was co-dominated by Chironomidae and Lumbriculidae (45% and 44%, respectively), while Stations FL-1 and FL-4 were co-dominated by Chironomidae (47% and 39%, respectively) and Pisidiidae (41% and 59%, respectively). Ostracoda was present in all three sampling areas, in relative densities up to 23%. Empididae accounted for up to 22% of the community by density in Reference Area 2 and 12% of the relative density at Station FL-5, but made up only a small fraction of the community at the other stations in Reference Area 1 and was almost entirely absent from the Exposure Area. Together, Lebertiidae, Ceratopogonidae, and the groups in the Others category made up less than 10% of the benthic community by density. At this level of evaluation, there is no obvious difference between the Exposure Area and Reference Area communities that would suggest a Mine-related effect on the benthic community.

Figure 5.3-4: Family Level Relative Density of Invertebrates at each Replicate Station, 2016



At the sub-family level, the relative density of invertebrates varied among stations and areas (Figure 5.3-5). Chironomini (collectors and gatherers) was present at all stations in the Exposure and Reference Areas, but the highest relative densities were found in Reference Area 2 (up to 65%). Together, Tanytarsini (filterers and gatherers) and Orthocladiinae (gatherers and scrapers) made up between 41% and 71% of the benthic community composition by density at Stations SCP-1 to SCP-4 in the Exposure Area. The composition at Station SCP-5 differed from the other stations in the Exposure Area. Station SCP-5 was dominated by Lumbriculidae (44%) and to a lesser extent, Pseudochironomini (23%). The dominance of benthic invertebrate sub-families varied among stations in Reference Area 1. Pisidiidae made up between 41% and 59% of the relative density at Stations FL-1 and FL-4, while Station FL-2 was dominated by the predator, Tanypodinae (41%). The community composition at Station FL-5 was similar to that of the Exposure Area, with Chironomini (29%), Tanytarsini (29%), and Orthocladiinae (16%) contributing the majority of the relative density. At the sub-family level, the benthic invertebrate community in Reference Area 2 varied among stations. Stations R2-1 and R2-4 were Chironomini-dominated (65% at both), Station R2-3 was Pseudochironomini-dominated (51%; gatherers), and Stations R2-2 and R2-5 were made up a mix of sub-families. The Others category made up less than 10% of the relative density in the Exposure and Reference Areas. Community composition at this level also provided no indication of a Mine-related effect on the benthic invertebrate community of the Exposure Area.

Figure 5.3-5: Sub-Family Level Relative Density of Invertebrates at each Replicate Station, 2016



Note: The dominant Chironomidae taxa were split up into sub-families and tribes.

5.3.4 Comparison of Phase 2 and Phase 5 EEM Statistical Analysis

The Phase 2 EEM field program was conducted in 2008, more than three years after the last treated Mine effluent discharge period. Results of the Phase 2 EEM program indicated that benthic invertebrates may have been influenced by historical Mine contamination (AECOM 2009). Although differences were detected in the benthic invertebrate community, they did not confirm the Phase 1 results and benthic invertebrates were excluded from the Phase 3 IOC study. The next benthic invertebrate study was conducted as part of the Phase 5 EEM program in 2016 (the current study). A summary of the benthic invertebrate results for the Phase 2 and Phase 5 EEM programs is provided in Table 5.3-10.

The sampling areas differed between the Phase 2 and Phase 5 EEM programs. Sampling for the Phase 2 EEM was conducted within the same sampling areas as the Phase 1 EEM program, which included the Exposure Area (Seep Creek Ponds) and one reference area (Fingers Lake). Reference Area 2 was sampled for the first time during the Phase 5 EEM program.

In Phase 2, mean invertebrate density was higher in the Exposure Area compared to the Reference Area. There was no significant difference between areas for taxonomic richness, diversity, and evenness. Total density and the dissimilarity index (BCI) were significantly higher in the Exposure Area compared to the Reference Area, indicating a potential Mine-related effect. For total density, the direction of difference between the Exposure and Reference Areas (i.e., higher in the Exposure Area) was inconsistent with an adverse effect (i.e., lower in the Exposure Area) on the benthic invertebrate community.

In Phase 5, no statistically significant differences were detected for the any of the invertebrate endpoints compared statistically among sampling areas. The magnitudes of effects were below the CES for all endpoints, suggesting that differences among areas were not biologically significant. Visual evaluation of community composition and invertebrate presence-absence also suggested no Mine-related effects on the benthic invertebrate community in the Exposure Area.

Overall, results of the Phase 2 and Phase 5 EEMs indicate a potential improvement in environmental quality in the Exposure Area, with no statistically significant results during Phase 5, compared to some effects noted in Phase 2. Results of these programs indicate that Mine effluent had a low effect (Phase 2) to no effect (Phase 5) on the benthic invertebrate community in the Exposure Area.

Table 5.3-10: Summary of Phase 2 and Phase 5 Environmental Effects Monitoring Benthic Invertebrate Community Survey Results, 2008 and 2016

Endpoint	Phase 2 EEM (2008)			Phase 5 EEM (2016)		
	Effect?[a]	Direction[b]	Magnitude >CES?	Effect?[a]	Direction[b]	Magnitude >CES?
Effect Endpoint						
Total Density	Y	EXP >REF1	-	N	n/a	N
Family Level Richness	N	n/a	-	N	n/a	N
Family Level SEI	N	n/a	-	N	n/a	N
Family Level BCI[c]	Y	EXP >REF1	-	N	n/a	N
Supporting Endpoint						
Lowest Taxonomic Level Richness	-	-	-	N	n/a	N
Family Level SDI	N	n/a	-	N	n/a	N
Chironomidae Density	-	-	-	N	n/a	N
Family Level BCI[d]	-	-	-	N	n/a	N
Family Level Community Composition[e]	All areas were dominated by Chironomidae (Orthocladiinae, Tanypodinae, Tanytarsini, and Chironomini).			All areas were dominated by Chironomidae (Chironominae, Orthocladiinae, and Tanypodinae) and to a lesser extent, Ostracoda. Diptera (Empididae) made up a noticeable proportion of the community composition in the Reference Areas, but not in the Exposure Area.		
Family Level Presence/Absence	Number of families was slightly higher in the Reference Area (18 families) compared to the Exposure Area (16 families).			Number of families was highest in the Exposure Area (15 families), followed by Reference Area 2 (12 families), and then Reference Area 1 (9 families).		
Lowest Taxonomic Level Presence/Absence	-			Total number of taxa was similar among the Exposure and References Areas (36 to 37 taxa).		

(a) Effect designations are based on statistical significance (P<0.10), as required by Environment Canada (EC 2012a) and the Metal Mining Effluent Regulations (EC 2002a).

(b) EXP >REF = Exposure Area mean greater than Reference Area mean.

(c) Bray-Curtis Index calculated using the standard EEM method using Reference Area medians (EC 2012a).

(d) Bray-Curtis Index calculated using the pairwise comparison method (Huebert et al. 2011).

(e) Community composition based on percentages of major invertebrate groups.

CES = critical effect size; SEI = Simpson's Evenness Index; BCI = Bray-Curtis Index; SDI = Simpson's Diversity Index; EXP = Exposure Area; REF1 = Reference Area 1; REF2 = Reference Area 2; P-value = probability value; - = not available; n/a = not applicable.

5.4 Summary

Supporting water quality results (Section 4.3.4) indicated the presence of residual treated effluent in the Exposure Area (effluent is no longer discharged), with decreasing concentrations of some parameters from upstream to downstream. Compared to the Reference Areas, concentrations of a number of parameters differed by more than a factor of two in the Exposure Area, including specific conductivity, TDS, chloride, sulphate, most nutrients, and most metals. In general, pH was below minimum CWQG range in every area in 2016. Cadmium, copper, nickel, zinc in the Exposure Area exceeded CWQGs in 2016, consistent with previous EEMs. In 2016, aluminum exceeded the CWQG at five out of six Reference Area stations, and copper exceeded the CWQG at one Reference Area 2 station. Since 2005, there has been a general decreasing trend in concentrations in the Exposure Area.

Sediment quality results (Section 4.3.5) indicated that arsenic, beryllium, cadmium, cobalt, copper, manganese, mercury, molybdenum, nickel, sulphur, and zinc were elevated in the Exposure Area sediments compared to the Reference Area sediments, with the exception of concentrations at SCP-2 (Exposure Area), which were similar to those measured in the Reference Areas. Arsenic concentrations were above the PEL at all sampling stations but one (i.e., SCP-2) within the Exposure Area. Copper concentrations were below the PEL at all sampling stations and below the ISQG at all but one station in the Exposure Area (i.e., SCP-5). A clear spatial pattern was not evident for any sediment parameter; however, there was a general decrease in concentrations of some parameters in sediments in the Exposure Area from upstream to downstream.

Statistical analyses of invertebrate effect endpoints identified no significant differences among areas in density, and family level richness, SEI, and BCI. The effect endpoints were higher in the Exposure Area compared to the Reference Areas, with the exception of evenness which was lower in the Exposure Area. The magnitude of the difference did not exceed the CES for any of the EEM effect endpoints, suggesting that observed differences were not biologically significant. Statistically significant differences were not identified for any supporting endpoints. Lowest taxonomic level richness was higher in the Exposure Area compared to the Reference Areas, while the opposite was true for diversity. The magnitude of the difference did not exceed the CES for either supporting endpoint, suggesting that the differences in the supporting endpoints were also not biologically significant.

Overall, the results of the Phase 5 EEM benthic invertebrate community survey indicate that Mine-related effects on the benthic invertebrate community were not present in the Exposure Area.

6.0 FISH SURVEY – PHASE 5

6.1 Introduction and Objectives

The objective of the Phase 5 EEM fish survey was to evaluate potential biological effects of effluent in the Exposure Area on fish health by assessing abundance (catch-per-unit-effort), length, weight, age distribution, energy use, and energy storage of the exposure and reference areas fish populations. Fish tissue analysis was not required because mercury concentrations in the effluent have been consistently below 0.1 µg/L (AECOM 2011; EC 2002a). The survey included a lethal and a non-lethal component, with two different species considered suitable. The survey included sampling of Arctic Grayling as occurred in Phases 1 through 3. The design also considered the MMER, and comments from the TAP review of the Phase 5 EEM Study Design (Golder 2016a, 2016c).

6.2 Methods

6.2.1 Species

Adult and juvenile Ninespine Stickleback were approved as the sentinel species for the lethal component and YOY and juvenile Arctic Grayling as the sentinel species for the non-lethal component. Arctic Grayling were used for a lethal study for the Phase 1 ad Phase 2 EEMs, and for a non-lethal study for the Phase 3 IOC. Ninespine Stickleback were used for the Phase 2 EEM lethal study.

Sample The proposed target sample sizes were 30 adult males, 30 adult females, and 30 juveniles Ninespine Stickleback, and a minimum of 100 YOY, and a maximum of 400 YOY Arctic Grayling from each of the Exposure and Reference Areas. Only fish that were not infected with cestode parasites were targeted for the lethal survey. Inclusion of these fish in data analyses could increase the variability of the data; therefore, this source of variability was removed by excluding fish infected with cestode parasites during lethal sampling using a visual, internal assessment.

6.2.2 Study Area and Sampling Size

6.2.3 Locations

The lethal and non-lethal components of the fish survey were conducted at the Exposure Area along approximately 2.1 km of Seep Creek, which began approximately 3.0 km from the base of the dam and extended to the outflow of Seep Creek Pond 1 (Figure 2.2-2). Reference Area 1 was sampled for the lethal and non-lethal components along the entire length of Fingers Creek, including one unnamed pond, and Reference Area 2 was sampled along the unnamed creek and pond identified within the Phase 5 EEM Study Design (Figure 2.2-2; Golder 2016a). The selection of sampling sites was based on the presence of habitat suitable for Ninespine Stickleback (i.e. slow moving water with associated aquatic vegetation) or Arctic Grayling (i.e., moving water with abundant cover available) and the abundance of target fish (i.e., areas where target fish were captured were visited multiple times). Sampling was conducted in areas in the shallow (wadeable) watercourse habitat, dominated by large cobbles and boulders and flowing water, as well as the non-flowing, ponded areas dominated by fines with varying amounts of aquatic vegetation. Effort was made to sample habitat in the reference areas similar to that available in the exposure area. The wadeable area sampled was not predetermined, but depended on fish abundance. Sampling continued at sites in each area until the required number of fish were captured (Table 6.2-1).

Table 6.2-1: Fish Sampling Sites at Lupin Mine, 2016

Area	Site Description	UTM Coordinates[a]				Length Sampled (m)
		Start		End		
		Easting	Northing	Easting	Northing	
Exposure Area	Watercourse	482946	7290070	481359	7290687	1,800
Reference Area 1	Watercourse	494457	7288874	496041	7291102	2,800
Reference Area 1	Pond	494922	7289046	495251	7289194	645
Reference Area 2	Watercourse	499490	7285899	495688	7287617	4,100
Reference Area 2	Pond	494945	7287556	495741	7287664	955

(a) All UTM coordinates are Zone 12, NAD 83.

UTM = Universal Transverse Mercator coordinate system; NAD = North American Datum.

6.2.4 Study Timing

The fish survey was conducted from 25 August to 11 September 2016. The timing of the Phase 5 EEM fish survey was consistent with the timing of surveys in Phase 1 through 3. Sampling typically occurred from early to mid-day (i.e., 9:00 to 14:00).

6.2.5 Fish Collection Methods

Several fishing methods were used to capture both Ninespine Stickleback and Arctic Grayling at sampling locations throughout the field program (Table 6.2-2). Fish were captured by qualified field staff, following methods detailed in the Golder's Technical Procedure TP 8.1-3, Fish Inventory Methods (Golder internal document). The following equipment was used to capture fish:

- a single panel seine net that was 10 m long by 1.0 m deep with a 5 mm Delta Knotless mesh
- galvanized steel minnow traps with 6.4 mm mesh size
- a Smith-Root backpack electrofisher (Smith-Root 12B, Smith-Root Inc., Vancouver, WA, USA) with an 11" aluminum ring (Appendix A, Photograph 29)
- a fyke net with a main opening measuring 30" diameter, wings 10' length, and with a 4 mm mesh size (Appendix A, Photograph 30)

The fishing effort was conducted according to the conditions detailed in the Fisheries and Oceans Canada (DFO) Licence to Fish for Scientific Purposes (S-16/17-1034-NU) and Animal Use Protocol (FWI-ACC-2016-000).Each fishing effort was assigned a unique number. Multiple fishing methods were used at each sampling event (Table 6.2-2).A fyke net was only used in Reference Area 2 to capture Arctic Grayling because the proposed minimum sample size for non-lethally sampled YOY Arctic Grayling (i.e., 100 fish per area) was not met after 5 days of fishing effort using minnow traps, electrofishing, and seine netting.

Table 6.2-2: Fish Survey Sampling Schedule for Lupin Mine Phase 5 Environmental Effects Monitoring Program, 2016

Date	Area Sampled	Sampling Method			
		Seine Net	Minnow Traps	Backpack Electrofisher	Fyke Net
25-Aug-16	Exposure Area	X			
26-Aug-16	Reference Area 1		X	X	
27-Aug-16	Exposure Area	X		X	
28-Aug-16	Exposure Area	X		X	
29-Aug-16	Reference Area 1	X	X	X	
30-Aug-16	Reference Area 1	X		X	
31-Aug-16	Exposure Area	X	X		
1-Sep-16	Exposure Area			X	
2-Sep-16	Reference Area 2		X	X	
3-Sep-16	Reference Area 2			X	
4-Sep-16	Reference Area 1	X	X	X	
	Reference Area 2		X		
5-Sep-16	Reference Area 1			X	
	Reference Area 2	X	X		
6-Sep-16	Exposure Area	X		X	
	Reference Area 2		X		
7-Sep-16	Reference Area 2		X	X	
8-Sep-16	Reference Area 1		X	X	
9-Sep-16	Reference Area 2			X	X
10-Sep-16	Reference Area 1		X		
	Reference Area 2				X
11-Sep-16	Reference Area 2				X

X = sampling method was used; blank = sampling method not used.

For each fishing effort, the following information was collected:

- sampling date, start and end time
- UTM coordinates for both start and end locations for electrofishing and seining, and placement locations for minnow traps and fyke nets
- general habitat description
- the distance or area sampled (electrofishing and seine netting)
- fishing effort, as electrofishing duration in seconds, fyke net in hours and minnow traps in hours
- backpack electrofishing settings (voltage [V], frequency [Hz], and pulse width [ms])
- the number of each fish species captured and observed

Captured fish were held in aerated buckets until processing (Appendix A, Photograph 31). Non-target fish were measured for length: fork length and total length for fish with a forked tail; only total length for fish without a distinguishable fork) and body weight, and live-released to the capture area. If mortalities occurred, fish were

weighed and measured before being disposed of. If the mortality was an Arctic Grayling, the otoliths were also removed. Ninespine Stickleback retained for the lethal study were held in buckets filled with ambient, well-oxygenated water and transported to the on-site laboratory where they were processed. Fish captured for each effort were kept in separate containers. The time that fish were held between capture and sacrifice was kept as short as possible, and no fish used in the lethal sampling program were held overnight.

6.2.6 Catch-per-unit-effort

Catch-per-unit-effort (CPUE) was calculated for each species captured and was summarized by area and sampling method. This calculation also provides a measure of relative abundance among sampling areas by standardizing the catch data for the Exposure and Reference areas.

6.2.7 Lethal Study – Ninespine Stickleback

Ninespine Stickleback captured for the lethal fish health assessment were assigned an individual fish identification number. Fish were measured for total length (±1 mm) using a Mastercraft electronic caliper; body weight was measured (±0.001 g) using an Acculab Vicon VIC-123 Electronic Precision Scale (capacity 120 g, readability 0.001 g). Both external and internal assessments were completed on lethally sampled Ninespine Stickleback according to methods outlined in the Phase 5 EEM Study Design (Golder 2016a) and according to the Technical Guidelines Document (EC 2012a) and Golder's Technical Procedure TP 8.16-0, Fish Health Assessment – Metals (Golder internal document; Appendix A, Photographs 32 and 33).

6.2.7.1 External Examination

Detailed observations were made of any abnormal features of the fish, such as wounds, lesions, tumours, parasites, fin fraying, or gill parasites. Following the external observations, Ninespine Stickleback were sacrificed by a sharp blow to the back of the head.

6.2.7.2 Internal Examination

A complete internal examination was completed on lethally sampled Ninespine Stickleback, immediately after the external examination was complete, according to Golder *Technical Procedure 8.16-0 Fish Health Assessment – Metals* (Golder internal document). Fish were dissected on a cutting board wrapped with a clean sheet of plastic wrap that was changed between each fish. Dissecting equipment was cleaned between each fish with phosphate-free soap, a new scalpel blade was used for each individual, and clean surgical gloves were used for each examination. The internal examination collected the following information:

- sex
- state-of-maturity (as described in Table 6.2-3)
- life stage (YOY, juvenile, adult)
- abnormalities observed in liver, spleen, gall bladder, kidney, and gonads
- liver weight (±0.001 g)
- total gonad weight (±0.001 g)parasite load
- carcass weight (±0.001 g)

- stomach fullness estimate (%) and content

Because there are no visible external differences between male and female Ninespine Stickleback in the fall, and due to the small size and post-spawn condition of the gonads, field sex and state-of-maturity were determined by microscopic examination of the whole gonad tissue. Gonads were placed into individually-labelled cryovials and preserved in 10% buffered formalin. Photographs were taken of most of the gonads and gonad samples were archived at Golder Associates, Edmonton office.

If parasites were present and were large enough to remove from the viscera, they were removed and parasite weight was recorded. Any Ninespine Stickleback found to contain a parasite of adequate size (e.g., *Ligula intestinalis*) were excluded, and did not receive a full internal examination.

6.2.7.3 Stomach Content

Ninespine Stickleback stomachs that were more than 50% full were placed into individually-labelled cryovials and preserved in 10% buffered formalin and analyzed for stomach content enumeration and taxonomic identification. Analysis were conducted by Dr. Jack Zloty (Summerland, BC). Organisms within the stomach were identified to genus using recognized taxonomic keys. Organisms that could not be identified to the desired taxonomic level were reported as “other”. The taxonomic composition within each individual stomach was determined as percentages of major invertebrate groups by abundance.

6.2.7.4 Ageing

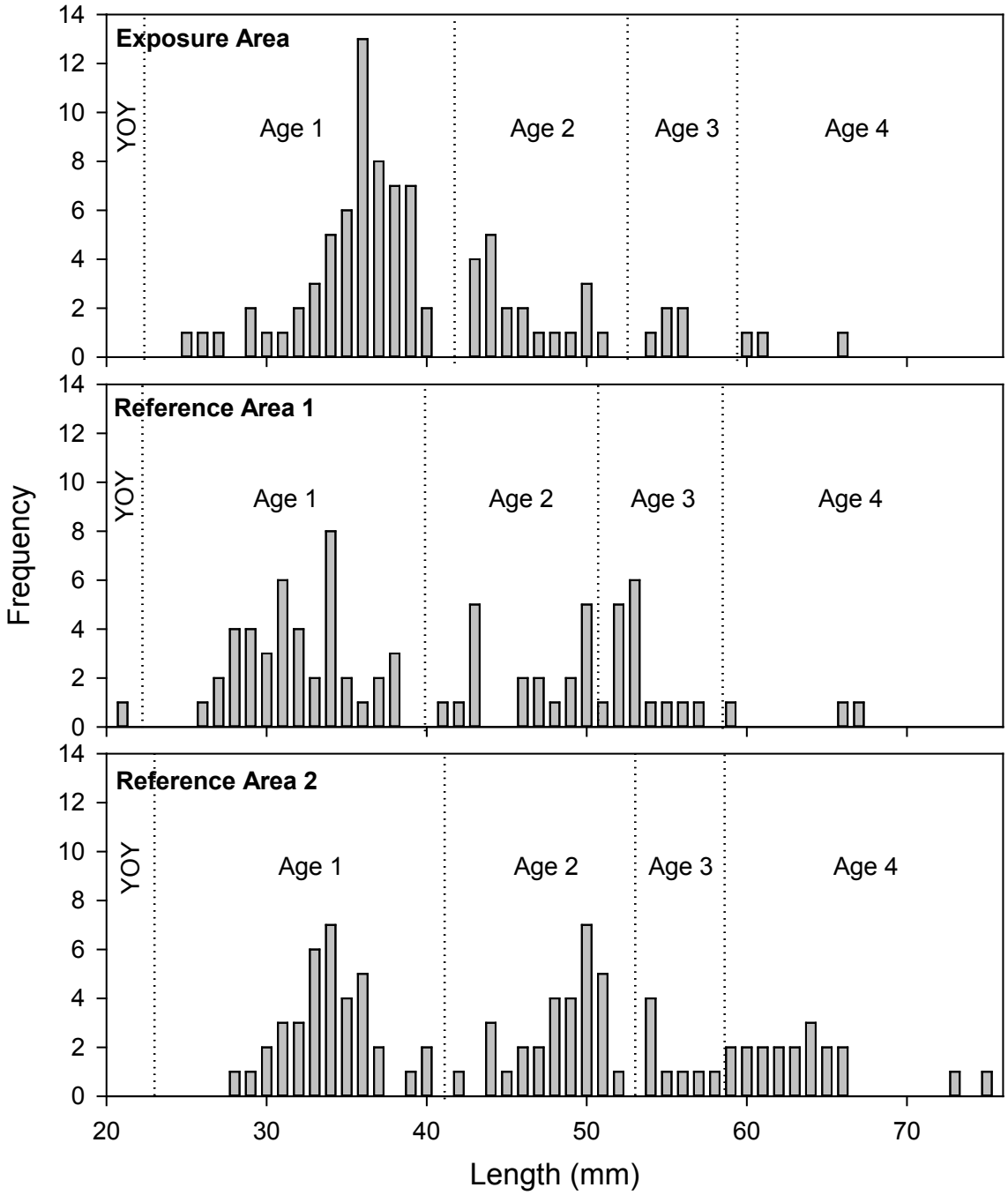
Ageing structures (i.e., sagittal otolith pairs) were collected from each lethally sampled Ninespine Stickleback using jewelers’ tweezers and a microscope; the otoliths were folded into parafilm and placed in a pre-labelled aging envelope. Aging structures were shipped to North South Consultants Inc. (Winnipeg, MB) for age determination.

A generalized determination of age was used in the field while collecting individual Ninespine Sticklebacks for the lethal study (Section 6.2.5). Based on previous EEM studies conducted by Golder, including those conducted at Con Mine, on average a fish that was less than 25 mm was determined to be a YOY, a fish that was between 25 mm and 34 mm was a juvenile, and a fish that was 35 mm or greater was determined to be an adult (Golder 2016c). There was the possibility of overlap between categories, where fish less than 35 mm in length may be adults and fish greater than 35 mm may be juveniles. These size categories were used as a general guideline used in the field when collecting individuals for the lethal study. Upon the completion of the study, ages were assigned based on length modes, with a total of four ages assigned.

After aging structure results were returned from the lab, Ninespine Stickleback otolith ageing data was confirmed to be invalidated and length frequency distributions were used to determine age categories. The length frequency distributions provided similar results for the three areas. Based on total length, Ninespine Stickleback were assigned the following cut-offs:

- Exposure Area: <23 mm = YOY; ≥23 to <41 mm = juvenile or age 1; >41 mm = adult or age 2+
- Reference Area 1: <23 mm = YOY; ≥23 to <39 mm = juvenile or age 1; >39 mm = adult or age 2+
- Reference Area 2: <23 mm = YOY; ≥23 to <40 mm = juvenile or age 1; >40 mm = adult or age 2+

Figure 6.2-1: Length Frequency Distribution and Age Assignment of Ninespine Stickleback from Exposure Area, Reference Area 1, and Reference Area 2



A secondary determination of age was conducted during the internal examination, and was based on observations of the gonads outlined in Table 6.2-3. Ninespine Stickleback of age 1+ (as determined by the length frequency modes) represented a combination of juveniles and adults, as this is the age that they reach sexual maturity.

Table 6.2-3 Gonad Maturity Categories for Male and Female Ninespine Stickleback

State of Maturity	Maturity Code	Definition
Unknown sex	0X	Unable to determine sex.
Unknown stage	10	Unable to determined stage.
Male		
Immature	21	Small testes, often clear and threadlike.
Early Stage Development	22	Small testes, semi-translucent, but easily identified.
Late Stage Development	23	Testes large, firm and lobate. White to purplish in colour. Granular appearance.
Ripe	24	Milt released with gentle pressure on abdomen.
Spent	25	Small and deflated testes. Blood vessels obvious. Violet-pink in colour.
Reabsorbing	26	Not typically observed in males.
Resting	27	Small testes, often threadlike.
Female		
Immature	11	Small ovaries, often clear, blood vessels indistinct.
Early Stage Development	12	Enlarging ovaries, blood vessels more distinct. Granular in appearance.
Late Stage Development	13	Large ovaries filling the body cavity, prominent blood vessels. Individual oocytes visible.
Ripe	14	Eggs released with gentle pressure on abdomen.
Spent	15	Deflated ovaries, blood vessels prominent.
Reabsorbing	16	Small atretic oocytes throughout the ovaries, which are hard and white.
Resting	17	Small ovaries, blood vessels reduced but present.

X = the stage code, if stage can be determined, when sex is unknown;

6.2.8 Non-lethal Study – Arctic Grayling

For the non-lethal study, captured Arctic Grayling were assigned an individual fish identification number, measured for total and fork length (±1 mm) and body weight (±0.001 g). Fish lengths were measured using an appropriate graduated measuring board; body weight was measured using an Ohaus Scout Pro S*P*-123 electronic scale (capacity 120 g, readability 0.001 g) and an Ohaus CS200 compact scale (capacity 200 g, readability 0.1 g).

6.2.8.1 External Examination

An external health assessment was completed according to methods outlined in the Phase 5 EEM Study Design (Golder 2016a) and according to the Technical Guidelines Document (EC 2012; Appendix A, Photograph 33). Individual Arctic Grayling that underwent an external examination were given a unique fish identification number. Detailed observations of the fish were conducted and included analysis of the eyes, gills, pseudobranchs, thymus, skin, fins and opercula. Notes were made of any abnormal features (i.e., wounds, lesions, tumours, parasites, fin

fraying, or gill parasites). Handling times were kept as minimal as possible, and all fish caught for the non-lethal study were released back into the area where they were captured.

6.2.8.2 Ageing

Scales were collected from immediately above the pectoral fin along the lateral line system from all released Arctic Grayling in the non-lethal study. A subset of 10 YOY Arctic Grayling were sacrificed and the sagittal otoliths removed to be analysed and used to validate the use of scales as the primary ageing structure.

In 2016, the target size ranges for YOY and fish aged 1+ years were based on previous Lupin Mine EEM results (AECOM 2009, 2011) and the size at outmigration of the Baker Creek YOY grayling population in Yellowknife (Golder 2010b). All size categories from these previous studies were verified using age structures. The size differentiation of YOY and juvenile fish was at a fork length of 70 mm.

6.2.9 Data Analyses

Fish health data collected in 2016 were analyzed to determine whether or not significant differences in effect endpoints occurred between the Exposure Area and two reference areas (i.e., Reference Area 1 and Reference Area 2). Data were entered into the Excel Spreadsheet Template downloaded from the Environment Canada website (EC 2017), and were uploaded to Environment Canada when completed. The data were independently reviewed for quality control purposes prior to submission to Environment Canada.

6.2.9.1 Data Screening

Prior to analysis, fish health data were plotted as box plots and scatterplots to visually examine data for potential data entry errors or unusual data. Outliers detected during this qualitative screening were removed from the dataset if errors resulted from sampling or measurement errors. These were checked against field data sheets as part of the screening process, prior to removal from the dataset.

6.2.9.2 Descriptive Statistics

Descriptive statistics were calculated for lethally-sampled Ninespine Stickleback by location (i.e., exposure and reference areas) and sex (i.e., male, female, and juvenile). Sample size, median, arithmetic mean, standard deviation (SD), standard error (SE), and minimum and maximum values were calculated for fish age, body weight, total length, condition factor, liver weight, liversomatic index (LSI), gonad weight, and gonadosomatic index (GSI). Fish health indices were calculated as follows:

condition factor (K) $$K = \left(\frac{body\ weight}{total\ length^3}\right) \times 100{,}000; \quad \text{[Equation 6.1]}$$

gonadosomatic index (GSI) $$GSI = \left(\frac{gonad\ weight}{body\ weight}\right) \times 100; \quad \text{[Equation 6.2]}$$

liversomatic index (LSI) $$LSI = \left(\frac{liver\ weight}{body\ weight}\right) \times 100; \quad \text{[Equation 6.3]}$$

where body weight represented either total body weight or carcass weight (i.e., the weight of the fish with all organs removed). Weights and total length measurements were reported in grams and millimetres, respectively.

Descriptive statistics were calculated for non-lethally sampled Arctic Grayling by location (i.e., exposure and reference areas) and life stage (young-of-the-year, age 1+ and all fish combined). Sample size, median, arithmetic

mean, standard deviation (SD), standard error (SE), and minimum and maximum values were calculated for fish age, body weight, total length, and condition factor.

6.2.9.3 Statistical Analysis

Statistical comparisons were conducted among the exposure and reference areas for effect endpoints presented in Tables 6.2-4 and 6.2-5. Differences in age, total body weight, carcass weight and total length were compared among areas using analysis of variance (ANOVA). Differences in size-at-age, condition, relative gonad weight, and relative liver weight among areas were assessed using analysis of covariance (ANCOVA). If significant differences were detected among areas, differences between areas were compared using Tukey's Test (Sokal and Rohlf 1995).

The difference in length-frequency distributions between areas was assessed using the non-parametric, two-sample Kolmogorov-Smirnov (K-S) test. The K-S test compares the cumulative distributions of total length between areas by comparing the maximum difference between the two cumulative frequency distributions to a critical value, which is determined by the sample sizes, to assess whether the difference is large enough to indicate that the two distributions came from different populations. The calculation of descriptive statistics and statistical analyses were conducted using Systat 13.1 (SYSTAT 2009). A significance level (α) of 0.1 was used for assessing differences between areas.

Table 6.2-4: Statistical Procedures Used in the Lethal Ninespine Stickleback Survey for Identifying Differences Between the Exposure and Reference Areas

Indicator	Effect Endpoint	Response Variable (Y)	Covariate (X)	Statistical Procedure
Survival	Length frequency distribution	n/a	n/a	K-S test
	Age	Age	n/a	ANOVA
Growth (energy use)	Body weight	Body weight	n/a	ANOVA
	Weight-at-age 1+	Body weight	n/a	ANOVA
	Weight-at-age 2+	Body weight	n/a	ANOVA
	Weight-at-age 3+	Body weight	n/a	ANOVA
	Total length	Total length	n/a	ANOVA
	Length-at-age 1+	Total length	n/a	ANOVA
	Length-at-age 2+	Total length	n/a	ANOVA
	Length-at-age 3+	Total length	n/a	ANOVA
Reproduction (energy use)	Relative gonad size	Gonad weight	Body weight	ANCOVA
Condition (energy storage)	Condition	Body weight	Total length	ANCOVA
	Relative liver size	Liver weight	Body weight	ANCOVA

Note: Body weight represents either total body weight or carcass weight.

n/a = not applicable; K-S test = Kolmogorov-Smirnov test; ANOVA = analysis of variance; ANCOVA = analysis of covariance.

Table 6.2-5: Statistical Procedures Used in the Non-lethal Arctic Grayling Survey for Identifying Differences Between the Exposure and Reference Areas

Indicator	Effect Endpoint	Response Variable (Y)	Covariate (X)	Statistical Procedure
Survival	Length frequency distribution	n/a	n/a	K-S test
Growth (energy use)	Body weight (YOY)	Body weight	n/a	ANOVA
	Total length (YOY)	Total length	n/a	ANOVA
	Body weight 1+	Body weight	n/a	ANOVA
	Total length 1+	Total length	n/a	ANOVA
Reproduction (energy use)	Relative abundance of YOY[(a)]	n/a	n/a	K-S test
Condition (energy storage)	Condition	Body weight	Total length	ANCOVA

(a) Relative abundance of YOY tested by comparing length-frequency distributions among areas with and without YOY included.

YOY = young-of-the-year; n/a = not applicable; K-S test = Kolmogorov-Smirnov test; ANOVA = analysis of variance; ANCOVA = analysis of covariance.

6.2.9.4 Testing Assumption for Statistical Analysis

The assumptions of ANOVA and ANCOVA are that the residuals of the data after being fit to the model are normally distributed and have equal variance between groups. The assumption of normality was assessed using the Shapiro-Wilk test with an α of 0.05. Levene's test was used to assess equality of variances between areas with an of 0.05. If the assumption of normality and equality of variance were not met, the data were $\log_{10}$-transformed and the assumptions were re-assessed. If assumptions were not met using $\log_{10}$-transformed data, the non-parametric Kruskal-Wallis (K-W) test was used instead of ANOVA and the non-parametric rank ANCOVA was used instead of ANCOVA (Barrett 2011).

An additional assumption of the ANCOVA is the homogeneity of regression slopes. This was evaluated by first fitting separate regression models for each area using a general linear model that included an interaction term between the area and covariate:

full ANCOVA model $$y = \beta_0 + \beta_1(x) + \beta_2(Area) + \beta_3(x) \times (Area) + \varepsilon$$ [Equation 6.4]

where y is the response variable, x is the covariate, $Area$ is an indicator variable, and ε is the error term. If the coefficient β_3 of the $(x) \times (Area)$ interaction term was not significant (α = 0.05), then the slopes were considered parallel and the ANCOVA proceeded by testing the significance of the coefficient β_2 of the $(Area)$ term in the reduced ANCOVA model that fits separate regressions for each area, but with a common regression slope:

reduced ANCOVA model $$y = \beta_0 + \beta_1(x) + \beta_2(Lake) + \varepsilon.$$ [Equation 6.5]

When a significant interaction was observed, the regression slopes were considered significantly different. When the covariate is a strong predictor of the response variable, and the ANCOVA has a high (>0.8) coefficient of determination (r^2), the test for parallel slopes has high power to detect a difference that may not be practically significant. In this case, when the interaction term in the full ANCOVA model was significant, the slopes were forced parallel by fitting the reduced ANCOVA model because the reduced model explained almost as much (within 2%) of the variability in the response variable as the full model. In this case, the ANCOVA proceeded under the assumption that the regression slopes between groups were practically the same (Barrett et al. 2010).

Statistical outliers were evaluated using studentized residuals (SR) from the ANOVA and ANCOVA models. A magnitude of 3.0 for the SR was used to identify observations that may be unusual. When an outlier was detected, the validity of the data point was examined. If the outlier was determined to be the result of a data entry error, it was corrected; if the outlier was not the result of data entry errors and could not be resolved otherwise, the analysis was completed with and without the outlying data point(s).

6.2.9.5 Magnitude of Difference

The magnitude of differences in effect endpoints between the exposure and reference areas were calculated by expressing the difference as a percentage of the mean of the reference areas as follows:

Magnitude $$Magnitude = \frac{\bar{x}_{Exposure} - \bar{x}_{Reference}}{\bar{x}_{Reference}} * 100$$ [Equation 6.7]

where $\bar{x}$ is the mean of the exposure area and reference area effect endpoints.

If the statistical comparison was conducted on $\log_{10}$-transformed data, then the percent difference was calculated using back transformed means. For effect endpoints analyzed using ANCOVA, least squares means were calculated on the $\log_{10}$-transformed scale and back-transformed before calculating the percent difference (i.e., equivalent to a geometric mean). When comparisons were made among reference areas, the relative percent difference (RPD) was calculated instead, using the following formula:

Relative Percent Difference $$RPD = \frac{\bar{x}_{Reference\ Area\ 1} - \bar{x}_{Reference\ Area\ 2}}{\bar{x}_{Reference}} * 100$$

[Equation 6.8]

where $\bar{x}$Reference Area 1 and $\bar{x}$Reference Area 2 are the means of the individual reference areas, and $\bar{x}$Reference is the mean of both reference areas. The percent difference in length-frequency distributions between areas was defined as the maximum percent difference between the two cumulative relative frequency distributions. Magnitude was only calculated for effect endpoints that were significantly different between areas. If two areas were not significantly different from each other, the magnitude was presented as zero (i.e., no difference).

6.2.9.6 Power Analysis

In cases where significant differences were not observed, power analyses were performed to determine the power and sample size requirements to detect a magnitude of change that is equivalent to the critical effect sizes (CES) defined in the *Metal Mining Technical Guidance for Environmental Effects Monitoring* (Munkittrick et al. 2009; EC 2012a). The CES defined for condition is a 10% change in the mean relative to reference, and a 25% change in the mean relative to reference for weight at age, relative gonad size, relative liver size and age. Type I (α) and Type II (β) error rates were set to 0.1.

For endpoints where data were $\log_{10}$-transformed, the effect size was transformed to reflect a 25% or 10% change in the untransformed means as outlined in Munkittrick et. al. (2010). This is because the effect sizes on a $\log_{10}$-transformed scale are different to detect an increase versus a decrease in the reference mean. The impact hypotheses include both increases and decreases in the key fish health effect endpoints, so the power analyses

were performed to detect both possibilities. Power analyses were conducted using the power and sample size function in G*Power 3.1 (Faul et al. 2007).

6.2.10 Quality Assurance and Quality Control

As part of practices for field operations for this program, the following QA/QC procedures were undertaken:

- Detailed specific work instructions outlining each field task were provided to the field personnel prior to the field program.
- A pre-field meeting with the field crew and team lead was conducted to review the specific work instructions so that procedures were understood.
- Samples were collected by experienced personnel and were collected, labelled, preserved and shipped according to laboratory instructions and Golder Technical Procedures (Golder internal document).
- Field equipment (i.e., electronic scales, water quality meter) was regularly calibrated according to manufacturer's recommendations.
- Detailed field notes were recorded in pencil in waterproof field notebooks, on waterproof pre-printed field data sheets, or directly entered electronically into an excel spreadsheet.
- Field data (i.e., datasheets, notebook, and electronic spreadsheets) were checked at the end of the day for completeness and accuracy.
- Samples were documented and tracked using chain-of-custody forms, and receipt of samples by the analytical laboratory was confirmed. Field crews were responsible for managing sample shipping to the analytical laboratory. Prior to sample shipping, field crews confirmed the following:
  - required samples were collected and accounted for
  - chain-of-custody and analytical request forms were completed and correct
  - proper bottle labelling and documentation procedures were followed

Field-collected data, datasheets, and the field notebook were reviewed for completeness and unexpected values and trends.

Upon receipt of stomach content data, a check was undertaken to determine if each sample submitted was analyzed and that the sum of percentages of taxon composition within each individual stomach was 100%.

A minimum of 10% of the ageing structures re-analyzed by a different North/South Consultants technician to determine variability between age assessments. Upon receipt of ageing data from North/South consultants, the internal laboratory QA/QC results were reviewed and age results were plotted against fish lengths to screen out any outliers.

A subset of 10 Arctic Grayling were sacrificed and the sagittal otoliths removed to be analysed and used to validate the use of scales as the primary ageing structure. All aging structures were placed into individually labelled envelopes, sealed and submitted to North/South Consultants (Winnipeg, MB) for age determination.

At least 10% of the data entered electronically were verified by a second person to identify transcription errors. Results of statistical data analyses were reviewed by an independent biologist with appropriate technical

qualifications. Tables containing data summaries and statistical results were reviewed and values were verified by a second, independent individual.

6.3 Results and Discussion

6.3.1 Fish Catch

A total of 1,418 fish were captured during the 2016 fish survey: 497 in the Exposure Area, 535 in Reference Area 1 and 385 fish were captured in Reference Area 2 (Table 6.3-1; Appendix E, Tables E-1 to E-7). Arctic Char, Arctic Grayling, Burbot and Ninespine Stickleback were captured at the Exposure Area and Reference Area 1 and 2. In addition, Lake Trout, Round Whitefish and Slimy Sculpin were captured at both Reference Areas. Raw catch data, including fish lengths and weights, are provided in Appendix E, Table E-7.

Table 6.3-1: Total Number and Species of Fish Captured in Exposure Area, Reference Area 1, and Reference Area 2, 2016

Common Name	Latin Name	Exposure Area			Reference Area 1			Reference Area 2		
		# of Fish Captured	# of Fish Processed[a]	# of Fish Analyzed[b]	# of Fish Captured	# of Fish Processed[a]	# of Fish Analyzed[b]	# of Fish Captured	# of Fish Processed[a]	# of Fish Analyzed[b]
Ninespine Stickleback	*Pungitius pungitius*	252	88	86	250	81	78	235	94	93
Arctic Grayling	*Thymallus arcticus*	136	136	126	219	219	176	12	12	0
Arctic Char	*Salnelinus alpinus*	2	0	0	2	0	0	12	0	0
Burbot	*Lota lota*	1	0	0	49	0	0	13	0	0
Lake Trout	*S. namaycush*	0	0	0	5	0	0	12	0	0
Round Whitefish	*Prosopium cylidraceum*	0	0	0	1	0	0	2	0	0
Slimy Sculpin [c]	*Cottus cognatus*	0	0	0	7	0	0	5	0	0
Total		**391**	**224**	**212**	**533**	**300**	**254**	**291**	**106**	**93**

(a) Includes only the fish processed for lethal and non-lethal program. All fish captured were measured for length and/or weight (Appendix C).

(b) Total number of processed fish used in statistical analysis (after removal of outliers).

(c) Sculpins captured were assumed to be Slimy based on field observations.

For the lethal component of the fish survey, a total of 358 Ninespine Stickleback were captured in the Exposure Area, 252 in Reference Area 1 and 329 in Reference Area 2 (Table 6.3-1; Appendix E, Tables E-1 to E-3). The length frequency distributions for each area exhibited similar modal distributions (Figure 6.2-1). Ninespine Stickleback are typically considered sexually mature at age 2 (Jones and Hynes (1950) and Griswold and Smith (1973)). As mentioned in Section 6.2.7.4, these values were used as a general baseline for age determination, with gonad observations during the internal examination taken into account as well when determining maturity in individual fish. These combined factors altered the realized sample sizes collected during this study (Table 6.3-2).

For the non-lethal component of the fish survey, a total of 136 Arctic Grayling were captured in the Exposure Area, 219 were captured in Reference Area 1 and 12 were captured in Reference Area 2 (Table 6.3-1; Appendix E, Tables E-4 to E-6). Captured Arctic Grayling were measured for length and weight. As stated in Section 6.2.8.2, based on previous EEM work conducted at Lupin Mine and studies conducted at Giant Mine in Baker Creek, the size differentiation of YOY to juvenile fish was at a fork length of 70 mm. However, based on the results of the analysis of aging structures, the largest YOY caught had a fork length of 90 mm. When using the lab aging data to determine life history, 95% of the Arctic Grayling captured in the Exposure Area were YOY and 84% captured in Reference Area 1 were YOY. None of the fish captured in Reference Area 2 were YOY. The number of scales and otoliths collected during the non-lethal survey for laboratory analysis are detailed in Table 6.3-2.

Table 6.3-2: Lethal and Non-Lethal Survey Structure Collection – Target Sample Sizes versus Actual Sample Sizes, 2016

Area	NNST Fish Health (lethal survey)	ARGR Fish Health (non-lethal survey)	ARGR Aging Structures (SC)	ARGR Aging Structures (OT)	NNST Aging Structures (OT)		Gonad Archive	Stomach Contents
Exposure Area								
Target[a]	**90**	**Minimum of 100 YOY**	**≥ 100**	**10**	**90**		**90**	**Any that were >50% full**
	M = 30				**NNST**	M = 30	M = 30	
	F = 30					F = 30	F = 30	
	J = 30					J=30	J = 30	
Total Collected[a]	**88**	**136**	**136**	**9**	**88**		**88**	**24**
	M = 32				**NNST**	M = 32	M = 32	M = 15
	F = 29	**YOY = 126**				F = 29	F = 29	F = 8
	J = 27	**Age 1+ = 6**				J = 27	J = 27	J = 1
Total Analyzed[b]	**86**	**132**	**132**	**9**	**86**		**No Analysis**	**24**
	M = 32				**NNST**	M = 32		M = 15
	F = 29	**YOY = 126**				F = 29		F = 8
	J = 25	**Age 1+ = 6**				J = 25		J = 1
Reference Area 1								
Target[a]	**90**	**Minimum of 100 YOY**	**≥ 100**	**10**	**90**		**90**	**Any that were >50% full**
	M = 30				**NNST**	M = 30	M = 30	
	F = 30					F = 30	F = 30	
	J = 30					J=30	J = 30	
Total Collected[a]	**80**	**219**	**219**	**4**	**80**		**80**	**26**
	M = 19				**NNST**	M = 19	M = 19	M = 5
	F = 31	**YOY = 186**				F = 31	F = 31	F = 8
	J = 30	**Age 1+ = 33**				J = 30	J = 30	J = 13
Total Analyzed[b]	**78**	**209**	**209**	**4**	**78**		**No Analysis**	**26**
	M = 17				**NNST**	M = 17		M = 5
	F = 31	**YOY = 176**				F = 31		F = 8
	J = 30	**Age 1+ = 33**				J = 30		J = 13

Table 6.3-2: Lethal and Non-Lethal Survey Structure Collection – Target Sample Sizes versus Actual Sample Sizes, 2016

Area	NNST Fish Health (lethal survey)	ARGR Fish Health (non-lethal survey)	ARGR Aging Structures (SC)	ARGR Aging Structures (OT)	NNST Aging Structures (OT)		Gonad Archive	Stomach Contents
Reference Area 2								
Target[(a)]	**90**	**Minimum of 100 YOY**	**≥ 100**	**10**	**90**		**90**	**Any that were >50% full**
	M = 30				**NNST**	M = 30	M = 30	
	F = 30					F = 30	F = 30	
	J = 30					J=30	J = 30	
Total Collected[(a)]	**94**	**12**	**12**	**0**	**94**		**94**	**37**
	M = 33				**NNST**	M = 33	M = 33	M = 15
	F = 31	**YOY = 0**				F = 31	F = 31	F = 13
	J = 30	**Age 1+ = 12**				J = 30	J = 30	J = 9
Total Analyzed[(b)]	**93**	**136**	**12**	**0**	**93**		**No Analysis**	**37**
	M = 33				**NNST**	M = 33		M = 15
	F = 31	**YOY = 0**				F = 31		F = 13
	J = 29	**Age 1+ = 12**				J = 29		J = 9

(a) Target size ranges were collected based on proposed numbers found in the Lupin Mine Phase 5 EEM Study Design (Golder 2016).

(b) total analyzed for non-lethal survey included only individuals that had both accurate length and weight collected.

n/a = not applicable; A = adult; M = male; F = female; J = juvenile; YOY = young-of-the-year; > = greater than; ≥ greater than or equal to.

6.3.2 Catch-Per-Unit-Effort and Species Richness

Relative fish abundance (standardized as CPUE) was higher in the Exposure Area than in Reference Area 1 and Reference Area 2, for each fishing method used (Table 6.3-3). At both the Exposure Area and Reference Area 1, a larger number of fish were collected with the backpack electrofisher than with seine nets and minnow traps, while at Reference Area 2, fyke nets captured the most fish. In the Exposure Area and Reference Area 2, CPUE was highest for Ninespine Stickleback (Table 6.3-3). In Reference Area 1, CPUE was highest for Arctic Grayling (Table 6.3-3). Overall, Ninespine Stickleback and Arctic Grayling were the most abundant species at the Exposure Area and Reference Area 1. The most abundant species at Reference Area 2 were Ninespine Stickleback, with Arctic Grayling, Arctic Char and Lake Trout tied for being next most abundant (Table 6.3-3).

Table 6.3-3: Catch-per-unit-effort for Fish Species from Exposure Area, Reference Area 1 and Reference Area 2, 2016

Site	Backpack Electrofishing				Seine				Minnow Traps				Fyke Net			
	Effort (s)	Species	# of Fish Caputred	CPUE (# fish/100s)	Effort (m^2)	Species	# of Fish Caputred	CPUE (# fish/m^2)	Effort (h)	Species	# of Fish Caputred	CPUE (# fish/h)	Effort (h)	Species	# of Fish Caputred	CPUE (# fish/h)
Exposure Area	5,481	NNST	182	3.32	795	NNST	163	0.21	29.5	NNST	13	0.44	Not Used in Exposure Area			
		ARGR	127	2.32		ARGR	3	0.004		ARGR	6	0.20				
		ARCH	2	0.04		ARCH	0	0		ARCH	0	0				
		BURB	1	0.02		BURB	0	0		BURB	0	0				
		LKTR	0	0		LKTR	0	0		LKTR	0	0				
		RNWH	0	0		RNWH	0	0		RNWH	0	0				
		SLSC	0	0		SLSC	0	0		SLSC	0	0				
		TOTAL	**312**	**5.69**		**TOTAL**	**166**	**0.21**		**TOTAL**	**19**	**0.64**				
Reference Area 1	8,239	NNST	177	2.15	720	NNST	47	0.07	1,135	NNST	28	0.02	Not Used in Reference Area 1			
		ARGR	212	2.57		ARGR	0	0		ARGR	7	0.01				
		ARCH	2	0.02		ARCH	0	0		ARCH	0	0				
		BURB	33	0.40		BURB	0	0		BURB	16	0.01				
		LKTR	4	0.05		LKTR	0	0		LKTR	1	0.00				
		RNWH	1	0.01		RNWH	0	0		RNWH	0	0				
		SLSC	4	0.05		SLSC	0	0		SLSC	3	0.00				
		TOTAL	**433**	**5.26**		**TOTAL**	**47**	**0.07**		**TOTAL**	**55**	**0.05**				
Reference Area 2	5,095	NNST	72	1.41	180	NNST	0	0	775.0	NNST	68	0.09	48.5	NNST	189	3.90
		ARGR	12	0.24		ARGR	0	0		ARGR	0	0		ARGR	0	0
		ARCH	12	0.24		ARCH	0	0		ARCH	0	0		ARCH	0	0
		BURB	3	0.06		BURB	0	0		BURB	6	0.01		BURB	4	0.08
		LKTR	12	0.24		LKTR	0	0		LKTR	0	0		LKTR	0	0
		RNWH	0	0.00		RNWH	0	0		RNWH	0	0		RNWH	2	0.04
		SLSC	5	0.10		SLSC	0	0		SLSC	0	0		SLSC	0	0
		TOTAL	**116**	**2.28**		**TOTAL**	**0**	**0.00**		**TOTAL**	**74**	**0.10**		**TOTAL**	**195**	**4.02**

CPUE = catch-per-unit-effort; m^2 = square metre; #fish/m^2 = number of fish per square metre; spp. = species.

The total number of fish captured at the exposure and reference areas was greater in 2016 when compared to previous years (i.e., Phase 1 to 3 EEMs) (Table 6.3-4). Ninespine Stickleback have consistently been the dominant species in the Exposure Area and Reference Area 2. Both Arctic Grayling and Ninespine Stickleback were the dominant fish species captured at Reference Area 1 in 2016, where the dominant species have varied in previous years (i.e., Arctic Grayling in 2005, Ninespine Stickeback and Burbot in 2008, Burbot and Arctic Grayling in 2010. (Table 6.3-4).

Species richness in the Exposure Area was the highest in 2008, although only one individual of each of the five non-dominant species was captured during that sampling program (i.e., Arctic Char, Burbot, Lake Trout, Round Whitefish, Slimy Sculpin). In 2005, 2010 and 2016, only three to four species were captured (Table 6.3-4) in the Exposure Area. In Reference Area 1, Arctic Char was collected for the first time in 2016; however, Arctic Char were captured in Fingers Lake over 30 years ago (Moore 1978). The same seven species were consistently captured in Reference Area 2 since 2005.

Table 6.3-4: Total Fish Captured per Area – 2005, 2008, 2010 and 2016

Common Name	Exposure Area				Reference Area 1				Reference Area 2			
	2005[a]	2008[b]	2010[c]	2016	2005[a]	2008[b]	2010[c]	2016	2005[a]	2008[b]	2010[c]	2016
Ninespine Stickleback	99	130	193	358	1	96	16	252	n/a	n/a	158	329
Arctic Grayling	86	112	28	136	52	53	25	219	n/a	n/a	33	12
Arctic Char	1	1	0	2	0	0	0	2	n/a	n/a	11	12
Burbot	0	1	2	1	7	60	30	49	n/a	n/a	4	13
Lake Trout	2	1	0	0	2	30	15	5	n/a	n/a	22	12
Round Whitefish	0	1	0	0	10	15	2	1	n/a	n/a	7	2
Slimy Sculpin	0	1	0	0	2	41	73	7	n/a	n/a	66	5
Total	**188**	**247**	**223**	**497**	**74**	**295**	**161**	**535**	**n/a**	**n/a**	**301**	**385**

(a) Golder 2004

(b) AECOM 2009

(c) AECOM 2011

In 2016, the fish survey used different methods than previous years. Seine nets were used for the first time in 2016, gill nets were not used as they had been in previous years and a fyke net was used in Reference Area 2. (Table 6.3-5). Minnow traps and electrofishing have been used in multiple years, with the most effort in 2008, but the highest CPUE in 2016 (Table 6.3-5). Reference Area 1 required more total effort than the Exposure Area or Reference Area 2 to approach the target sample size; this is consistent with programs conducted in previous years. The CPUE in the Exposure Area, Reference Area 1 and Reference Area 2 for backpack electrofishing was the highest when compared with other techniques, indicating that it was the most effective fishing method. (Table 6.3-5).

Table 6.3-5: Total Catch-per-unit-effort per Site – 2005, 2008, 2010 and 2016

Area	Year	Effort						Number of Fish Captured	CPUE						Species Richness[a]
		Seine (m^2)	Gill Net (h)	Hoop Net (h)	Fyke Net (h)	Minnow Traps (h)	Backpack Electrofisher (s)		Seine (fish/m^2)	Gill Net (fish/h)	Hoop Net (fish/h)	Fyke Net (fish/h)	Minnow Trap (fish/h)	Backpack Electrofisher (fish/100s)	
Exposure Area	2005	n/a	102	n/a	91	589	2,935	188	n/a	0.47	n/a	0.14	0.15	1.26	4
	2008	n/a	819	n/a	n/a	681	28,656	247	n/a	0.05	n/a	n/a	0.007	0.7	7
	2010	2 hours 52 minutes 20 seconds[(b)]						223	78 fish/hour[(b)]						3
	2016	795	n/a	n/a	n/a	30	5,481	497	0.21	n/a	n/a	n/a	0.64	5.69	4
Reference Area 1	2005	n/a	4	189	n/a	760	5,044	74	n/a	2.75	0.02	n/a	0.003	1.07	6
	2008	n/a	1,031	n/a	n/a	2,140	42,372	295	n/a	0.027	n/a	n/a	0.01	0.55	6
	2010	4 hours 25 minutes 45 seconds[(b)]						161	36 fish/hour[(b)]						6
	2016	720	n/a	n/a	n/a	1,135	8,239	535	0.07	n/a	n/a	n/a	0.05	5.26	7
Reference Area 2	2008	2 hours 52 minutes 245 seconds[(b)]						301	104 fish/hour[(b)]						7
	2016	180			49	775	5,095	385	0	n/a	n/a	4.02	0.1	2.28	7

(a) Species richness is the total number of fish species captured.

(b) Effort was not separated by method in 2010 report, but was presented as a total for all efforts.

CPUE = catch-per-unit-effort; m2 = square metre; h = hour; fish/m2 = fish captured per square metre; fish/h = fish captured per hour; fish/100s = fish captured per 100 seconds.

6.3.3 Fish Health Assessment

6.3.3.1 Sample Size

A total of 263 Ninespine Stickleback and 367 Arctic Grayling were processed during the 2016 fish survey. For the lethal survey, target sample sizes (i.e., 30 adult male, 30 adult female and 30 juvenile fish from each area) were achieved for male fish at the Exposure Area, and Reference Area 2, for female fish at Reference Area 1 and Reference Area 2, and for juvenile fish at Reference Area 1 (Table 6.3-6). Samples sizes meet EEM minimum sample size requirements for lethal sampling, with the exception of male fish from Reference Area 1 (i.e., 20 adult male, 20 adult female fish from each area; EC 2012a).

For the non-lethal survey, Arctic Grayling YOY target samples sizes were achieved at the Exposure Area and Reference Area 1; however, no YOY were captured at Reference Area 2.

Table 6.3-6: Target and Achieved Sample Sizes for the Lethal and Non-lethal Fish Surveys, 2016

Survey	Species	Life Stage	Sex	Target n	Achieved n		
					EXP	REF1	REF2
Lethal	Ninespine Stickleback	Adult[(a)]	Male	30	32	19	33
			Female	30	29	31	31
		Juvenile	-	30	27	30	29
		YOY		n/a	0	1	0
		Total			88	81	93
Non-lethal	Arctic Grayling	YOY	-	100	130	186	0
		Juvenile		n/a	6	33	12
		Total			136	219	12

a) One adult Ninespine Stickleback of unknown sex excluded from table.

n = sample size; EXP = Exposure Area; REF1 = Reference Area 1; REF2 = Reference Area 2; YOY = young-of-the-year; n/a = not applicable.

6.3.3.2 Lethal Survey – Ninespine Stickleback

A total of 263 Ninespine Stickleback were processed from the study area in September 2016. Of these, 38 fish exhibited external abnormalities (14.4%), primarily consisting of pale gills and mild forms of fin erosion (Table 6.3-6). The most severe external abnormalities observed included the loss or scarring of the caudal fins from five fish collected at Reference Area 1, which likely resulting from predation. Of the 38 affected fish, nine were from the Exposure Area (23.7%), 16 from Reference Area 1 (42.1%), and 13 from Reference Area 2 (34.2%). No external parasites were observed on captured Ninespine Stickleback. Internal abnormalities were observed in a total of 41 Ninespine Stickleback (15.6%), and included pale livers, enlarged spleens, and internal cysts (Table 6.3-7). Of the 41 affected fish, seven were from the Exposure Area (17.1%), 22 from Reference Area 1 (53.7%) and 12 from Reference Area 2 (29.3%). The internal and external abnormalities observed in the study area are commonly observed in fish populations, and given the low rate of incidence, were not considered further.

Table 6.3-7: Summary of Ninespine Stickleback Internal and External Abnormalities, 2016

Area	Assessment	Abnormality	n	Occurrence	Prevalence	Description
EXP	External	Eyes	88	0	0.0%	
		Gills		1	1.1%	Gills pale in colour.
		Thymus		0	0.0%	
		Skin		1	1.1%	Discolouration on left dorsal side.
		Opercula		0	0.0%	
		Fins		5	5.7%	Mild fin erosion.
		Body Deformities		1	1.1%	Injury to caudal peduncle.
	Internal	Liver		6	6.8%	Pale liver; coffee cream in colour.
		Spleen		0	0.0%	
		Gall Bladder		0	0.0%	
		Kidney		0	0.0%	
		Parasites		1	1.1%	Cysts in stomach.
REF1	External	Eyes	81	0	0.0%	
		Gills		4	4.9%	Gills pale in colour.
		Thymus		0	0.0%	
		Skin		0	0.0%	
		Opercula		2	2.5%	Mild shortening of opercula.
		Fins		10	12.3%	Four fish with mild fin erosion. Five fish with caudal fin injuries. One fish missing left pectoral fin.
		Body Deformities		0	0.0%	
	Internal	Liver		16	19.8%	Pale liver; coffee cream in colour.
		Spleen		1	1.2%	Enlarged spleen.
		Gall Bladder		0	0.0%	
		Kidney		1	1.2%	Kidney inflammation.
		Parasites		1	1.2%	Cysts in stomach.
REF2	External	Eyes	94	0	0.0%	
		Gills		5	5.3%	Gills pale in colour.
		Thymus		0	0.0%	
		Skin		1	1.1%	Mild abrasion.
		Opercula		1	1.1%	Mild shortening of opercula.
		Fins		6	6.4%	Mild fin erosion.
		Body Deformities		0	0.0%	
	Internal	Liver		5	5.3%	Pale liver; coffee cream in colour.
		Spleen		7	7.4%	Spleen enlarged or pale in colour.
		Gall Bladder		0	0.0%	
		Kidney		0	0.0%	
		Parasites		0	0.0%	

n = sample size; EXP = Exposure Area; REF1 = Reference Area 1; REF2 = Reference Area 2.

Of the 263 fish captured, 32 (12.2%) had endpoints removed from consideration in the statistical analyses. Three of these fish were removed due to an absence of a caudal fins, which prevented accurate measurements of length and weight. Three adult fish were removed as their sex could not be determined with adequate confidence, and 26 fish had either gonad or liver weights removed from the analysis due issues with measurement precision (Appendix E, Tables E-1 to E3).

Of the remaining fish, 86 were captured from the Exposure Area, 78 from Reference Area 1, and 93 from Reference Area 2. Ninespine Stickleback ranged in age from 1 to 4 years on the basis of length-frequency assessment. Juvenile fish ranged in size from 25 to 38 mm, and 0.098 to 0.332 g. Adult fish ranged in size from 31 to 75 mm, and 0.162 to 2.334 g. Summary statistics for Ninespine Stickleback are provided in Table 6.3-8. The results of statistical comparisons among areas are presented in Table 6.3-9. ANCOVA scatterplots are provided in Appendix E, Figures E-1 to E-9.

The following statistical outliers were identified during analysis (i.e., SR ≥3.0):

- LU16FEXPNNST102 – male length adjusted weight (carcass)
- LU16FEXPNNST7 – male relative liver size (length)
- LU16FREF1NNST2038, LU16FREF1NNST2122 – female relative gonad size (body, carcass, length)
- LU16FREF1NNST2053 – female length adjusted weight (body)
- LU16FEXPNNST31 – female length adjusted weight (carcass)
- LU16FEXPNNST54, LU16FREF1NNST2009 – female relative liver size (length)
- LU16FREF2NNST5102 – juvenile relative liver weight (length)

Table 6.3-8: Summary Statistics for Lethally Sampled Ninespine Stickleback, 2016

Sex	Parameter	Exposure Area							Reference Area 1							Reference Area 2						
		n	Mean	Median	SD	SE	Min	Max	n	Mean	Median	SD	SE	Min	Max	n	Mean	Median	SD	SE	Min	Max
Male	Age	32	1.7	2	0.7	0.1	1	3	17	1.8	2	0.4	0.1	1	2	33	2.3	2	0.8	0.1	1	4
	Total body weight	32	0.466	0.375	0.295	0.052	0.162	1.242	17	0.485	0.489	0.172	0.042	0.227	0.740	33	0.780	0.689	0.301	0.052	0.348	1.514
	Carcass weight	32	0.388	0.310	0.258	0.046	0.125	1.078	17	0.408	0.391	0.150	0.036	0.187	0.633	33	0.665	0.587	0.257	0.045	0.307	1.319
	Total length	32	42.3	43	7.2	1.3	32	56	17	43.8	43	4.6	1.1	37	50	33	50.8	50	5.9	1.0	40	64
	K (carcass)	32	0.45	0.44	0.06	0.01	0.37	0.65	17	0.46	0.47	0.06	0.01	0.37	0.57	33	0.48	0.48	0.03	<0.01	0.43	0.54
	K (body)	32	0.55	0.54	0.06	0.01	0.47	0.75	17	0.55	0.55	0.06	0.01	0.45	0.67	33	0.57	0.57	0.04	0.01	0.49	0.64
	Liver weight	32	0.013	0.009	0.011	0.002	0.001	0.049	17	0.017	0.022	0.009	0.002	0.003	0.029	33	0.026	0.022	0.014	0.002	0.006	0.063
	LSI (carcass)	32	3.26	3.05	1.21	0.21	0.80	6.09	17	4.03	3.89	1.47	0.36	1.51	7.42	33	3.81	3.97	1.01	0.18	1.95	5.65
	LSI (body)	32	2.68	2.54	1.01	0.18	0.62	4.78	17	3.37	3.29	1.21	0.29	1.22	5.93	33	3.24	3.43	0.84	0.15	1.72	4.72
	Gonad weight	31	0.010	0.007	0.007	0.001	0.002	0.030	16	0.008	0.008	0.002	0.001	0.004	0.012	33	0.013	0.012	0.005	0.001	0.007	0.023
	GSI (carcass)	31	2.40	2.40	0.64	0.11	1.27	4.06	16	1.99	1.87	0.65	0.16	1.02	3.46	33	2.09	1.98	0.56	0.10	1.26	3.41
	GSI (body)	31	1.96	1.97	0.52	0.09	1.03	3.19	16	1.67	1.61	0.53	0.13	0.82	2.90	33	1.78	1.69	0.46	0.08	1.04	2.84
Female	Age	29	1.6	1	0.9	0.2	1	4	31	2.5	3	0.9	0.2	1	4	31	2.9	3	1.2	0.2	1	4
	Total body weight	29	0.439	0.284	0.296	0.055	0.202	1.264	31	0.698	0.716	0.381	0.068	0.173	1.843	31	0.946	0.874	0.549	0.099	0.222	2.334
	Carcass weight	29	0.364	0.238	0.247	0.046	0.164	1.076	31	0.579	0.599	0.312	0.056	0.136	1.488	31	0.794	0.739	0.462	0.083	0.181	1.905
	Total length	29	42.7	38	8.7	1.6	33	66	31	49.3	52	9.3	1.7	31	67	31	54.4	55	11.4	2.1	35	75
	K (carcass)	29	0.42	0.42	0.04	0.01	0.33	0.50	31	0.43	0.43	0.04	0.01	0.31	0.52	31	0.43	0.43	0.03	<0.1	0.37	0.48
	K (body)	29	0.51	0.51	0.04	0.01	0.40	0.59	31	0.53	0.53	0.05	0.01	0.38	0.63	31	0.52	0.52	0.03	0.01	0.45	0.57
	Liver weight	29	0.016	0.008	0.023	0.004	0.001	0.120	31	0.026	0.023	0.021	0.004	0.001	0.097	31	0.037	0.036	0.025	0.004	0.005	0.088
	LSI (carcass)	29	3.44	3.33	2.36	0.44	0.53	12.77	31	3.90	3.99	1.39	0.25	0.61	6.53	31	4.51	4.54	1.15	0.21	2.36	6.92
	LSI (body)	29	2.85	2.82	1.92	0.36	0.45	10.37	31	3.23	3.28	1.14	0.21	0.51	5.33	31	3.77	3.66	1.00	0.18	1.98	6.67
	Gonad weight	23	0.008	0.006	0.008	0.002	0.002	0.030	26	0.016	0.014	0.010	0.002	0.002	0.043	31	0.022	0.018	0.018	0.003	0.002	0.072
	GSI (carcass)	23	1.88	1.84	0.68	0.14	0.84	3.19	26	2.33	2.26	0.75	0.15	1.04	4.20	31	2.42	2.53	0.79	0.14	0.92	3.87
	GSI (body)	23	1.55	1.54	0.55	0.11	0.71	2.59	26	1.94	1.89	0.62	0.12	0.86	3.46	31	2.03	2.12	0.67	0.12	0.76	3.21
Juvenile	Age	25	1.0	1	<0.1	<0.1	1	1	30	1.0	1	<0.1	<0.1	1	1	29	1.0	1	<0.1	<0.1	1	1
	Total body weight	25	0.218	0.241	0.064	0.013	0.098	0.332	30	0.159	0.149	0.044	0.008	0.098	0.252	29	0.196	0.196	0.037	0.007	0.115	0.265
	Carcass weight	25	0.174	0.195	0.057	0.011	0.074	0.261	30	0.128	0.123	0.036	0.007	0.084	0.205	29	0.159	0.159	0.029	0.005	0.084	0.210
	Total length	25	33.6	36	3.8	0.8	25	38	30	30.8	31	2.7	0.5	26	36	29	33.0	33	2.1	0.4	28	37
	K (carcass)	25	0.44	0.44	0.03	0.01	0.37	0.50	30	0.43	0.43	0.04	0.01	0.35	0.54	29	0.44	0.44	0.03	<0.01	0.38	0.51
	K (body)	25	0.56	0.55	0.04	0.01	0.50	0.63	30	0.53	0.53	0.05	0.01	0.43	0.66	29	0.54	0.54	0.04	0.01	0.48	0.65
	Liver weight	24	0.005	0.005	0.003	0.001	0.001	0.012	30	0.004	0.003	0.002	<0.001	0.001	0.008	29	0.005	0.005	0.003	0.001	0.001	0.015
	LSI (carcass)	24	2.97	2.69	1.31	0.27	1.02	5.91	30	2.77	2.60	1.48	0.27	0.66	7.95	29	3.17	2.86	1.80	0.33	0.64	7.46
	LSI (body)	24	2.35	2.08	1.01	0.21	0.86	4.51	30	2.22	2.07	1.16	0.21	0.53	6.19	29	2.57	2.42	1.45	0.27	0.56	6.18

Note: gonad weight endpoints were excluded for juvenile fish.
n = sample size; SD = standard deviation; SE = standard error; min = minimum; max = maximum; K = condition factor; LSI = liversomatic index; GSI = gonadosomatic index.

Table 6.3-9: Statistical Analyses for Ninespine Stickleback, 2016

Sex	Parameter	Endpoint	Dependent Variable	Covariate	Test	Interaction		Main Effect		MSE	LSM			Contrasts			Magnitude (% difference)			SSD	Direction REF1 / REF2	Power Analysis	
						$Fstat_{(\alpha, df1, df2)}$	*P*-value	$Fstat_{(\alpha, df1, df2)}$	*P*-value		EXP	REF1	REF2	EXP* REF1	EXP* REF2	REF1* REF2	EXP* REF1	EXP* REF2	REF1* REF2			Power	n
Male	Survival	Length-frequency distribution	n/a	n/a	KS	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.428	**<0.001**	**0.002**	0.0[a]	-63.1[a]	-52.8[a]	Yes	- / ↓↓	n/a	n/a
		Age	Age	n/a	K-W	n/a	n/a	$12.017_{(0.1, 2, 79)}$	**0.002**	n/a	1.7	1.8	2.3	0.535	**0.001**	**0.021**	0.0	-26.1	-24.4	Yes	- / ↓↓	n/a	n/a
	Growth (energy use)	Body weight	Body weight	n/a	$ANOVA_{log}$	n/a	n/a	$16.007_{(0.1, 2, 79)}$	**<0.001**	0.038	0.398	0.454	0.731	0.587	**<0.001**	**0.002**	0.0	-45.5	-46.8	Yes	- / ↓↓	n/a	n/a
		Carcass weight	Carcass weight	n/a	K-W	n/a	n/a	$25.921_{(0.1, 2, 79)}$	**<0.001**	n/a	0.388	0.408	0.665	0.273	**<0.001**	**<0.001**	0.0	-41.7	-47.9	Yes	- / ↓↓	n/a	n/a
		Weight-at-age (body) 1+	Body weight	n/a	K-W	n/a	n/a	$5.218_{(0.1, 2, 18)}$	**0.074**	n/a	0.260	0.266	0.355	0.844	**0.022**	**0.055**	0.0	-26.8	-28.7	Yes	- / ↓↓	n/a	n/a
		Weight-at-age (body) 2+			K-W	n/a	n/a	$17.668_{(0.1, 2, 45)}$	**<0.001**	n/a	0.468	0.553	0.665	**0.045**	**<0.001**	**0.007**	-15.4	-29.6	-18.4	Yes	↓ / ↓↓	n/a	n/a
		Weight-at-age (body) 3+			ANOVA	n/a	n/a	$0.321_{(0.1, 2, 6)}$	0.592	0.014	1.078	n/a	1.029	n/a	n/a	n/a	n/a	0.0	n/a	No	- / -	0.836	5
		Weight-at-age (carcass) 1+	Carcass weight	n/a	K-W	n/a	n/a	$5.256_{(0.1, 2, 18)}$	**0.072**	n/a	0.210	0.217	0.309	0.767	**0.021**	**0.060**	0.0	-32.0	-35.0	Yes	- / ↓↓	n/a	n/a
		Weight-at-age (carcass) 2+			K-W	n/a	n/a	$18.608_{(0.1, 2, 45)}$	**<0.001**	n/a	0.387	0.467	0.558	**0.030**	**<0.001**	**0.006**	-17.1	-30.6	-17.8	Yes	↓ / ↓↓	n/a	n/a
		Weight-at-age (carcass) 3+			ANOVA	n/a	n/a	$0.434_{(0.1, 2, 6)}$	0.534	0.010	0.926	n/a	0.877	n/a	n/a	n/a	n/a	0.0	n/a	No	- / -	0.841	5
		Total length	Total length	n/a	K-W	n/a	n/a	$26.708_{(0.1, 2, 79)}$	**<0.001**	n/a	42.3	43.8	50.8	0.508	**<0.001**	**<0.001**	0.0	-16.9	-15.0	Yes	- / ↓	n/a	n/a
		Length-at-age 1+	Total length	n/a	K-W	n/a	n/a	$4.341_{(0.1, 2, 18)}$	0.114	n/a	36.2	37.5	40.0	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	1.000	5
		Length-at-age 2+			K-W	n/a	n/a	$18.817_{(0.1, 2, 18)}$	**<0.001**	n/a	44.4	45.7	48.8	0.256	**<0.001**	**<0.001**	0.0	-9.0	-6.6	Yes	- / ↓	n/a	n/a
		Length-at-age 3+			ANOVA	n/a	n/a	$0.667_{(0.1, 2, 6)}$	0.445	1.80	55.2	n/a	56.0	n/a	n/a	n/a	0.0	n/a	0.0	No	- / -	1.000	2
	Reproduction (energy use)	Relative gonad size	Gonad weight	Body weight	$ANCOVA_{rank}$	$5.153_{(0.05, 2, 74)}$	**0.008**	$3.522_{(0.1, 2, 76)}$	**0.034**	164	42	31	38	**0.026**	0.593	0.241	33.5	0.0	0.0	Yes	↑↑ / -	n/a	n/a
				Carcass weight	$ANCOVA_{rank}$	$4.827_{(0.05, 2, 74)}$	**0.011**	$3.533_{(0.1, 2, 76)}$	**0.034**	170	42	31	38	**0.026**	0.569	0.256	34.3	0.0	0.0	Yes	↑↑ / -	n/a	n/a
				Length	$ANCOVA_{rank}$	$4.163_{(0.05, 2, 74)}$	**0.019**	$1.834_{(0.1, 2, 76)}$	0.167	178	41	33	38	n/a	n/a	n/a	23.6	0.0	0.0	Yes	↑ / -	0.711	45
	Condition (energy storage)	Length-adjusted weight	Carcass weight	Length	$ANCOVA_{rank}$	$4.411_{(0.05, 2, 76)}$	$0.015^{[b]}$	$3.908_{(0.1, 2, 78)}$	**0.024**	14	40	44	41	**0.074**	0.766	0.338	-7.1	0.0	0.0	Yes	↓ / -	n/a	n/a
					$ANCOVA_{log}$	*$3.209_{(0.05, 2, 75)}$*	*$0.047^{[b]}$*	*$0.393_{(0.1, 2, 77)}$*	*0.676*	*0.001*	*0.427*	*0.435*	*0.436*	*n/a*	*n/a*	*n/a*	*0.0*	*0.0*	*0.0*	*No*	*- / -*	*1.000*	*10*
			Body weight	Length	$ANCOVA_{log}$	$1.229_{(0.05, 2, 76)}$	0.298	$0.305_{(0.1, 2, 78)}$	0.738	0.001	0.527	0.522	0.518	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	1.000	9
		Relative liver size	Liver weight	Body weight	ANCOVA	$1.228_{(0.05, 2, 76)}$	0.299	$2.170_{(0.1, 2, 78)}$	0.121	<0.001	0.019	0.022	0.019	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	0.999	11
				Carcass weight	ANCOVA	$1.448_{(0.05, 2, 76)}$	0.241	$1.961_{(0.1, 2, 78)}$	0.148	<0.001	0.019	0.022	0.019	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	0.999	12
				Length	$ANCOVA_{log}$	$0.692_{(0.05, 2, 76)}$	0.504	$1.379_{(0.1, 2, 78)}$	0.258	0.029	0.014	0.017	0.015	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	0.866	31
					$ANCOVA_{log}$	*$1.023_{(0.05, 2, 75)}$*	*0.365*	*$1.069_{(0.1, 2, 77)}$*	*0.349*	*0.026*	*0.015*	*0.017*	*0.015*	*n/a*	*n/a*	*n/a*	*0.0*	*0.0*	*0.0*	*No*	*- / -*	*0.863*	*31*
Female	Survival	Length-frequency distribution	n/a	n/a	KS	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	**0.002**	**0.001**	**0.016**	-47.7[a]	-50.9[a]	-38.7[a]	Yes	↓↓ / ↓↓	n/a	n/a
		Age	Age	n/a	K-W	n/a	n/a	$20.965_{(0.1, 2, 88)}$	**<0.001**	n/a	1.6	2.5	2.9	**<0.000**	**<0.001**	0.163	-37.0	-45.4	0.0	Yes	↓↓ / ↓↓	n/a	n/a
	Growth (energy use)	Body weight	Body weight	n/a	K-W	n/a	n/a	$15.964_{(0.1, 2, 88)}$	**<0.001**	n/a	0.439	0.698	0.946	**0.009**	**<0.001**	0.100	-37.1	-53.6	0.0	Yes	↓↓ / ↓↓	n/a	n/a
		Carcass weight	Carcass weight	n/a	K-W	n/a	n/a	$15.782_{(0.1, 2, 88)}$	**<0.001**	n/a	0.364	0.579	0.794	**0.010**	**<0.001**	**0.097**	-37.1	-54.2	-38.7	Yes	↓↓ / ↓↓	n/a	n/a
		Weight-at-age (body) 1+	Body weight	n/a	$ANOVA_{log}$	n/a	n/a	$21.417_{(0.1, 2, 26)}$	**<0.001**	0.002	0.258	0.189	0.240	**<0.001**	**0.324**	**0.002**	36.5	0.0	-37.7	Yes	↑↑ / -	n/a	n/a
		Weight-at-age (body) 2+			ANOVA	n/a	n/a	$0.787_{(0.1, 2, 18)}$	0.470	0.009	0.561	0.587	0.521	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	0.418	25
		Weight-at-age (body) 3+			ANOVA	n/a	n/a	$2.343_{(0.1, 2, 19)}$	0.142	0.006	n/a	0.771	0.831	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	n/a[c]	n/a[c]
		Weight-at-age (body) 4+			$ANOVA_{log}$	n/a	n/a	$1.163_{(0.1, 2, 17)}$	0.336	0.008	1.186	1.510	1.419	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	0.435	15
		Weight-at-age (carcass) 1+	Carcass weight	n/a	ANOVA	n/a	n/a	$17.046_{(0.1, 2, 26)}$	**<0.001**	<0.001	0.214	0.154	0.196	**<0.001**	0.247	0.010	39.0	0.0	0.0	Yes	↑↑ / -	n/a	n/a
		Weight-at-age (carcass) 2+			ANOVA	n/a	n/a	$0.878_{(0.1, 2, 18)}$	0.433	0.007	0.469	0.489	0.429	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	0.886	8
		Weight-at-age (carcass) 3+			ANOVA	n/a	n/a	$2.251_{(0.1, 2, 19)}$	0.150	0.004	n/a	0.643	0.692	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	n/a[c]	n/a[c]
		Weight-at-age (carcass) 4+			$ANOVA_{log}$	n/a	n/a	$1.469_{(0.1, 2, 17)}$	0.258	0.007	0.982	1.245	1.202	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	0.439	14
		Total length	Total length	n/a	ANOVA	n/a	n/a	$10.500_{(0.1, 2, 88)}$	**<0.001**	97.9	42.7	49.3	54.4	**0.031**	**<0.001**	0.112	-13.4	-21.5	0.0	Yes	↓ / ↓	n/a	n/a
		Length-at-age 1+	Total length	n/a	ANOVA	n/a	n/a	$15.525_{(0.1, 2, 26)}$	**<0.001**	2.1	36.9	33.2	36.0	**<0.001**	0.408	**0.009**	11.4	0.0	-37.7	Yes	↑ / -	n/a	n/a
					ANOVA	*n/a*	*n/a*	*$23.917_{(0.1, 2, 25)}$*	*<0.001*	*1.5*	*37.2*	*33.2*	*36.0*	*<0.001*	*0.162*	*0.002*	*12.1*	*0.0*	*-37.7*	*Yes*	*↑ / -*	*n/a*	*n/a*
		Length-at-age 2+			K-W	n/a	n/a	$1.226_{(0.1, 2, 18)}$	0.542	n/a	48.3	47.7	46.7	0.711	0.295	0.532	0.0	0.0	0.0	No	- / -	1.000	3
		Length-at-age 3+			K-W	n/a	n/a	$5.006_{(0.1, 2, 19)}$	**0.025**	n/a	n/a	52.9	54.8	n/a	n/a	**0.021**	n/a	n/a	-37.7	No	- / -	n/a	n/a
		Length-at-age 4+			ANOVA	n/a	n/a	$0.345_{(0.1, 2, 17)}$	0.713	19.3	62.3	64.0	64.6	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	1.000	4
	Reproduction (energy use)	Relative gonad size	Gonad weight	Body weight	$ANCOVA_{rank}$	$1.196_{(0.05, 2, 74)}$	0.308	$0.969_{(0.1, 2, 76)}$	0.384	65	37	39	41	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	0.998	12
					$ANCOVA_{rank}$	*$0.470_{(0.05, 2, 72)}$*	*0.627*	*$1.086_{(0.1, 2, 74)}$*	*0.343*	*43*	*37*	*39*	*40*	*n/a*	*n/a*	*n/a*	*0.0*	*0.0*	*0.0*	*No*	*- / -*	*1.000*	*9*

Table 6.3-9: Statistical Analyses for Ninespine Stickleback, 2016

Sex	Parameter	Endpoint	Dependent Variable	Covariate	Test	Interaction		Main Effect		MSE	LSM			Contrasts			Magnitude (% difference)			SSD	Direction REF1 / REF2	Power Analysis	
						Fstat$_{(\alpha, df1, df2)}$	*P*-value	Fstat$_{(\alpha, df1, df2)}$	*P*-value		EXP	REF1	REF2	EXP* REF1	EXP* REF2	REF1* REF2	EXP* REF1	EXP* REF2	REF1* REF2			Power	n
				Carcass weight	ANCOVA$_{rank}$	1.038$_{(0.05, 2, 74)}$	0.359	1.169$_{(0.1, 2, 76)}$	0.316	64	37	40	41	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	0.999	12
					ANCOVA$_{rank}$	*0.311$_{(0.05, 2, 72)}$*	*0.734*	*1.338$_{(0.1, 2, 74)}$*	*0.269*	*44*	*37*	*39*	*40*	*n/a*	*n/a*	*n/a*	*0.0*	*0.0*	*0.0*	*No*	*- / -*	*1.000*	*9*
				Length	ANCOVA$_{rank}$	0.892$_{(0.05, 2, 74)}$	0.414	1.965$_{(0.1, 2, 76)}$	0.147	60	36	40	40	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	0.999	11
					ANCOVA$_{rank}$	*0.669$_{(0.05, 2, 72)}$*	*0.515*	*2.270$_{(0.1, 2, 74)}$*	*0.110*	*40*	*37*	*40*	*40*	*n/a*	*n/a*	*n/a*	*0.0*	*0.0*	*0.0*	*No*	*- / -*	*1.000*	*8*
	Condition (energy storage)	Length-adjusted weight	Body weight	Length	ANCOVA$_{rank}$	1.132$_{(0.05, 2, 85)}$	0.327	0.395$_{(0.1, 2, 87)}$	0.675	21	46	47	46	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	0.994	16
					ANCOVA$_{bg}$	*2.874$_{(0.05, 2, 84)}$*	*0.062*	*2.490$_{(0.1, 2, 86)}$*	***0.089***	*0.001*	*0.547*	*0.571*	*0.558*	***0.073***	*0.536*	*0.501*	*-4.3*	*0.0*	*0.0*	*Yes*	*↓ / -*	*n/a*	*n/a*
			Carcass weight	Length	ANCOVA$_{rank}$	1.243$_{(0.05, 2, 85)}$	0.294	0.333$_{(0.1, 2, 87)}$	0.717	21	46	46	46	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	0.990	17
					ANCOVA$_{bg}$	*0.719$_{(0.05, 2, 83)}$*	*0.490*	*1.171$_{(0.1, 2, 85)}$*	*0.315*	*0.001*	*0.457*	*0.468*	*0.457*	*n/a*	*n/a*	*n/a*	*0.0*	*0.0*	*0.0*	*No*	*- / -*	*1.000*	*10*
		Relative liver size	Liver weight	Body weight	ANCOVA$_{rank}$	4.749$_{(0.05, 2, 85)}$	0.011[b]	1.875$_{(0.1, 2, 87)}$	0.159	72	43	45	48	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	1.000	9
				Carcass weight	ANCOVA$_{rank}$	4.806$_{(0.05, 2, 85)}$	0.011[b]	1.943$_{(0.1, 2, 87)}$	0.149	73	43	45	48	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	1.000	9
					ANCOVA$_{bg}$	*7.402$_{(0.05, 2, 83)}$*	*0.001[b]*	*0.597$_{(0.1, 2, 85)}$*	*0.552*	*0.025*	*0.017*	*0.018*	*0.019*	*n/a*	*n/a*	*n/a*	*0.0*	*0.0*	*0.0*	*No*	*- / -*	*0.781*	*42*
				Length	ANCOVA$_{rank}$	2.912$_{(0.05, 2, 85)}$	0.060	1.515$_{(0.1, 2, 87)}$	0.226	89	43	45	47	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	1.000	11
Juvenile	Survival	Length-frequency distribution	n/a	n/a	KS	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	**0.001**	**0.015**	**0.019**	53.3[a]	41.7[a]	-39.2[a]	Yes	↑↑ / ↑↑	n/a	n/a
		Age	Age	n/a	K-W	n/a	n/a	<0.001$_{(0.1, 2, 84)}$	1.000	n/a	1.0	1.0	1.0	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	1.000	2
	Growth (energy use)	Body weight	Body weight	n/a	K-W	n/a	n/a	16.917$_{(0.1, 2, 84)}$	**<0.001**	n/a	0.218	0.159	0.196	**<0.001**	0.168	**0.003**	37.1	0.0	-20.8	Yes	↑↑ / -	n/a	n/a
		Carcass weight	Carcass weight	n/a	K-W	n/a	n/a	14.992$_{(0.1, 2, 84)}$	**0.001**	n/a	0.174	0.128	0.159	**<0.001**	0.293	**0.003**	35.9	0.0	-21.6	Yes	↑↑ / -	n/a	n/a
		Weight-at-age (body) 1+	Body weight	n/a	K-W	n/a	n/a	16.917$_{(0.1, 2, 84)}$	**<0.001**	n/a	0.218	0.159	0.196	**<0.001**	0.168	**0.003**	37.1	0.0	-20.8	Yes	↑↑ / -	n/a	n/a
		Weight-at-age (carcass) 1+	Carcass weight	n/a	K-W	n/a	n/a	14.992$_{(0.1, 2, 84)}$	**0.001**	n/a	0.174	0.128	0.159	**<0.001**	0.293	**0.003**	35.9	0.0	-21.6	Yes	↑↑ / -	n/a	n/a
		Total length	Total length	n/a	K-W	n/a	n/a	14.562$_{(0.1, 2, 84)}$	**0.001**	n/a	33.6	30.8	33	**<0.001**	0.176	**0.007**	9.1	0.0	-6.9	Yes	↑ / -	n/a	n/a
		Length-at-age 1+	Total length	n/a	K-W	n/a	n/a	14.562$_{(0.1, 2, 84)}$	**0.001**	n/a	33.6	30.8	33	**<0.001**	0.176	**0.007**	9.1	0.0	-6.9	Yes	↑ / -	n/a	n/a
	Condition (energy storage)	Length-adjusted weight	Body weight	Length	ANCOVA$_{rank}$	1.137$_{(0.05, 2, 78)}$	0.326	0.982$_{(0.1, 2, 80)}$	0.379	44	43	41	43	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	0.842	34
			Carcass weight	Length	ANCOVA$_{rank}$	0.961$_{(0.05, 2, 78)}$	0.387	0.773$_{(0.1, 2, 80)}$	0.465	48	42	41	44	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	0.799	38
		Relative liver size	Liver weight	Body weight	ANCOVA$_{log}$	0.104$_{(0.05, 2, 77)}$	0.902	0.147$_{(0.1, 2, 79)}$	0.864	0.052	0.004	0.004	0.004	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	0.447	94
				Carcass weight	ANCOVA$_{log}$	0.166$_{(0.05, 2, 77)}$	0.847	0.005$_{(0.1, 2, 79)}$	0.995	0.054	0.004	0.004	0.004	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	0.431	98
				Length	ANCOVA$_{log}$	0.106$_{(0.05, 2, 77)}$	0.900	0.052$_{(0.1, 2, 79)}$	0.949	0.058	0.004	0.004	0.004	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	0.420	102
					ANCOVA	*0.245$_{(0.05, 2, 76)}$*	*0.784*	*0.209$_{(0.1, 2, 78)}$*	*0.812*	*<0.001*	*0.005*	*0.004*	*0.005*	*n/a*	*n/a*	*n/a*	*0.0*	*0.0*	*0.0*	*No*	*- / -*	*0.768*	*40*

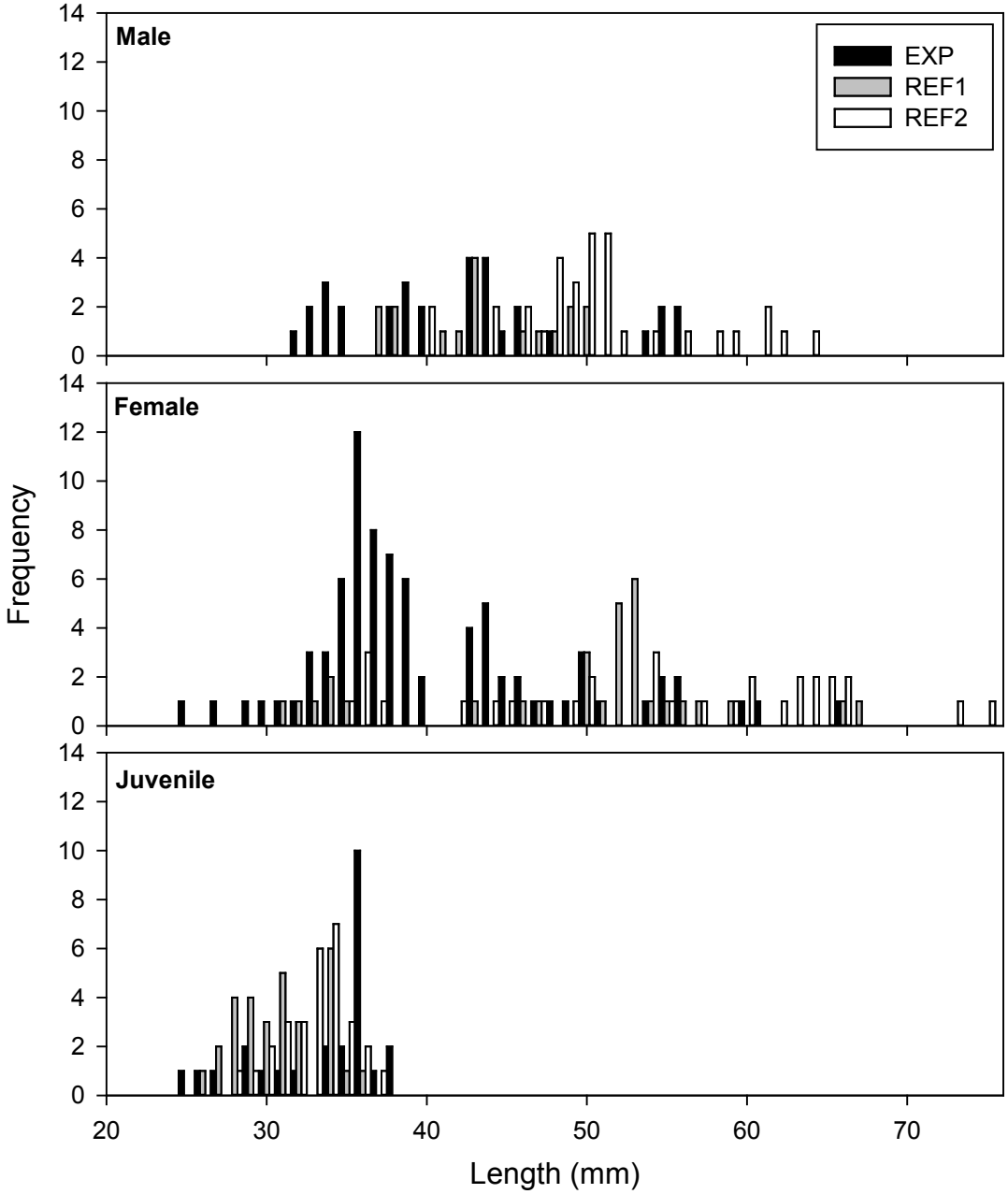
Note: Values in *italics* indicate results were calculated with statistical outliers removed (i.e., studentized residuals greater than 3.0). Significant differences are indicated in **bold**. Gonad endpoints were excluded for juvenile fish.
a) Magnitude based on maximum percent difference between length-frequency distributions.
b) Not practically different (Barrett et al. 2010).
c) Power analysis not completed, as no fish were captured from the Exposure Area.
Fstat = test statistic; *P*-value = probability value; MSE = mean squared error; LSM = least squares mean; SSD = statistically significant difference (α= 0.1); EXP = Exposure Area; REF1 = Reference Area 1; REF2 = Reference Area 2; n = sample size; ANOVA = Analysis of Variance; ANCOVA = Analysis of Covariance; log = log 10 transformed data; rank = rank transformed data; K-W = Kruskal-Wallis; K-S = Kolmogorov–Smimov; <= less than; n/a = not applicable; ↓ = significantly lower at the Exposure Area; ↓↓ = significantly lower at the Exposure Area and magnitude exceeded 25% (10% for length-adjusted weight); ↑ = significantly greater at the Exposure Area; ↑↑ = significantly lower at the Exposure Area and magnitude exceeded 25% (10% for length-adjusted weight).

6.3.3.2.1 Survival

Survival of male, female and juvenile Ninespine Stickleback were compared among sampling areas based on measures of size (i.e., length-frequency distributions) and age. Length-frequency distributions of Ninespine Stickleback were significantly different among areas, for male, female and juvenile fish (Table 6.3-9; Figure 6.3-2). For males, Ninespine Stickleback sampled from the Exposure Area had a similar distribution to fish collected from Reference Area 1; however, both areas were significantly different from Reference Area 2, which had a greater proportion of larger fish (Figure 6.3-2). For female Ninespine Stickleback, significant differences in length-frequency distributions were observed among all three areas, with a greater proportion of smaller fish collected from the Exposure Area when compared to the two reference areas. For juvenile fish, significant differences in length-frequency distributions were observed among all three areas, with a greater proportion of larger fish collected from the Exposure Area.

The age of male and female Ninespine Stickleback sampled during the lethal survey also varied among sampling areas (Table 6.3-9). Male fish sampled from the Exposure Area did not vary significantly in age from Reference Area 1; however, fish sampled from the Exposure Area and reference Area 1 were younger than male fish sampled from Reference Area 2. Female fish collected from the Exposure Area were younger than fish collected from Reference Areas 1 and 2, with no significant differences observed between the reference areas. No significant differences were observed in age among juvenile fish, which were 1 year of age across areas (Table 6.3-9).

Figure 6.3-1: Length Frequency Distribution of Male, Female, and Juvenile Ninespine Stickleback, 2016



EXP = Exposure Area; REF1 = Reference Area 1; REF2 = Reference Area 2.

6.3.3.2.2 Energy Use

Energy use of male, female and juvenile Ninespine Stickleback was compared among areas based on measures of growth (i.e., length, weight and size-at-age) and reproduction (i.e., relative gonad weight). Growth endpoints varied significantly among areas. Male Ninespine Stickleback sampled from the Exposure Area had similar body weight, carcass weight and total length, compared to males sampled from Reference Area 1; however, fish from the Exposure Area and Reference Area 1 were significantly smaller than individuals sampled from Reference Area

2 (Table 6.3-8). A similar pattern was observed for weight-at-age of 1 year old (i.e., 1+) male fish. Male Ninespine Stickleback sampled from the Exposure Area had similar body weight and carcass weight compared to males sampled from Reference Area 1; however, fish from the Exposure Area and Reference Area 1 were significantly smaller than individuals sampled from Reference Area 2. No significant differences were observed in total length. For 2 year old male fish (i.e., 2+), significant differences were observed in weight-at-age among all three areas based on body weight and carcass weight. The length-at-age of 2+ male fish from the Exposure Area were similar to Reference Area 1 and significantly shorter than male fish from Reference Area 2. For 3 year old male fish (i.e., 3+), no significant differences were observed among areas in weight or length-at-age; however no 3+ were sampled from Reference Area 1 for comparison. Four year old male fish (i.e., 4+) were only collected from Reference Area 2, and could not be compared to the other areas.

Size also varied among areas for female Ninespine Stickleback. Female fish from the Exposure Area had significantly lower body weight and carcass weight than individuals sampled from the reference areas (Reference Area 1 and Reference Area 2), while length was significantly greater when compared to Reference Area 1 (Table 6.3-9). Weight and length-at-age were significantly greater for 1+ female fish from the Exposure Area when compared to Reference Area 1, but not significantly different from Reference Area 2. No significant differences were observed between the Exposure Area and the reference areas for weight and length-at-age of 2+ and 4+ female fish. No 3+ fish were collected from the Exposure Area for comparison.

Juvenile Ninespine Stickleback collected from the Exposure Area had significantly greater body weight, carcass weight and length, when compared to Reference Area 1 (Table 6.3-9). No significant differences were observed between the Exposure Area and Reference Area 2. Juvenile fish sampled from Reference Area 1 were significantly smaller than individuals sampled from Reference Area 2. Weight and length-at-age could not be compared among juvenile Ninespine Stickleback, as individuals were all the same age (i.e., 1+).

Relative gonad weight was similar among areas for female fish, but was significantly different for males (Table 6.3-9). Relative gonad weight was significantly greater for males at the Exposure Area when compared to Reference Area 1, but did not vary significantly from Reference Area 2. Differences in gonad weight between the Exposure Area and Reference Area 1 were of similar magnitude relative to body weight, carcass weight and length. No significant differences were observed in relative gonad weight for female Ninespine Stickleback (Table 6.3-8).

6.3.3.2.3 Energy Storage

Energy storage of male, female and juvenile Ninespine Stickleback was compared among areas based on measures of condition (i.e., length-adjusted weight) and liver size (i.e., relative liver weight). For male fish, condition based on body weight was significantly different between the Exposure Area and Reference Area 1, with condition of fish collected from the Exposure Area significantly lower than Reference Area 1; however, these differences were not observed when statistical outliers were omitted from the analysis (Table 6.3-9). No significant differences were observed between the Exposure Area and Reference Area 2, and no significant differences were observed for condition based on carcass weight (Table 6.3-9). For female fish, no significant differences were observed in condition based on body weight and carcass weight among areas; however, the condition of female fish from the Exposure Area was significantly lower than Reference Area 1 when statistical outliers were removed from the analysis. No significant differences were observed among areas in the condition of juvenile fish based on body weight and carcass weight.

Relative liver size of male, female and juvenile Ninespine Stickleback were similar among areas based on relative body weight, carcass weight and length (Table 6.3-8). Statistical power was low for juvenile fish compared to adults, ranging from 0.420 to 0.768. This relative decrease in statistical power resulted from greater error between liver weight and the covariates (i.e., body weight, carcass weight and length).

6.3.3.3 Non-lethal Survey – Arctic Grayling

A total of 367 Arctic Grayling were captured in the study area in September 2016. Of these, seven fish exhibited external abnormalities (1.9%), which included mild forms of fin erosion and skin abrasion (Table 6.3-10). Five of the affected fish were from the Exposure Area, one from Reference Area 1 and one from Reference Area 2. These types of abnormalities are commonly observed in fish populations, and given the low rate of incidence, were not considered further. No external parasites were observed on captured Arctic Grayling. Of the 367 fish captured, 15 (4%) were removed from consideration in statistical analyses due to measurement errors (i.e., inaccurate field measurements of weight; Appendix E, Tables E-4 to E-6).

Of the remaining fish, 132 were captured from the Exposure Area, 176 from Reference Area 1, and 11 from Reference Area 2. Captured Arctic Grayling were predominantly YOY at the Exposure Area (95%) and Reference Area 1 (84%). No YOY were captured at Reference Area 2 (0%). Young-of-the-year ranged in length among areas from 55 to 90 mm, while juvenile fish (age 1+) ranged from 109 to 182 mm. Three larger fish from a separate cohort (age 2+) were also captured, ranging in length from 212 to 253 mm. These fish were included in data analyses, but removed as statistical outliers for comparisons of weight. Descriptive statistics for YOY and juvenile Arctic Grayling are provided in Table 6.3-11. The results of statistical comparisons among areas are presented in Table 6.3-12. ANCOVA scatterplots are provided in Appendix E, Figure E-10. The following statistical outliers were identified during analysis (i.e., SR ≥3.0):

- LU16FREF1ARGR3007 – total body weight
- LU16FREF1ARGR3123 – total body weight (Age 2+)
- LU16FREF2ARGR6001 – total body weight (Age 2+)
- LU16FREF2ARGR6003 – total body weight (Age 2+)

Table 6.3-10: Summary of Arctic Grayling External Abnormalities, 2016

Area	External Abnormality	Total Number of Fish Processed	Number of Fish with Observed Abnormality	Prevalence	Description
EXP	Eyes	136	0	0%	
	Gills		0	0%	
	Thymus		0	0%	
	Skin		1	1%	Mild ventral abrasion
	Operculums		0	0%	
	Fins		4	3%	Minor fin erosion
	Body Deformities		0	0%	
REF1	Eyes	219	0	0%	
	Gills		0	0%	
	Thymus		0	0%	
	Skin		0	0%	
	Operculums		0	0%	
	Fins		1	0%	Minor fin erosion
	Body Deformities		0	0%	
REF2	Eyes	12	0	0%	
	Gills		0	0%	
	Thymus		0	0%	
	Skin		0	0%	
	Operculums		0	0%	
	Fins		1	8%	Minor fin erosion
	Body Deformities		0	0%	

EXP = Exposure Area; REF1 = Reference Area 1; REF2 = Reference Area 2.

Table 6.3-11: Summary Statistics for Non-lethally Sampled Arctic Grayling, 2016

Life Stage	Parameter	Exposure Area							Reference Area 1							Reference Area 2						
		n	Mean	Median	SD	SE	Min	Max	n	Mean	Median	SD	SE	Min	Max	n	Mean	Median	SD	SE	Min	Max
YOY	Age	126	0	0	-	-	0	0	176	0	0	-	-	0	0	0	-	-	-	-	-	-
	Total body weight	126	2.7	2.7	0.7	0.1	1.2	5.3	176	2.9	2.8	0.7	0.1	1.0	5.2	0	-	-	-	-	-	-
	Total length	126	73.3	73	6.1	0.5	55	90	176	75.4	76	4.6	0.4	61	90	0	-	-	-	-	-	-
	K	126	0.69	0.69	0.08	0.01	0.44	0.84	176	0.67	0.68	0.10	0.01	0.44	0.92	0	-	-	-	-	-	-
Juvenile	Age	6	1.0	1	-	-	1	1	33	1.0	1	0.2	<0.1	1	2	11	1.2	1	0.4	0.1	1	2
	Total body weight	6	21.8	19.4	8.3	3.4	12.9	37.0	33	22.4	20.6	14.9	2.6	11.1	101.2	11	44.5	29.6	44.5	13.4	19.9	166.0
	Total length	6	143.7	142	25.0	10.2	109	182	33	141.4	140	19.0	3.3	115	234	11	160.6	151	37.9	11.4	131	253
	K	6	0.74	0.77	0.18	0.07	0.48	1.00	33	0.74	0.77	0.11	0.02	0.50	0.96	11	0.88	0.89	0.07	0.02	0.80	1.03
All	Age	132	0.0	0	0.2	<0.1	0	1	209	0.2	0	0.4	<0.1	0	2	11	1.2	1	0.4	0.1	1	2
	Total body weight	132	3.6	2.7	4.4	0.4	1.2	37.0	209	6.0	3.0	9.2	0.6	1.0	101.2	11	44.5	29.6	44.5	13.4	19.9	166.0
	Total length	132	76.5	73	16.6	1.4	55	182	209	85.8	77	25.6	1.8	61	234	11	160.6	151	37.9	11.4	131	253
	K	132	0.69	0.69	0.08	0.01	0.44	1.00	209	0.69	0.69	0.10	0.01	0.44	0.96	11	0.88	0.89	0.07	0.02	0.80	1.03

n = sample size; SD = standard deviation; SE = standard error; min = minimum; max = maximum; YOY = young-of-the-year; K = condition factor; - = not applicable.

Table 6.3-12: Statistical Analyses for Arctic Grayling, 2016

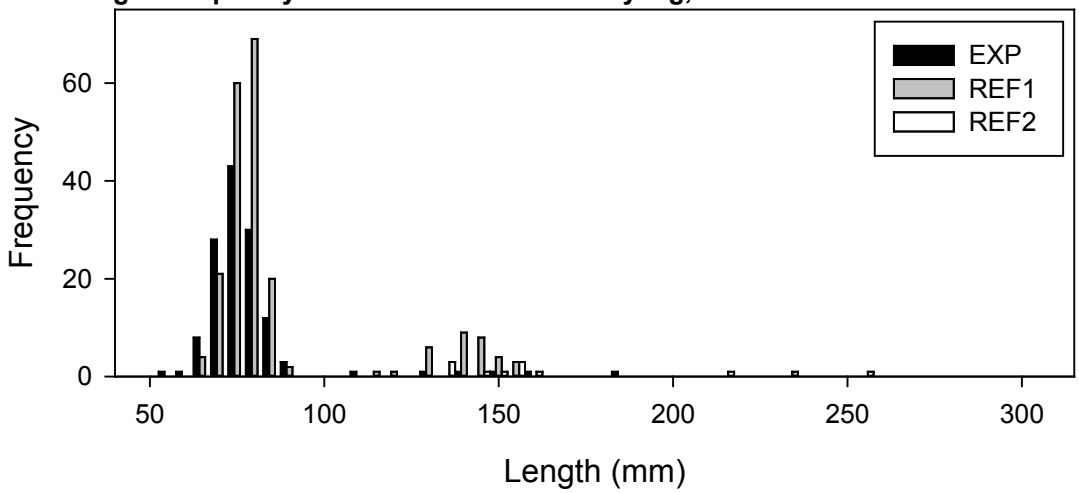
Parameter	Endpoint	Test	Interaction		Main Effect		MSE	LSM			Contrasts			Magnitude (% difference)			SSD	Direction REF1 / REF2	Power Analysis	
			$Fstat_{(\alpha, df1, df2)}$	*P*-value	$Fstat_{(\alpha, df1, df2)}$	*P*-value		EXP	REF1	REF2	EXP* REF1	EXP* REF2	REF1* REF2	EXP* REF1	EXP* REF2	REF1* REF2			Power	n
Survival	Length-frequency distribution (All)	K-S	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	**<0.001**	**<0.001**	**<0.001**	-23.4[a]	-97.0[a]	-88.0[a]	Yes	↓ / ↓↓	n/a	n/a
Growth (energy use)	Total body weight (YOY)	K-W	n/a	n/a	n/a	**0.017**	n/a	2.74	2.92	-	**0.017**	-	-	-6.3	-	-	Yes	↓ / -	n/a	n/a
		$ANOVA_{log}$	*n/a*	*n/a*	*$7.194_{(0.1, 1, 299)}$*	***0.008***	*0.011*	*2.65*	*2.86*	-	***0.008***	-	-	*-7.3*	-	-	*Yes*	↓ / -	*n/a*	*n/a*
	Total length (YOY)	K-W	n/a	n/a	n/a	**<0.001**	n/a	73.3	75.4	-	**<0.001**	-	-	-2.8	-	-	Yes	↓ / -	n/a	n/a
	Total body weight (Juveniles)	K-W	n/a	n/a	n/a	**0.006**	n/a	21.8	22.4	44.5	0.840	**0.016**	**0.001**	0.0	-51.0	-66.1	Yes	- / ↓↓	n/a	n/a
		ANOVA	*n/a*	*n/a*	*$5.312_{(0.1, 2, 44)}$*	***0.009***	*26.5*	*21.7*	*19.9*	*26.2*	*0.695*	*0.240*	***0.006***	*0.0*	*0.0*	*-27.4*	*Yes*	*- / -*	*n/a*	*n/a*
	Total length (Juveniles)	K-W	n/a	n/a	n/a	**0.090**	n/a	143.7	141.4	160.6	0.638	0.261	**0.028**	0.0	0.0	-12.7	Yes	- / -	n/a	n/a
Reproduction (energy use)	Relative abundance of YOY[b]	K-S	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0.533	0.778	**0.069**	0.0[a]	0.0[a]	-46.2[a]	Yes	↓ / ↑	n/a	n/a
Condition (energy storage)	Length-adjusted weight (All)	$ANCOVA_{rank}$	$0.037_{(0.05, 2, 346)}$	0.963	$1.246_{(0.1, 2, 348)}$	0.289	2308	176	172	194	n/a	n/a	n/a	0.0	0.0	0.0	No	- / -	0.600	263
	Length-adjusted weight (YOY)	$ANCOVA_{rank}$	$1.625_{(0.05, 1, 298)}$	0.203	$0.655_{(0.1, 1, 299)}$	0.419	2619	151	146	-	n/a	n/a	-	0.0	0.0	-	No	- / -	0.789	216
	Length-adjusted weight (Juveniles)	$ANCOVA_{rank}$	$0.358_{(0.05, 2, 44)}$	0.701	$3.821_{(0.1, 2, 46)}$	**0.029**	72	21	24	32	0.640	**0.045**	**0.057**	0.0	-34.1	-26.4	Yes	- / ↓↓	n/a	n/a

Note: Values in *italics* indicate results were calculated with statistical outliers removed (i.e., studentized residuals greater than 3.0). Significant differences are indicated in **bold**.
a) Magnitude based on maximum percent difference between length-frequency distributions.
b) Relative abundance of YOY tested by comparing length-frequency distributions among areas with and without YOY included.
Fstat = test statistic; *P*-value = probability value; MSE = mean squared error; LSM = least squares mean; SSD = statistically significant difference (α= 0.1); EXP = Exposure Area; REF1 = Reference Area 1; REF2 = Reference Area 2; n = sample size; ANOVA = Analysis of Variance; ANCOVA = Analysis of Covariance; log = log 10 transformed data; rank = rank transformed data; K-W = Kruskal-Wallis; K-S = Kolmogorov–Smimov; <= less than; n/a = not applicable; ↓ = significantly lower at the Exposure Area; ↓↓ = significantly lower at the Exposure Area and magnitude exceeded 25% (10% for length-adjusted weight); ↑ = significantly greater at the Exposure Area; ↑↑ = significantly lower at the Exposure Area and magnitude exceeded 25% (10% for length-adjusted weight).

6.3.3.3.1 Survival

Arctic Grayling length-frequency distributions were significantly different among areas (Table 6.3-12; Figure 6.3-3). A maximum difference in distribution of 23.4% was observed between the Exposure Area and Reference Area 1, and 97.0% between the Exposure Area and Reference Area 2. Length-frequency distributions were also significantly different among reference areas with a maximum difference of 88.0%. Differences among areas resulted from differences in the size and abundance of YOY. Young-of-the- year collected from the Exposure Area were generally smaller than individuals collected from Reference Area 1. Differences in the proportion of YOY was also responsible for differences among reference areas, with YOY absent from the Reference Area 2.

Figure 6.3-2: Length Frequency Distribution of Arctic Grayling, 2016



EXP = Exposure Area; REF1 = Reference Area 1; REF2 = Reference Area 2.

6.3.3.3.2 Energy Use

Significant differences were observed in growth of YOY between the Exposure Area and Reference Area 1 (Table 6.3-12). Reference Area 2 was not included in the comparison, as no YOY were collected from that location (Table 6.3-11). Body weight was 6.3% lower at the Exposure Area when compared to Reference Area 1 (7.3% when outliers were omitted), and length was 2.8% shorter. When outliers were omitted from the analysis, there were no significant differences in the length and weight of juvenile fish sampled from the Exposure Area when compared to Reference Area 1 and Reference Area 2; however, there were significant differences among reference areas. Fish collected from Reference Area 1 had body weights 27.4% lower than Reference Area 2, and were 12.7% shorter in length. When outliers were included in the analysis, juvenile Arctic Grayling from the Exposure Area weighed 51% less than fish collected from Reference Area 2, resulting from the inclusion of two large fish from Reference Area 2 (>85 g; Table 6.3-11).

The relative abundance of YOY varied significantly among areas, with a greater proportion of YOY observed at the Exposure Area when compared to Reference Area 1 and Reference Area 2 (Table 6.3-12). A greater proportion of YOY was also observed at Reference Area 1 compared to Reference Area 2, where no YOY were collected.

6.3.3.3.3 Energy Storage

Length-adjusted weight was not significantly different among areas when all Arctic Grayling were considered, or when YOY were compared separately (Table 6.3-12). Juvenile fish were not significantly different between the

Exposure Area and Reference Area 1, but juvenile fish from the Exposure Area weighed 34.1% less than juvenile fish collected from Reference Area 2 (Table 6.3-12). Juvenile fish from Reference Area 1 weighed 26.4% less than juvenile fish from Reference Area 2.

6.3.4 Supporting Data

Detailed results for the supporting environmental information are provided in Section 4.0.

6.3.5 Quality Assurance and Quality Control

North South Consultants had difficulties both removing the structures from the parafilm and, once removed, accurately distinguishing the yearly annuli from daily growth annuli on the submitted otoliths. The confidence in age estimations was rated 'fair' at best. Upon review of the results, several of the ages did not correlate to the expected size of fish, as had been observed for other northern EEM programs (e.g., Con Mine 2010), and so it was determined that the aging data was unreliable. Since ages could not be reliably assessed using otoliths, length-frequency distributions were used to assign age classes as per the TGD (EC 2012a).

6.4 Summary

The results of the lethal Ninespine Stickleback survey identified significant differences in EEM effect endpoints between the Exposure Area and one or more reference areas for both male and female fish. For male fish, significant differences were observed in body weight-at-age for age 1+ and 2+ fish, relative gonad size and age, with differences exceeding EEM critical effects sizes (Table 6.4-1). For female fish, significant differences were observed for body weight-at-age for age 1+ fish and age. A significant difference was also observed for female condition when outliers were omitted from the comparison. These differences exceeded critical effect sizes for both body weight-at-age and age. Based on these results, Ninespine Stickleback sampled from the Exposure Area may have invested less energy in growth and may have reduced survival when compared to the reference areas; however, differences of a similar magnitude were also observed between the reference areas for age of males and body weight-at-age of males and females (Table 6.3-9), suggesting these differences may reflect variation in habitat, rather than Mine related effects. Female age was the only EEM effect endpoint that was significantly different between the Exposure Area and both reference areas, without significant differences between the reference areas.

The results of the non-lethal Arctic Grayling YOY survey identified significant differences in EEM effect endpoints between the Exposure Area and Reference Area 1. Arctic Grayling YOY were significantly shorter and weighed less than YOY sampled from Reference Area 1, however the magnitude of these differences were small and below EEM critical effect sizes (Table 6.4-2). The relative abundance of YOY also varied between sampling areas, with a smaller proportion of YOY occurring at the Exposure Area when compared to Reverence Area 1. No YOY were captured from Reference Area 2S for comparison.

Table 6.4-1: Summary of Key Results for Lethal Fish Survey EEM Effect Endpoints

Parameter	Endpoints	Male			Female		
		SSD	Magnitude (%)	>CES	SSD	Magnitude (%)	>CES
Growth	Body weight-at-age 1+	REF2	-26.8	Yes	REF1	36.5	Yes
	Body weight-at-age 2+	REF1 / REF2	-15.4 / -29.6	No / Yes	No	0.0	No
	Body weight-at-age 3+	No	0.0	No	No	0.0	No
	Body weight-at-age 4+	n/a	n/a	n/a	No	0.0	No
Reproduction	Relative gonad size (gonad weight to body weight)	REF1	33.5	Yes	No	0.0	No
Condition	Condition (body weight to length)	No	0.0	No	No (REF1)	0.0 (-4.3)	No (No)
	Relative liver size (liver weight to body weight)	No	0.0	No	No	0.0	No
Survival	Age	REF2	-26.1	Yes	REF1 / REF2	-37.0 / -45.4	Yes/Yes

Notes: Comparisons are between the Exposure Area and Reference Area 1 and Reference Area 2.
Comparisons with outliers omitted from the analysis are indicated in brackets.
SSD = statistically significant difference; >CES = greater than critical effect size; REF1 = Reference Area 1; REF2 = Reference Area 2; n/a = not applicable.

Table 6.4-2: Summary of Key Results for Non-Lethal Fish Survey EEM Effect Endpoints.

Parameter	Endpoint	SSD	Magnitude (%)	>CES
Growth	Length of YOY (age 0) at end of growth period	REF1[(a)]	-2.8	No
	Weight of YOY (age 0) at end of growth period	REF1[(a)]	-7.3	No
Reproduction	Relative abundance of YOY (% composition of YOY)	REF1 / REF2	n/a	n/a
Condition	Body weight at length	No	0.0	No
Survival	Length-frequency distribution	No	0.0	No

a) No young-of-the-year were collected from Reference Area 2 for comparison.
Note: Comparisons are between the Exposure Area and Reference Area 1 and Reference Area 2.
SSD = statistically significant difference; >CES = greater than critical effect size; REF1 = Reference Area 1; REF2 = Reference Area 2; n/a = not applicable.

7.0 GENERAL FINDINGS

7.1 Benthic Invertebrate Community

Overall, the results of the Phase 5 EEM benthic invertebrate community survey indicate that Mine-related effects on the benthic invertebrate community were not present in the Exposure Area, the Seep Creek Ponds. Based on the Phase 5 EEM results, none of the effect or supporting endpoints have confirmed effects between two consecutive benthic invertebrate surveys.

For all EEM effect endpoints and supporting endpoints, the magnitude of differences did not exceed the CES, indicating that observed non-significant differences between the Exposure and Reference Areas are also not biologically significant. Therefore, based on the Phase 5 EEM results, no effect of the residual effluent was found on the benthic invertebrate community in the Exposure Area. This is generally consistent with the Phase 2 EEM results, which indicated a potential effect on two benthic invertebrate variables (total density and BCI; AECOM 2009), but with a direction of effect on total density that was inconsistent with an adverse effect.

7.2 Fish Survey

A lethal and non-lethal fish survey was conducted in 2016 for the Lupin Mine Phase 5 EEM program. Ninespine Stickleback were the target fish species for the lethal fish survey and YOY Arctic Grayling were the target fish species for the non-lethal fish survey in the Exposure Area, Reference Area 1 and Reference Area 2. Lethal analysis of the Ninespine Stickleback and non-lethal analysis of the Arctic Grayling allowed for the detection of effects on survival, energy storage, and energy use between the exposure and reference areas.

Ninespine Stickleback from both areas were generally in good overall health. Ninespine Stickleback from the Exposure Area may have invested less energy in growth and may have reduced survival when compared to both reference areas. However, the habitat identified in each area varies greatly. Therefore these differences in growth and survival may be a reflection of the variation in habitat, rather than Mine related effects. The only endpoint with significant difference between the Exposure Area and both Reference Areas was the age of females, in that the Exposure Area appears to have younger female Ninespine Stickleback than both Reference Areas.

Arctic Grayling from both areas were generally in good health. However, YOY captured in the Exposure Area were shorter, weighted less and were less abundant than those captured in Reference Area 1. This finding is consistent with findings from all previous year studies (AECOM 2009). No Arctic Grayling YOY were captured in Reference Area 2. This absence of YOY in Reference Area 2 is likely due to the difference in available habitat and increased presence of competition from large-bodied salmonids, such as Arctic Char and Lake Trout. No Arctic Grayling spawning habitat was available in Reference Area 2, which may also contribute to the absence of YOY.

As in previous years, Ninespine Stickleback and Arctic Grayling were the most abundant species in all three sampling areas. Backpack electrofishing was the most effective fishing method for both species in all sample areas, with the exception of the fyke net for the capture of Ninespine Stickleback in Reference Area 2. Species richness was the lowest in the Exposure Area, with four species being captured in comparison to seven species captured in both Reference Areas. This difference in species richness was similar to that seen in previous years. In general, CPUEs were higher in 2016 than in previous years, though not all fishing techniques were utilized during each EEM Phase.

8.0 RECOMMENDATIONS FOR PHASE 6

The Phase 5 EEM program was a requirement of the MMER under the federal *Fisheries Act*. Phase 1 was conducted in 2005 as a Periodic Monitoring – Surveillance program and Phase 2 was conducted in 2008 as a Periodic Monitoring – Confirmation program. Both programs identified significant differences in fish growth endpoints between the Exposure and Reference areas. Phase 3 was conducted in 2010 as an Investigation of Cause (IOC) to explore the possibility of temperature as a factor in size differences; the results of the study did not verify that hypothesis. A Phase 4 study was developed and set to be carried out in 2013 as a Periodic Monitoring - Surveillance program; the program was never completed due to the suspension of all activities at the site in August 2013. The Phase 5 EEM biological monitoring program was conducted in 2016 as a Periodic Monitoring – Surveillance program.

Endpoints that exceeded critical effect sizes were observed in male fish for body weight-at-age for age 1+ and 2+, and in female fish for body weight-at-age for age 1+ fish and age between the Exposure Area and Reference Area 1 and 2. However, it is possible that these differences were as a result of difference in habitat, and not due to the effects of mine effluent. In addition, a difference between the size and weight of YOY Arctic Grayling between the Exposure Area and Reference Area 1 was observed, but the difference did not exceed the critical effect size. This observation was also consistent with findings from Phase 3. Due to the Phase 4 program not being completed as planned, there are not two consecutive EEM programs with the same effects. In addition, results of the Phase 2 and Phase 5 benthic invertebrate monitoring studies indicate no consistent effects on the benthic invertebrate community (i.e., no confirmed effect). The Phase 6 EEM program should be completed as a Periodic Monitoring – Surveillance program, designed similar fashion to Phase 5. The Phase 6 EEM program should be completed 36 months after completion of the Phase 5 EEM program (i.e., 2019 for the field program and 2020 for the interpretative report) with the Phase 6 study design submitted at least six months before the planned field program. After completion of the Phase 6 EEM, it can be established whether the exceedance of critical effect sizes are consistent. Efforts should also be made in Phase 6 to investigate possible causes of the difference in size of YOY Arctic Grayling between the Exposure Area and Reference Area 1.

Observations made during the Phase 5 EEM program support the elimination of Reference Area 2 from the non-lethal Arctic Grayling survey. No YOY Arctic Grayling were found during the field component of the study, and suitable spawning habitat was not located at this site. It is reasonable to conclude that due to the lack of spawning habitat present, in addition to the competition from larger salmonid species and life stages, that YOY Arctic Grayling do not utilize this watercourse.

Aside from the absence of YOY Arctic Grayling in Reference Area 2, the target sample sizes for the lethal (30 adult male, 30 adult female, 30 juvenile) Ninespine Stickleback survey and the non-lethal (100 to 400 YOY) Arctic Grayling survey seem achievable. The use of the fyke net in Reference Area 2 to capture Ninespine Stickleback was highly effective, and should be included from the start of the program.

The statistical analysis for the Phase 6 EEM program should only include those tests that are required under the MMER regulations. Additional tests can be included if they are deemed necessary.

9.0 CLOSURE

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10.0 REFERENCES

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12.0 UNITS OF MEASURES, ABBREVIATIONS AND ACRONYMS

Unit or Term	Definition
±	plus or minus
<	less than
>	greater than
≤	less than or equal to
≥	greater than or equal to
%	percent
% Sat	percent saturation
Bq/L	Becquerels per litre
ALS	Analytical Laboratory Services
ANCOVA	analysis of covariance
ANOVA	analysis of variance
BCI	Bray-Curtis index
CPUE	catch-per-unit-effort
CSQG	Canadian Sediment Quality Guidelines
CWQG	Canadian Water Quality Guidelines
DL	detection limit
DO	dissolved oxygen
EEM	Environmental Effects Monitoring
Golder	Golder Associates Ltd.
GSI	gonadosomatic index
IC_{25}	25% inhibition concentration
IOC	Investigation of Cause
ISQG	interim sediment quality guidelines
k	Fulton's Condition Factor
K-S	Kolmogorov-Smirnov test
LC_{50}	median lethal concentration
LMI	Lupin Mines Inc.
LSI	liversomatic index
MMER	Metal Mining Effluent Regulations
PEL	probable effects limit
QA	quality assurance
QC	quality control
R^2	coefficient of determination
r_s	Spearman rank correlations
SD	standard deviation
SDI	Simpson's Diversity Index

Unit or Term	Definition
SE	standard error
SEI	Simpson's Evenness Index
SR	studentized residuals
TAP	Technical Advisory Panel
TCA	tailings containment area
TDS	total dissolved solids
TGD	*Metal Mining Technical Guidance for Environmental Effects Monitoring*
TOC	total organic carbon
TSS	total suspended solids
UTM	Universal Transverse Mercator
YOY	young-of-the-year
α	Type I error
β	Type II error