Appendix 37

Whale Tail Addendum to First Biological Monitoring Study Design

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Addendum to Whale Tail Pit First Biological Monitoring Study Design

The following are Agnico Eagle's responses to comments and recommendations provided by the Technical Advisory Panel (TAP) in the letter entitled '*Whale Tail Pit 1st EEM Study Design – action items identified*' regarding the '*Environmental Effects Monitoring: Agnico Eagle Mines Ltd. – Whale Tail Pit, First Biological Monitoring Study Design*", submitted July 26, 2019.

Action Items

1. **P. 1 (NWB): The study design proposes that Lake D1 and Lake 8 be used as reference lakes for the EEM biological monitoring study. We have noted, however, that the request from Agnico Eagle Mines Ltd. (Agnico Eagle) to amend Water Licence No. 2AM-WTP1826 included potential discharge of mine site effluent to Lakes D1 and D5. Agnico Eagle indicated during the NWB Technical Meeting in October 2019 that an approval to discharge to D1 and D5 would not be requested from the Board; however, there have been additional submissions from the proponent indicating that the request still may arise as part of the Water Licence amendment process. Should the Water Licence be amended such that mine site effluent could be discharged to Lake D1, the Board recommends that Agnico Eagle modify the study design so that Lake D1 is not included as a reference area for the EEM biological monitoring study. Moreover, if the Water Licence is amended so that effluent could be discharged to any other additional locations not considered in the current study design, the Board recommends that Agnico Eagle consider monitoring effects at the additional discharge locations.**

   Response
   Unless effluent is discharged to Lake D1, it is considered to be a reference area. If, in the future, the Water License is amended and mine site effluent is discharged to Lake D1, then Lake D1 will become an exposure area. All active discharge points are considered during each EEM cycle and the biological studies examine the discharge point that is considered most likely to affect fish and benthic invertebrates.

2. **P. 4 (ECCC): The study design indicates that concentrations of mercury and selenium in effluent were below the values that would require studies of fish tissue concentrations. However, this was based on effluent samples taken on March 11 and May 6, 2019. Results from the effluent sample taken on July 3, 2019 were not included in the report; neither were results from samples collected in 2018. The requirement to conduct fish tissue studies is dependent on the results of effluent characterization for samples collected beginning on the day the mine became subject to section 7 of the *Metal and Diamond Mining Effluent Regulations* (MDMER) and ending on the day before the first study design is required to be submitted (Schedule 5, paragraph 9(3)(a)). Please ensure all effluent characterization results from this timeframe are reviewed to determine if fish tissue studies are required in accordance with Schedule 5, paragraphs 9(1)(c) and (d).**

   Response
   The results of all mercury and selenium analyses of effluent at Whale Tail Pit conducted prior to July 27, 2019, are presented in Table 1. Please note that in the Study Design Report the sampling date July 2, 2019, was incorrectly indicated to be July 3, 2019. Mercury concentrations were less than the detection

limit of 0.01 µg/L (0.00001mg/L) in all but one sample; in that sample the concentration was 0.02 µg/L. Therefore, a study of mercury in fish tissue is not required.

Selenium concentrations were less than the detection limit of 0.5 µg/L (0.0005 mg/L) in all but one sample; in that sample the concentration was 0.9 µg/L. Therefore, a study of selenium in fish tissue is not required.

Table 1. Mercury and selenium concentrations in effluent samples collected at Whale Tail Pit prior to July 27, 2019.

Discharge location	Date	Mercury		Selenium	
		mg/L		mg/L	
ST-MDMER-6-EEM Whale Tail North dewatering temporary diffuser in Mammoth Lake (65°23'54.43'' N 96°43'35.41'' W)	June 23, 2019	<	0.00001	<	0.0005
	July 2, 2019	<	0.00001		0.0009
ST-MDMER-5-EEM Whale Tail North dewatering temporary diffuser in Whale Tail South (65°23'51.30''N 96°40'49.00'' W)	March 11, 2019	<	0.00001	<	0.0005
	May 6, 2019		0.00002	<	0.0005
	July 17, 2019	<	0.00001	<	0.0005
ST-MDMER-4-EEM Temporary discharge of Whale Tail Lake water treated for TSS during dike construction (65°24'10.82''N 96°40'56.09'' W)	August 6, 2018	<	0.00001	<	0.0005

3. **P. 34 (CIRNAC): The study design report states:**

If the summer diffusers are installed and effluent is discharged in Q3 of 2019, a plume delineation study will be undertaken using specific conductance as a tracer. If this is not possible, after the diffusers are installed and their exact locations are known, the effluent concentrations at 100 m and 250 m from the diffusers will be predicted using the CORMIX model. The results of this plume delineation or the modelling will be used to refine the exposure zones for the EEM fish and benthic invertebrate studies and will be reported to Environment and Climate Change Canada by December 31, 2019.

CIRNAC recommends that the plume delineation results, if available, be used to justify or revise EEM sampling locations in Mammoth Lake.

<u>Response</u>
A plume delineation study was not conducted in Q3. A plume modelling study, conducted by Golder Associates Ltd. (2020), is provided in Appendix B. The results confirm that both fish and benthic invertebrate studies are required. The plume modelling results were used to select the fish and benthic invertebrate sampling locations. Those locations are provided in the response to Question 5 (below).

Reference: Golder Associate Ltd. April 2020. Predicted 1% water treatment plant effluent dilution

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location in Mammoth Lake. Technical Memorandum. Project No. 19136093-443-TM-Rev0. 8pp + appendices.

4. **P. 34 (ECCC): Further to comment #3, the MDMER require the first study design to include an estimate of the concentration of effluent in the exposure area at 100 m and 250 m from every point at which the effluent enters the area from a final discharge point (Schedule 5, subparagraph 10(a)(i)). The study design includes CORMIX model estimates for the distance at which the effluent will be at a dilution of 27, under different seasons and flow rates. However, the study design does not include estimates of effluent concentration based on measured or modeled values at 100 m and 250 m. The study design does indicate that plume delineation or modeling will be undertaken in 2019 and that results will be provided to Environment and Climate Change Canada. Please provide the required information regarding effluent mixing and effluent concentrations at 100 m and 250 m from the point(s) at which effluent enters the exposure area.**

Response
A plume modelling study, conducted by Golder Associates Ltd., is provided in Appendix B. The results (refer to Table 1 in Golder, 2020) indicate that after one week of effluent discharge the effluent concentration will exceed 1% at 100 m and potentially at 250 m from the point(s) at which effluent enters the exposure area. After two weeks of effluent discharge the effluent concentration will exceed 1% at 250 m from the point(s) at which effluent enters the exposure area.

5. **P. 35 (ECCC): The MDMER require that the first study design includes a description of and scientific rationale for the sampling areas selected for the fish population study (Schedule 5, subparagraph 10(b)(i)) and the benthic invertebrate study (Schedule 5, subparagraph 10(c)(i)). The selection of the sampling areas is one of the most critical components of the study design and should be considered carefully to maximize the quality of the information gained from the study (EC 2012, section 2.2.2.2). Please provide more specific information on the sampling locations for fish and benthic invertebrates. In particular, please indicate whether they differ from sampling locations used in the past, describe where sampling areas support habitat for benthic invertebrates and fish species, and detail the relationship between the effluent discharge locations and the exposure sampling areas.**

Response
It is currently proposed that fish studies be conducted using Lake Trout and Slimy Sculpin as sentinel species. Lake Trout will be captured with gill nets set within the area where the effluent concentration will exceed 1% at the surface (and bottom) within two weeks of effluent discharge beginning (Figure 1). Slimy sculpin will be captured by backpack electrofishing along the shoreline. This will occur along shorelines where the concentration of effluent at the bottom exceeds 1% within two weeks of effluent discharge beginning (Figure 1), which is deemed appropriate because Slimy Sculpin is a benthic fish. This area includes the locations where Slimy Sculpin were collected in 2018 and 2019 for an ongoing University of Waterloo study. The specific locations where gill nets will be set and electrofishing will be conducted will be determined based on conditions in the field, including wind velocity and direction. If the necessary numbers of fish cannot be captured with a reasonable amount of effort within these areas Environment and Climate Change will be consulted to determine if the sampling area should be

expanded or a lesser sample size accepted.

The benthic invertebrate sampling will occur within the area shown in Figure 1, below. This is the area where benthic invertebrate samples for the CREMP have been collected annually since 2015. This area was selected based on the depth, which matches the sampling depth for CREMP sampling, and the substrate, which is similar to the substrate for most CREMP sampling locations. This area will be exposed to effluent concentration of 1% or more within two weeks of the start of effluent discharge (refer to Figure 1 in Golder, 2020).

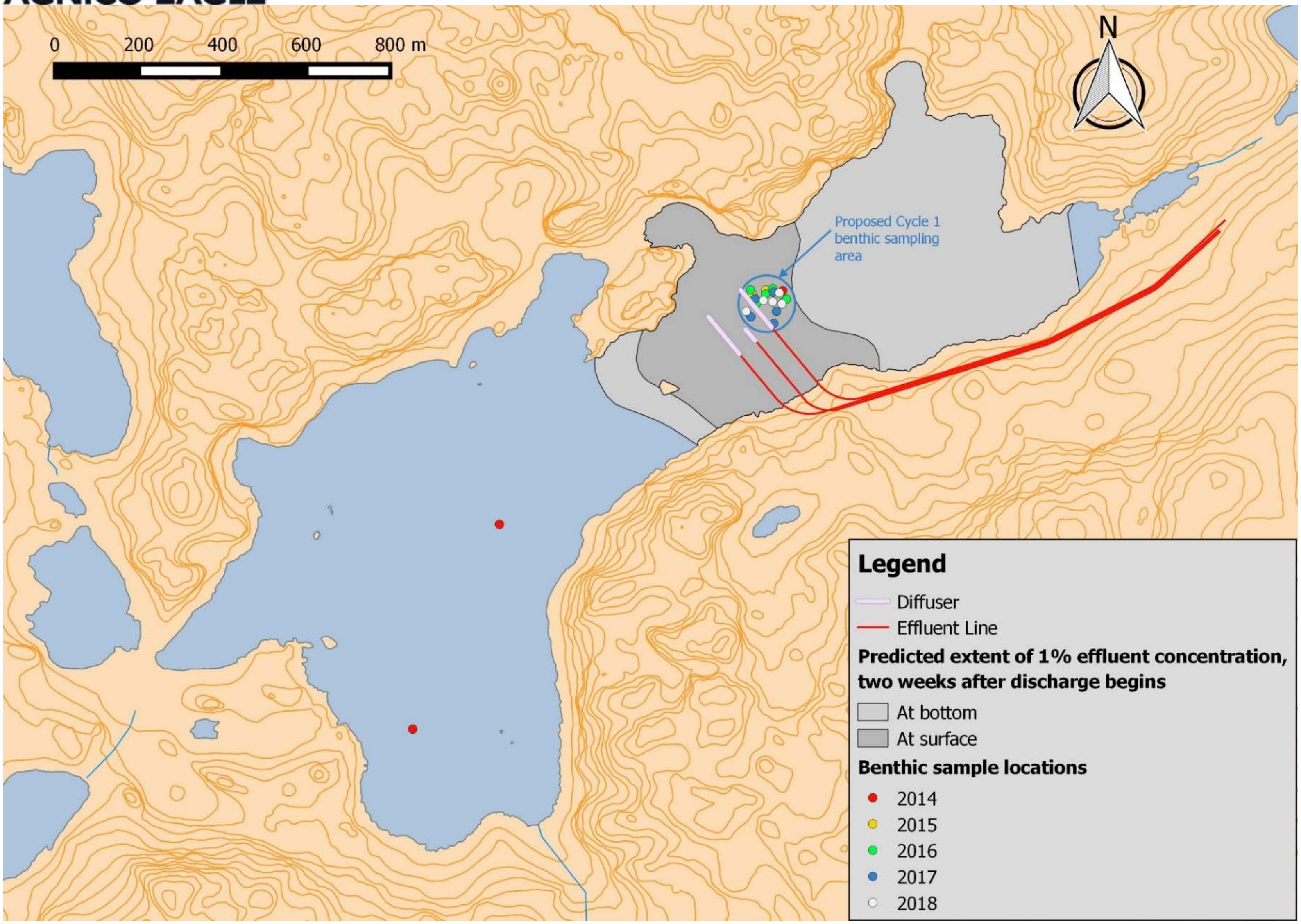

Figure 1. Extent of the 1% effluent plume two weeks after effluent discharge begins. The proposed benthic invertebrate sampling area, and the locations where benthic invertebrate samples were collected in previous years, are also shown.

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6. **Pp. 36-45 (ECCC): The MDMER require the first study design to include an explanation as to how the study will provide the information necessary to determine if the effluent has an effect on the fish population (Schedule 5, subparagraph 10(b)(ii)). The MDMER further elaborate that interpretative reports shall contain effect indicators of growth, reproduction, condition and survival that include, if practicable, the length, total body weight and age of the fish, the weight of its liver or hepatopancreas and, if the fish are sexually mature, the egg weight, fecundity and gonad weight of the fish (Schedule 5, subparagraph 12(1)(e)(i)). As detailed in comments below, the proposed lethal fish studies do not adequately assess all the required effect indicators. Please describe an approach that will provide all required information for the fish population study and be explicit in how the fish studies will determine if the effluent has an effect on growth, reproduction, condition and survival. If there are concerns about the potential impacts of sampling on small fish populations, non-lethal sampling may be warranted (EC 2012; section 3.4.2).**

Response

Please refer to Appendix A, which describes the revised proposed fish study. A non-lethal study is proposed for Slimy Sculpin.

7. **Pp. 36, 39 and 43 (ECCC): The study design indicates that reproductive investment will not be assessed in the lethal lake trout study since only a portion of the adult lake trout are expected to spawn each year. As well, the study design indicates that reproductive endpoints will not be assessed in slimy sculpin. Even though samples sizes for sexually mature lake trout may be small, note that the MDMER require an assessment of fish reproduction, that includes, if practicable, the egg weight, fecundity and gonad weight of sexually mature fish (Schedule 5, paragraphs 12(1)(e), (h), (i) and (j)). For a lethal fish survey, the recommended effect indicator for reproduction is gonad weight at total body weight (Table 3-3, EC 2012). Statistical analysis of gonad weight at total body weight can be done using ANCOVA (EC 2012; section 8.3.3.2). Additionally, the MDMER have defined a critical effect size of ±25% of the reference mean for gonad weight at total body weight (Schedule 5, subsection 1(2)); use of critical effect sizes to determine future monitoring requirements is contingent on analysis of effect indicators and calculation of effect sizes as specified in the MDMER. Please provide a description of any additional methods for the lake trout and slimy sculpin fish survey to ensure collection of all required information, including an assessment of fish reproduction, in accordance with Schedule 5, paragraph 10(b). Note that the proportion of fish developing to spawn can also be analysed (EC 2012, section 3.4.1), but is not regarded as an effect indicator for the fish survey (Table 3-3, EC 2012).**

Response

Agnico is aware of no additional methods that will ensure collection of all required information, including an assessment of fish reproduction. Please refer to Appendix A, which describes the revised proposed fish study.

8. **P. 37 (ECCC): The MDMER require that the first study design include a description of and the scientific rationale for the sampling period selected for the fish population study (Schedule 5, subparagraph 10(b)(i)). The study design indicates that lake trout will be collected in August 2020. The *Metal Mining Technical Guidance for EEM* (Table 3-4, EC 2012) suggests that synchronous fall spawners be sampled in early to mid-summer. Please note our previous comment regarding the requirement to measure egg weight, fecundity and gonad weight of sexually mature fish, and consider whether the timing of sample collection for lake trout is appropriate.**

<u>Response</u>
Agnico now propose to conduct the fish sampling in the second half of August.

Please refer to Appendix A, which discusses the proposed fish study and the impossibility of conducting a fish study that obtains a sufficient number of mature fish that will spawn in the current year to investigate egg weight, fecundity and gonad weight. Agnico note that Table 3-4 (EC, 2012) provides generalized guidance regarding sampling times, and Table 3-5 (revised April 2013) provides species-specific guidance.

For Lake Trout, Table 3-5 (revised April 2013) recommends collecting fish 4 – 6 weeks prior to spawning. The exact time of Lake Trout spawning at Whale Tail Pit is not known, however, based on three previous EEM fish studies using Lake Trout at the Meadowbank site, sampling in the second half of August is appropriate. In those studies, conducted in the latter part of August and into the beginning of September, eggs were well developed in the few mature females that would spawn in the current year that were captured in those studies, but the fish were not spontaneously expelling eggs during handling (i.e. were not ripe). Likewise, males were not expelling quantities of milt during handling.

For Slimy Sculpin, Table 3-5 (revised April 2013) recommends collecting fish 4 – 6 weeks prior to spawning, however the lakes in the Whale Tail Pit region are frozen at that time. Arciszewski et al (2010) recommend sampling Slimy Sculpin for EEM purposes in northern lakes in late August or even September. In the Whale Tail Pit region, the weather deteriorates around the beginning of September, with higher winds and precipitation falling as snow. Therefore, it is desirable to complete fish sampling by the end of August, if possible.

Reference: Arciszewski, T. M. Gray, K. Munkittrick and C. Baron. 2010. Guidance for the collection and sampling of slimy sculpin (Cottus cognatus) in northern Canadian lakes for environmental effects monitoring (EEM). Canadian Technical Report of Fisheries and Aquatic Sciences 2909.

9. **Pp. 39 and 43 (ECCC): The study design indicates that specific conductance, pH, dissolved oxygen and temperature will be determined in the field during fish sampling. Please note that the MDMER require that water quality monitoring be conducted at the same time as the biological monitoring studies, on samples of water collected from the fish and benthic invertebrate sampling areas (Schedule 5, section 7). Additionally, the MDMER identify the various parameters that are to be included in this water quality monitoring (Schedule 5, section 7). Please ensure this required water quality monitoring is included in the study design. Clarify whether the water quality monitoring described for the benthic invertebrate sampling (p. 51) will also apply for the**

fish sampling.

Response
Agnico will conduct the required water quality monitoring. In reference lakes, the water quality monitoring for the benthic invertebrate sampling will also serve as the sampling for the fish study, as there is no reason to expect significant spatial variation. In Mammoth Lake additional samples will be collected if conductivity indicates that there is significant spatial variability. Note that the lakes do not stratify, so variation with depth is not a concern.

10. **Pp. 39-40 and 43-44 (ECCC): The study design indicates that age will be estimated for each lake trout and slimy sculpin that is part of the lethal sample, and that length versus age will be analysed. However, the study design does not include age itself as an endpoint to assess survival. Note that the MDMER require an assessment of fish survival that includes, if practicable, the age of the fish (Schedule 5, paragraphs 12(1)(e), (h), (i) and (j)). Age is the recommended effect indicator to assess survival in a lethal fish survey (Table 3-3, EC 2012). Statistical analysis of age can be done using ANOVA (EC 2012; section 8.3.3.1). Moreover, the MDMER have defined a critical effect size for age of ±25% of the reference mean (Schedule 5, subsection 1(2)); use of critical effect sizes to determine future monitoring requirements is contingent on analysis of effect indicators and calculation of effect sizes as specified in the MDMER. Please provide a description of any additional methods for the lake trout and slimy sculpin fish survey to ensure collection of all required information, including an assessment of fish survival, in accordance with Schedule 5, paragraph 10(b).**

Response
Please refer to Appendix A, which describes the revised proposed fish study. The number of Lake Trout that would have to be sampled in order to achieve the desired power for total weight at age and mean age is considerably higher than proposed.

11. **P. 40 and 44 (ECCC): The study design indicates that growth in lake trout and slimy sculpin will be assessed using length at age. The report authors suggest that this is appropriate for lake trout since approximately twice as many fish per site would be required to achieve adequate power for the weight versus age relationship; no rationale is provided for this approach in the slimy sculpin study. Note that the MDMER require an assessment of fish growth that includes, if practicable, the length, total body weight and age of the fish (Schedule 5, paragraphs 12(1)(e), (h), (i) and (j)). Body weight at age is the recommended effect indicator to assess growth in a lethal fish survey (Table 3-3, EC 2012); length at age can be used as a supporting endpoint. Statistical analysis of body weight at age can be done using ANCOVA (EC 2012; section 8.3.3.2). Additionally, the MDMER have defined a critical effect size of ±25% of the reference mean for total body weight at age (Schedule 5, subsection 1(2)); use of critical effect sizes to determine future monitoring requirements is contingent on analysis of effect indicators and calculation of effect sizes as specified in the MDMER. Please provide a description of any additional methods for the lake trout and slimy sculpin fish survey to ensure collection of all required information, including an assessment of fish growth, in accordance with Schedule 5, paragraph 10(b). If there are concerns about the potential impacts of sampling on small fish populations, non-lethal sampling may be warranted (EC 2012; section 3.4.2).**

Response

Please refer to Appendix A, which describes the revised proposed fish study.

12. P. 41 (ECCC): The study design indicates that water levels in Whale Tail Lake and three upstream lakes are expected to increase during Whale Tail pit operations. Please provide additional information on the extent of this flooding and how this potentially confounding factor will be addressed by the study design.

Response

As shown in Figure 2, below, water levels have increased south of Whale Tail Dike and the water from south Whale Tail Lake and lakes upstream will be rerouted during mine operations to flow into the west end of Mammoth Lake. The benthic invertebrate and fish sampling locations are in the eastern portion of Mammoth Lake, which will minimize exposure to that inflow. These changes will be confounding factors. The CREMP benthic invertebrate data from 2019, prior to the diversion being constructed, maybe useful in parsing out these factors.

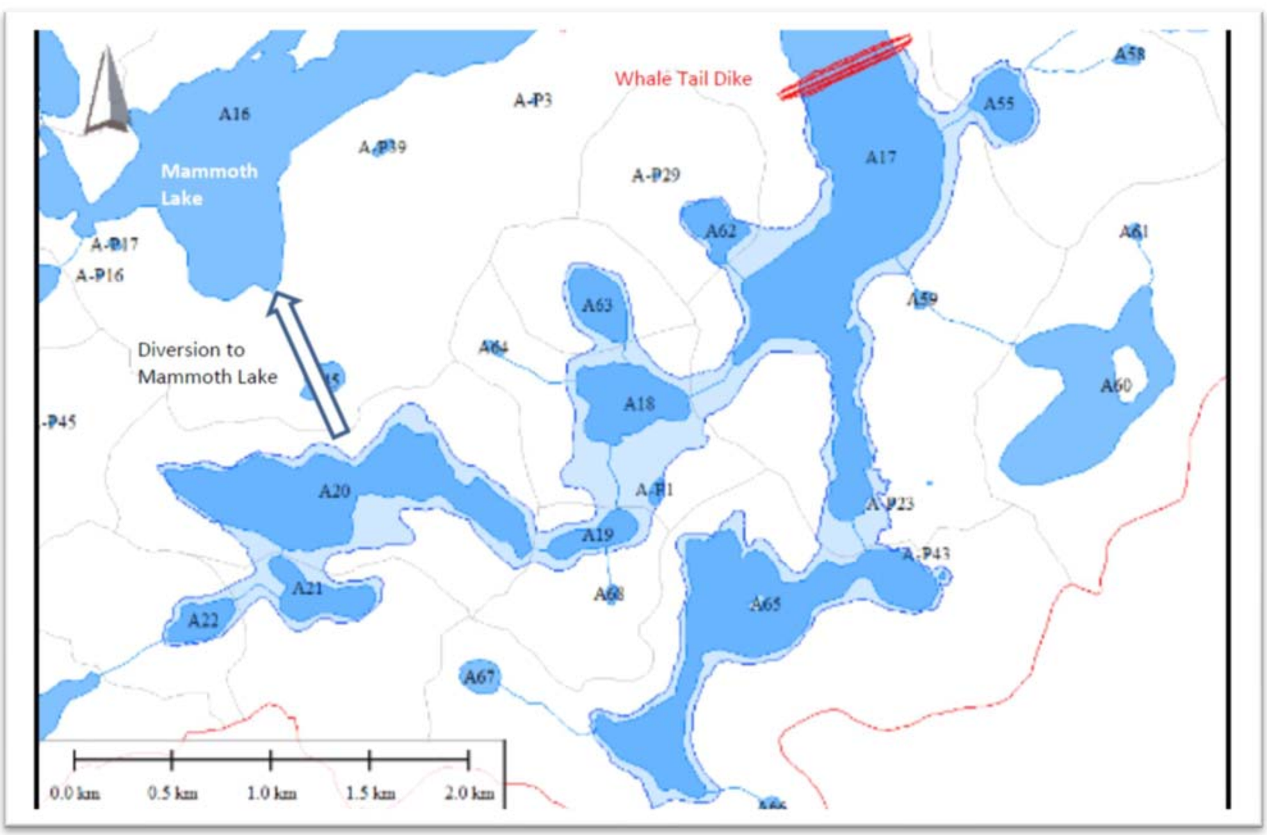


Figure 2. Whale Tail Lake (South Basin) Diversion Flooding occurring between June 2018 to July 2020, and during operation (July 2020 to 2026). Light blue shading shows flooded areas.

13. P. 42 (ECCC): The MDMER require that the first study design include a description of and the scientific rationale for the sampling period selected for the fish population study (Schedule 5, subparagraph 10(b)(i)). The study design indicates that slimy sculpin will be collected in late July or in August 2020. Note that the *Metal Mining Technical Guidance for EEM* (Table 3-4, EC 2012) suggests that synchronous spring spawners be sampled in late fall. Please consider whether the timing of the sculpin survey could be moved later in the year, particularly given our earlier comment (#7) regarding the requirement to measure egg weight, fecundity and gonad weight of sexually mature fish.

Response

Please refer to Appendix A, which describes the revised proposed fish study. A non-lethal study is now proposed for Slimy Sculpin and the sampling is now scheduled for the second half of August, which is consistent with the advice in Arciszewski et al (2010).

Reference: Arciszewski, T. M. Gray, K. Munkittrick and C. Baron. 2010. Guidance for the collection and sampling of slimy sculpin (Cottus cognatus) in northern Canadian lakes for environmental effects monitoring (EEM). Canadian Technical Report of Fisheries and Aquatic Sciences 2909.

14. P. 43-44 (ECCC): The study design indicates that slimy sculpin measurements will include length, body weight and age, and that total weight versus length will be analyzed to assess condition. Note the requirement to also provide, if practicable, the liver or hepatopancreas weight (Schedule 5, paragraphs 12(1)(e), (h), (i) and (j)). For a lethal fish survey, ECCC recommends that liver weight at body weight, as well as body weight at length, be used to assess condition (Table 3-3, EC 2012). Statistical analysis of body weight at length and liver weight at body weight can be done using ANCOVA (EC 2012; section 8.3.3.2). Additionally, the MDMER have defined a critical effect size of ±25% of the reference mean for liver weight at total body weight, and critical effect size of ±10% of the reference mean for total body weight at length (Schedule 5, subsection 1(2)). Use of critical effect sizes to determine future monitoring requirements is contingent on analysis of effect indicators and calculation of effect sizes as specified in the MDMER. Please provide a description of any additional methods for the slimy sculpin fish survey to ensure collection of all required information, including assessments of fish condition, in accordance with Schedule 5, paragraph 10(b). ECCC acknowledges that the study design does include liver weight at body weight and body weight at length to assess condition in lake trout.

Response

A non-lethal study of Slimy Sculpin is now proposed. Please refer to Appendix A, which describes the revised proposed fish study.

15. **P. 46 (ECCC): The study design indicates that there are differences in organic content in lake sediments – 2% in MAM, 9% in LK1, and 24% in LK8. The *Metal Mining Technical Guidance for EEM* (EC 2012, Chapter 2) identifies organic content in lake sediment as a potential confounding factor, and discusses the importance of reference areas having fish habitat that is most similar to that of the exposure area. Please consider whether the Lake 8 reference area is appropriate for the benthic invertebrate study, given differences in organic content of lake sediments; if warranted, please discuss how this potential confounding factor can be accounted for in the study design so that its influence can be assessed during data interpretation.**

<u>Response</u>
There is an error in Table 8-2: TOC and percent sand are interchanged. An updated Table 8-2 is provided here, with correct %TOC and % sand.

Table 8-2 Updated: Benthic community composition, effect indicators and sediment depth and character in Lake D1, Lake 8 and Mammoth Lake, 2017 and 2018

Family	**LK1**	**LK8**	**MAM**	
	2018	**2018**	**2017**	**2018**
Nemata	3	8	<1	7
Platyhelminthes	<1	<1	<1	1
Naididae	<1	1	1	1
Lumbriculidae	2	1	<1	2
Acarina		<1		
Acalyptonotidae	1	1	<1	
Lebertiidae	1	1	1	1
Oxidae	2	1	1	1
Pionidae			<1	<1
Limnephilidae			<1	
Chironomidae	70	39	64	61
Empididae		<1		
Sphaeriidae	18	25	18	17
Ostracoda	4	24	13	9
Notostraca			<1	<1
EEM Effect Indicators				
Abundance (#/m^2)	2326	3296	5148	4604
Family Richness	6.6	8.2	8.2	8.6
Family Equitability	0.32	0.44	0.28	0.30
Sediment Character				
% TOC	4	1	11	9
% Silt	79	66	81	85
% Clay	12	10	17	13
% Sand	8.7	24.0	2.1	1.7
Depth of sample (m)	7.7	7.7	8.1	8.2

Considering the corrected data, the sediments from MAM are finer (less sand) and have a higher organic content relative to the two proposed 'local' reference lakes. As ECCC points out, those differences have the potential to result in the benthic communities from MAM differing from those in Lake 1 and/or Lake 8. Those differences in texture and organic content, further, have the potential to modify the bioavailability of metals (see comment 16 below). With a higher organic content, metals loads in sediments in MAM can be anticipated to be less bioavailable (USEPA 2005).

The CREMP monitors additional lakes. Of those in the larger CREMP, Wally Lake (WAL) has a substrate that has a texture (with ~ 2% sand, ~ 86% silt) and organic content (4 to 9% TOC) like what is found in MAM. Wally Lake, however, was exposed to mine 'effluent' from 2013 to 2017, so it cannot be used as a temporal reference.

This EEM program has both temporal and spatial references. Agnico intends to use the baseline period data from MAM as a direct reference for the exposure-period data MAM, in a before-after context. Agnico proposed using Lake 1 and Lake 8 as local reference lakes and INUG and PDL as regional reference lakes. The data from Lake 1, Lake 8, INUG and PDL can be used as both spatial and temporal references, testing to see if MAM differs from the spatial references, and to see if changes over time in MAM differ from changes over time in the four reference lakes. Because there are data from INUG and PDL during MAM's baseline period, it will be possible to test whether changes in benthic indices from baseline to exposure periods in MAM have changed differently than from the baseline to exposure period in INUG and PDL in a classic BACI ANOVA. Given that the sediments of the four reference lakes differ in organic content relative to MAM, there is the potential that they will have a different trajectory over time than will MAM, but those variations can be interpreted using supporting environmental information. Further, variations in MAM can be judged relative to the baseline data in WAL, as a final means of evaluating whether variations are being caused by mine effluent or other factors.

Reference:

USEPA. 2005. Procedures for the derivation of equilibrium partitioning sediment benchmarks (ESBs) for the protection of benthic organisms: metal mixtures (cadmium, copper, lead, nickel, silver, and zinc). United States Environmental Protection Agency, Office of Research and Development, Washington, EPA-600-R-02-011.

16. **P. 48 (CIRNAC): Further to comment #15 from ECCC, CIRNAC has a comment regarding LK1 and LK8 as reference areas. Table 8-2 on Page 48 of the study design report summarized data collected on benthic community composition, effect indicators and sediment depth and character in LK1, LK8 and Mammoth Lake. The design proposes the LK1 and LK8 as reference areas for EEM. A review of the data indicates that both sediments in LK1 and LK8 have much higher total organic carbon contents than that of the Mammoth Lake. Sediment in LK8 seems to have unusually high total organic carbon content (i.e., 24.0%) for a typical arctic lake. Given the potential importance of organic carbon on sediment pore water chemistry, metal mobility, and fish habitat, CIRNAC recommends that the suitability of LK1 and LK8 as reference areas be reassessed.**

Response

Please see the response to Comment 15 above. There was an error in Table 8-2, and there are other reference data available that can be used to inform the interpretation of variations in the benthic community in MAM.

17. P. 48 (ECCC): The study design indicates that two sub-samples of the benthic community will be composited for each sampling station. Note that the Metal Mining Technical Guidance for EEM (EC 2012, section 4.3.3) recommends that each composite sample consist of three or more benthic invertebrate field sub-samples. As was done for the last EEM program for Third Portage Lake, please consider assessing within-station variability, given that this is a new receiving environment and the first EEM biological study at this site; see section 4.4.2 of the technical guidance (EC 2012) for details on determining sampling effort for field sub-sampling.

Response

Agnico understands that the EEM Guidance Document recommends that an analysis of precision be undertaken for new sites, in order to determine the number of subsamples required to obtain estimates of within-site means of density, family richness, and equitability within 20% of the true values with a reasonable likelihood (95%; reference). Further, Agnico understands that this exercise normally involves the collection of a minimum of three samples (Ekman grabs in the case of the Whale Tail project), with an assessment of precision based on the separate processing of the three samples. As ECCC is aware, the Whale Tail and associated Meadowbank projects have been monitoring for several years in reference lakes and lakes variously exposed to mine processes, and that sampling has generally followed a classic EEM design with five stations within lake areas. The Meadowbank/Whale Tail CREMP varies from the classic EEM program in collecting duplicate Ekman grabs.

Agnico is proposing not to undertake an assessment of within-station precision for the following reasons.

1. The 2-grab sample has been used by Agnico since 2006 pursuant to the company's CREMP requirements. CREMP was designed in response to requirements under the company's Nunavut Water License. Environment Canada provided input on the CREMP study design as part of the federal review process.

2. The Agnico Core Receiving Environment Monitoring Program (CREMP), which began in 2006 and was approved by regulatory agencies, uses 2-grab composites. The CREMP sampling program was designed with before-after-control-impact (BACI) comparisons in mind and has collected data annually from multiple exposure areas and multiple reference areas to support those ideal designs. There are four years of CREMP baseline data (2015 - 2018) and one year of historical exposure data (2019) from MAM that can be used to evaluate the 2020 exposure data in MAM, if 2-grab data samples are used. There are also CREMP baseline data from Lake 1 and Lake 8, in addition to multiple years of data from other referenced lakes (INUG and PDL) that were all sampled using the 2-grab protocol. The baseline data that are available from CREMP sampling for Mammoth Lake and the reference lakes data are very valuable for understanding natural variations in effect endpoints. The ANOVA designs supported by these data are also far more relevant for identifying true effects related to perturbation than the basic EEM reference-exposure design (Green, 1979; Underwood, 1991, 1992, 1993, 1994;

Kilgour et al., 2007; Somers et al., 2018). If three-grab samples are used in the Meadowbank EEM, the two-grab benthic samples collected for CREMP cannot be used in the analyses.

3. Environment Canada's (2012) guidance suggests that the number of replicate samples should be based on precision. Agnico demonstrated in the study design for Cycle 2 for TPN that 2 grabs were sufficient to result in estimates of precision for abundance and richness in INUG and PDL (C. Portt and Associates and Kilgour & Associate, 2014). If benthic communities in MAM were more variable than in INUG or PDL, then there may be some justification for collecting a third sample to achieve the recommended precision. Though it does not specifically evaluate precision within MAM, we computed the coefficient of variation (CVs) for density, family richness and equitability for samples from MAM, and compared them to CVs for indices from INUG, PDL, Lake 1 and Lake 8 (all available years for all of those lakes). The CVs combine among-grab and among-station variability and do not allow us to specifically evaluate the among-grab variance and precision. However, if the among-grab variance in MAM was appreciably higher than in INUG, PDL, Lake 1 or Lake 8, then the CV for MAM would be higher than in INUG, PDL, Lake 1 or Lake 8. The graph below (Figure 3) indicates that CVs for benthic indices from MAM were within the range of values observed in INUG and PDL. There is no variance in CV's for Lake 1 or Lake 8 because there is currently only one year of data. We infer from those graphs that variance among grabs in MAM must be generally similar to what has been observed in the historical EEM reference lakes INUG and PDL. These data support a conclusion that a 2-grab sample would be sufficient for estimating within-station estimates of indices of composition, and do not support a conclusion that a 3-grab sample would be required.

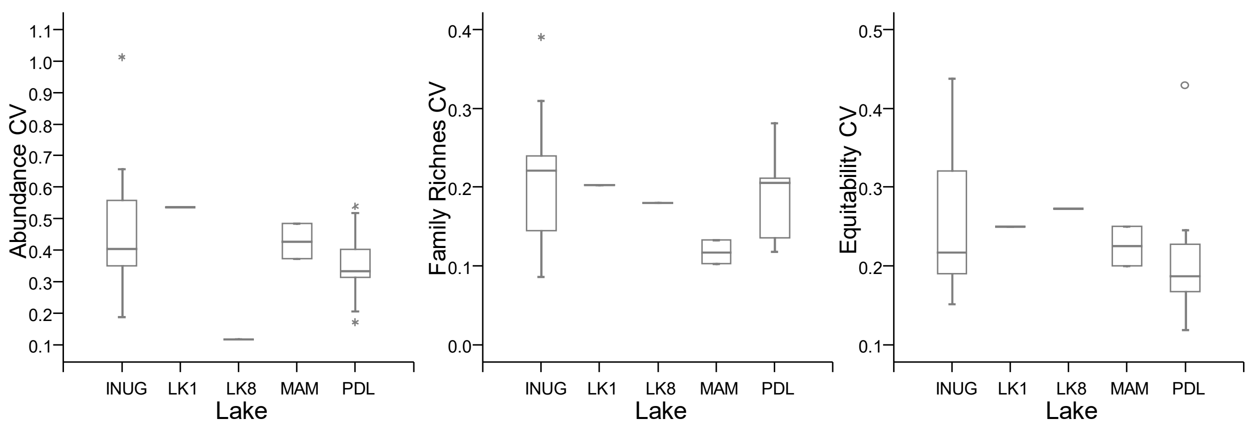


Figure 3. Box plots of coefficients of variation for indices of benthic community composition

4. Detailed power calculations (Appendix C) indicate that there is a negligible difference in power if 2 grabs per station are collected compared to 3 grabs per station. Further, the 3-Area (2 reference, 1 exposure), 2 grabs-per-station survey proposed by Agnico has marginally but still greater statistical power to detect effects equivalent to the benthic critical effect size compared to a conventional EEM program with 2 Areas (1 reference, 1 exposure) and 3 grabs per station.

18. P. 50 (CIRNAC): Further to comment # 5 from ECCC, CIRNAC has a comment regarding EEM sediment and benthos sampling locations and their relationships with the two effluent

discharge locations in Mammoth Lake. The EEM sediment and benthos sampling areas are found in Figure 8-1 on page 50. However, it is not clear where exactly the sampling locations are in relation to the two effluent discharge locations in Mammoth Lake.

Response
Please refer to Figure 1.

19. **P. 55 (ECCC): The study design indicates that ANOVA will be used "to test for changes in differences in average values of compositional indices between reference and exposure areas of the conventional effect variables abundance, richness and equitability". Note that the MDMER require that the first interpretative report also include a determination of whether there is statistical difference between areas for the similarity index effect indicator (Schedule 5, paragraph 12(1)(h)). Please provide a description of how the similarity index will be assessed to determine if the effluent has an effect on the benthic community, in accordance with Schedule 5, subparagraph 10(c)(ii).**

Response
Agnico indicated in Section 8.2.6.6 on Page 56 of the First Study Design Report that Agnico would use a Mantel test to assess whether there were differences in the similarity index (Bray-Curtis distances) between exposure and reference conditions. Agnico will use various 'hypothesis' matrices in the Mantel tests, to mimic the tests being carried out with the conventional indices (density, richness, equitability).

Other Items

20. **P. 4 (ECCC): The mercury and selenium concentrations included in the study design were from samples collected during lake dewatering. The study design does not include effluent samples taken during mine production, though such samples might have higher concentrations of metals than samples taken during dewatering. Please discuss whether mine production is expected to result in concentrations of mercury and selenium that are higher than those measured during lake dewatering.**

Response
The determination of whether a fish tissue study is required is based on the concentrations of mercury and selenium measured in effluent prior to the submission of the study design for each EEM cycle. The need for a fish tissue study in future cycles will be evaluated based on the more recent effluent analyses.

21. **P. 32 (CIRNAC): On p. ii of the study design report, it is stated that "The only mine effluent to be discharged at Whale Tail Pit during operations is treated contact water. Contact water from the major mine infrastructure will be directed to the Whale Tail Attenuation Pond (WTAP), which will be located in the dewatered north basin of Whale Tail Lake." A review of Figure 5-1 on page 32 of the study design report indicates that the WTAP is separated from the Whale Tail Lake South Basin only by the constructed Whale Tail dike. How is the contact water contained with the WTAP? Is there monitoring to determine if mine contact water from the WTAP could**

exchange with water in the south basin of Whale Tail Lake?

Response

Figure 5-1 shows mine infrastructure superimposed on baseline conditions. The future Whale Tail Attenuation Pond (WTAP) will be in the area outlined in blue on the figure, which will be in the dewatered area north of the Whale Tail dike. The WTAP will be approximately 0.5 km north of the Whale Tail dike and below the elevation of the south basin of Whale Tail Lake. It will not be possible for water from the WTAP to enter the south basin of Whale Tail Lake.

22. **P. 35 (ECCC): Please note that the MDMER require that the first interpretative report include the latitude and longitude of sampling areas (Schedule 5, paragraph 12(1)(b)). The study design provides UTM coordinates – but not latitude and longitude – for the general sampling areas.**

Response

The latitude and longitude of the sampling areas are provided in the table below.

EEM Sampling Area		Coordinates (NAD83)*	
Type	**Name**	**Latitude**	**Longitude**
Exposure	Mammoth Lake (MAM)	65° 24' 01.438''	-96° 43' 51.617''
Reference	Lake A44	65° 23' 10.759''	-96° 47' 22.932''
Reference	Lake D1 (LK1)	65° 21' 08.974''	-96° 41' 23.254''
Reference	Lake 8 (LK8)	65° 25' 51.857''	-96° 35' 43.666''

*indicates the approximate centre of the sampling area.

23. **Pp. 40 and 44 (ECCC): The MDMER require that the first interpretative report include median values as well as the mean, standard deviation, standard error, minimum and maximum (Schedule 5, paragraph 12(1)(e)). Please include the median in the list of summary statistics that will be included in the statistical analyses for lake trout and slimy sculpin.**

Response

Median will be included in the summary statistics provided for lake trout and slimy sculpin.

24. **Pp. 40 and 44 (ECCC): The MDMER require that the first interpretative report include summary statistics, if practicable, for length, total body weight, age, liver or hepatopancreas weight, egg weight, fecundity and gonad weight (Schedule 5, paragraph 12(1)(e)). Please add age, liver weight, egg weight, fecundity, and gonad weight to the list of endpoints for which you will provide summary statistics for lake trout and slimy sculpin.**

Response

Please refer to Appendix A, which describes the revised proposed fish study.

25. **Pp. 40 and 44 (ECCC): The study design presents a statistical approach for analyses of results from the lethal fish surveys. Note that the recommended approach for multiple control-impact designs is to pool the reference data for comparison to the exposure, provided the reference areas have comparable habitat characteristics to the exposure area. If the reference areas differ and the data cannot be pooled, the results for the reference area that best matches the**

characteristics of the exposure area should be used, subject to site-specific considerations.

Response
Agnico was not aware that pooling of reference area data was recommended provided that the reference areas' have similar habitat to the exposed area. A specific reference to this recommended approach would be appreciated.

26. **Pp. 40 and 44 (ECCC): *Metal Mining Technical Guidance for EEM* (EC 2012, section 8.3.1) suggests that sexes should initially be treated separately when conducting the analyses, for all parameters. In addition, sexually immature fish should not be mixed with sexually mature fish for analyses. Please take this into consideration when presenting descriptive statistics and conducting the statistical analyses on the fish population data for the interpretative report.**

Response
When there are adequate mature individuals that will spawn in the current year available, and reproductive endpoints including gonad weight and fecundity are being analyzed, mature and immature fish would not be mixed for analyses. When there are not, which means that gonad size and fecundity cannot be investigated, and when one of the objectives is not to kill fish unnecessarily, for parameters that are highly correlated (i.e. total weight with length, liver weight with total length) pooling immature and mature fish of both sexes does not result in a large decrease in statistical power. Please refer to Section 4.4 in the Revised Fish Survey Study Design for Whale Tail Pit First Biological Study in Appendix A.

27. **Pp. 40 and 44 (ECCC): The study design indicates that the assumption of normality will be tested before applying ANOVA to lake trout and slimy sculpin data. Homogeneity of variance should also be assessed; see Chapter 8, Appendix A1.3 of the *Metal Mining Technical Guidance for EEM* (EC 2012) for the various steps for ANOVA. Note that this would apply to analyses for site differences in age, as well as length and weight.**

Response
Agnico will also assess homogeneity of variance for age, length, and weight.

28. **P. 56 (ECCC): The study design presents an approach to assessing covariable effects. Please note that the *Metal Mining Technical Guidance for EEM* (EC 2012, section 8.5.1) suggests that ANCOVA can be used for control-impact or multiple control – impact designs to factor out covariates that may create noise that makes it difficult to make simple ANOVA comparisons of reference to exposure areas.**

Response
Agnico will use ANCOVA or similar methods to partition variability associated with non-mine related variables.

29. **P. 57 (ECCC): The study design presents effect size calculations for BA and BACI hypotheses. Please note that the MDMER specify a formula for the calculation of the magnitude of the effect**

for the benthic invertebrate community effect indicators in which the denominator is the standard deviation for the indicator in the reference area (Schedule 5, subsection 12(3)).

Response
Agnico will incorporate the within-reference area standard deviations in the computation of effect sizes.

References

Arciszweski, T., M.A. Gray, K.R. Munkittrick, and C. Baron. 2010. Guidance for the collection and sampling of slimy sculpin (Cottus cognatus) in northern Canadian Lakes for environmental effects monitoring (EEM). Can. Tech. Rep. Fish. Aquat. Sci. 2909: v + 21p.

Barrett, T.A., M.A. Tingley, K.R. Munkittrick, and R.B. Lowell. 2010. Environmental Effects Monitoring Data: Dealing with Heterogeneous Regression Slopes in Analysis of Covariance. Environmental Monitoring and Assessment, 166:279-291.

C. Portt and Associates and Kilgour & Associates Ltd. 2014. Environmental Effects Monitoring: Agnico-Eagle Mines Ltd.- Meadowbank Division Cycle 2 Study Design. Prepared for Agnico-Eagle Mines Ltd., Regional Office - 93, Rue Arseneault, suite 202, Val-d'Or, Québec, J9P 0E9. 55 p. + 4 appendices.

C. Portt and Associates and Kilgour & Associates Ltd. 2017. Environmental Effects Monitoring: Agnico-Eagle Mines Ltd.- Meadowbank Division Cycle 3 Study Design. Prepared for Agnico-Eagle Mines Ltd., Regional Office - 93, Rue Arseneault, suite 202, Val-d'Or, Québec, J9P 0E9. 62 p. + 5 appendices.

Environment Canada (EC) 2012. Metal Mining Technical Guidance for Environmental Effects Monitoring.

C. Portt and Associates and Kilgour & Associates. 2020. Revised Fish Survey Study Design for Whale Tail Pit EEM First Biological Study. Prepared for Agnico-Eagle Mines Ltd., Regional Office - 93, Rue Arseneault, suite 202, Val-d'Or, Québec, J9P 0E9. April 2020. 24 p.

USEPA. 2005. Procedures for the derivation of equilibrium partitioning sediment benchmarks (ESBs) for the protection of benthic organisms: metal mixtures (cadmium, copper, lead, nickel, silver, and zinc). United States Environmental Protection Agency, Office of Research and Development, Washington, EPA-600-R-02-011.

APPENDIX A

Revised Fish Survey Study Design for Whale Tail Pit EEM First Biological Study

Revised Fish Survey Study Design for Whale Tail Pit EEM First Biological Study

C. Portt and Associates and Kilgour & Associates.

June 7, 2020.

1 INTRODUCTION

This revised fish study design for Whale Tail Pit First EEM Biological Study has been prepared after review of the comments and recommendations that Agnico received from Environment and Climate Change Canada (ECCC), accompanying a letter dated February 10, 2020, regarding the fish study that was proposed in the July 2019 report “Environmental Effects Monitoring: Agnico Eagle Mines Ltd. – Whale Tail Pit, First Biological Monitoring Study Design” (the study design report). In preparation of this study design addendum, Agnico has considered the comments and recommendation received in the ECCC review, re-examined the background information considered while preparing the previous study design, and examined additional background information. A plume modeling study for Whale Tail Pit, appended to this report, also informed the revision. The fish study design presented in this document supersedes the design proposed in the July 2019 report.

2 SUMMARY OF ENVIRONMENT AND CLIMATE CHANGE CANADA COMMENTS AND RECOMMENDATIONS

The ECCC review of the study design report that accompanied the February 10, 2020, letter included seven action items requesting that the fish study be redesigned. Those action items are summarized below:

- Comment 6. Please describe an approach that will provide all required information for the fish population study and be explicit in how the fish studies will determine if the effluent has an effect on growth, reproduction, condition and survival.
- Comment 7. Please provide a description of any additional methods for the Lake Trout and Slimy Sculpin fish survey to ensure collection of all required information, including an assessment of fish reproduction, in accordance with Schedule 5, paragraph 10(b).
- Comment 8. Please note our previous comment regarding the requirement to measure egg weight, fecundity and gonad weight of sexually mature fish, and consider whether the timing of sample collection for Lake Trout is appropriate.
- Comment 10. Please provide a description of any additional methods for the Lake Trout and Slimy Sculpin fish survey to ensure collection of all required information, including an assessment of fish survival, in accordance with Schedule 5, paragraph 10(b).

- Comment 11. Please provide a description of any additional methods for the Lake Trout and Slimy Sculpin fish survey to ensure collection of all required information, including an assessment of fish growth, in accordance with Schedule 5, paragraph 10(b).
- Comment 13. Please consider whether the timing of the sculpin survey could be moved later in the year, particularly given our earlier comment (#7) regarding the requirement to measure egg weight, fecundity and gonad weight of sexually mature fish.
- Comment 14. Please provide a description of any additional methods for the Slimy Sculpin fish survey to ensure collection of all required information, including assessments of fish condition, in accordance with Schedule 5, paragraph 10(b).

The ECCC review included two additional comments relating to the fish study design, which are summarized below:

- Comment 24. Please add age, liver weight, egg weight, fecundity, and gonad weight to the list of endpoints for which you will provide summary statistics for Lake Trout and Slimy Sculpin.
- Comment 26. In addition, sexually immature fish should not be mixed with sexually mature fish for analyses. Please take this into consideration when presenting descriptive statistics and conducting the statistical analyses on the fish population data for the interpretative report.

These recommendations and additional comments, in aggregate, are interpreted to indicate that ECCC does not agree with the approach to the fish study that was used in the previous Meadowbank EEMs and proposed in the study design report for Whale Tail Pit, and is of the opinion that the fish survey must address all of the EEM fish survey endpoints related to growth, reproduction, condition and survival. In response, Agnico has reconsidered and, where considered appropriate, revised the fish study design.

3 Study Design Overview

3.1 Exposed and Reference Areas

The EEM First Biological study design for the Whale Tail Pit proposed to examine one exposure area, Mammoth Lake, and two reference areas, Lake D1 and Lake 8. No change is proposed to the exposed lake or the reference lakes, except that a third potential reference lake, A44, will be considered as an alternate reference lake for Slimy Sculpin, if required. Lake A44 drains to Lake A43, which in turn drains to Mammoth Lake (Figure 1). Slimy Sculpin were sampled from Lake A44 in 2019, and those data could contribute to trend-through-time analyses, if desired, in the future. The inter-lake connections between A44, A43 and Mammoth Lake are via boulder fields with sections where flow is interstitial (C. Portt, personal observation), so the potential for fish movement between A44 and Mammoth Lake is very low.

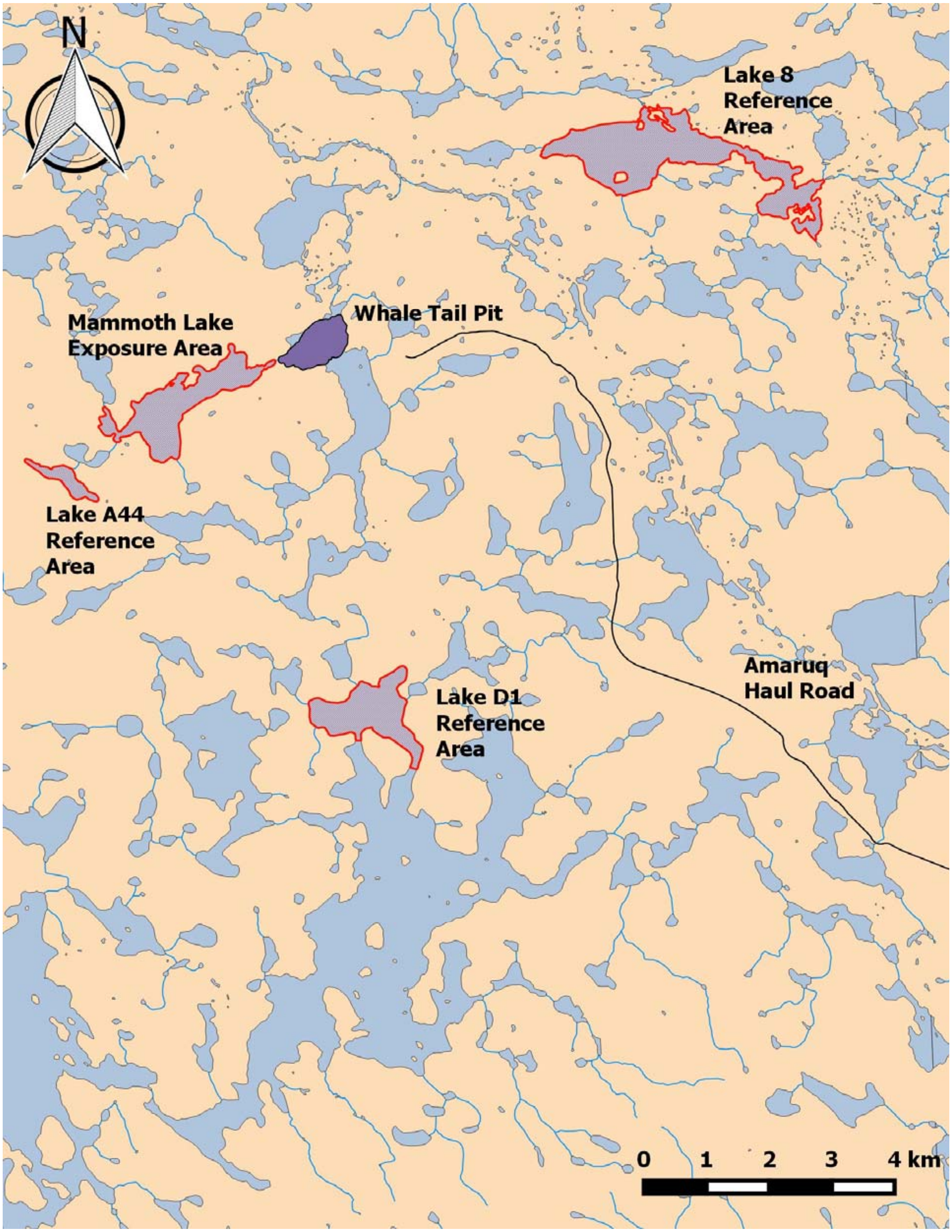


Figure 1. Location of Mammoth Lake, the exposed lake, reference lakes D1, Lake 8, and alternate Slimy Sculpin reference lake A44.

Lake A44 is not being considered as an alternate reference lake for Lake Trout because, given its size, Lake Trout numbers are expected to be low. Lake Trout are present however; one Lake Trout was captured in A44 by electrofishing in 2019 (C. Portt and Associates, 2019).

3.2 Fish Community and Selection of Sentinel Fish Species

A total of six fish species occur in the lakes in the Whale Tail Pit study area (C. Portt and Associates, 2018), comprised of four large-bodied species (Lake Trout, *Salvelinus namaycush*; Arctic Char, *S. alpinus*; Round Whitefish, *Prosopium cylindraceum*; Burbot, *Lota lota*) and two small-bodied species (Slimy Sculpin, *Cottus cognatus*; Ninespine Stickleback, *Pungitius pungitius*). In order to conduct a fish survey, it must be feasible to collect enough individuals of the sentinel species from the exposed site and one or more reference sites. It is generally considered undesirable to kill large numbers of non-target species in order to collect enough of the sentinel species. Consequently, catch-per-unit-effort (CPUE) is a key factor in determining the potential sentinel species.

At Whale Tail Pit the most effective methods of collecting fish have been gill netting for large-bodied fishes and backpack electrofishing for small-bodied fishes (C. Portt and Associates, 2018). Minnow traps have been effective for catching Ninespine Stickleback in small lakes and ponds but not in the larger lakes. Upstream migrations of Ninespine Stickleback into shallow tributary lake and ponds have been observed in the spring, suggesting that they may be too mobile to be a good choice for a sentinel species.

Lake Trout is the most abundant large-bodied fish species in gill net catches in Mammoth Lake and proposed reference lakes D1 and Lake 8 (Table 1). The CPUE of Lake Trout in Mammoth Lake was 2.4 times the CPUE of the next most abundant species, Round Whitefish, during baseline field investigations. The ratio of the Lake Trout CPUE to Round Whitefish or Arctic Char CPUE was even higher in the reference lakes. Based on relative CPUE, large numbers of Lake Trout would be captured if a fish survey was attempted using a different large-bodied species as a sentinel species. That was the experience in the Cycle 1 EEM at Meadowbank, when a lethal study using Round Whitefish as a second sentinel species was attempted and terminated, after consultation with Environment Canada, because it was evident that sufficient numbers of Round Whitefish could not be captured with a reasonable amount of effort and without killing large numbers of Lake Trout (Table 2; Azimuth, 2012). Based on the CPUE data and the previous Meadowbank experience, Agnico is of the opinion that Lake Trout is the only large-bodied species that can be used as a sentinel species.

Table 1. Summary of gill net catches and catch-per-unit-effort (CPUE; number of fish caught per hour of soak time) in lakes in the Whale Tail Pit area, by lake and species.

Lake	Number of sets	Total soak time (hours)	Lake Trout		Arctic Char		Round Whitefish	
			catch	CPUE	catch	CPUE	catch	CPUE
Mammoth	20	92.4	49	0.53	0	0.00	20	0.22
Lake D1	2	13.0	4	0.31	0	0.00	1	0.08
Lake 8	2	35.2	23	0.65	1	0.03	0	0.00

Table 2. Number of fish captured by gill netting in Cycle 1 at each location and the number of Lake Trout that were released alive or were dead (Source: Azimuth, 2012).

Location	Lake Trout			Round Whitefish	Arctic Char
	released alive	dead	total		
TPN (exposed)	62	63	125	2	33
INUG (reference)	45	43	88	39	5
PDL (reference)	26	32	58	7	8
total	133	138	271	48	46

Slimy Sculpin was the only species in 2018 and 2019 shoreline electrofishing catches in Mammoth Lake and in the proposed reference lakes that was sufficiently abundant to be used as a sentinel species (Table 3). In 2019, Mammoth Lake was electrofished again and Lake A44, a previously unfished lake that is tributary to Mammoth Lake, was electrofished for the first time. Again, Slimy Sculpin was the species with the highest CPUE, which was one to two orders of magnitude higher than the CPUE for any of the other species. Based on the 2018 and 2019 CPUEs, Slimy Sculpin is the only small-bodied fish that can be captured in Mammoth Lake and the reference lakes with a reasonable amount of effort.

Table 3. Electrofishing catches in Mammoth Lake, Lake D1 and Lake 8, in 2018 and Mammoth Lake and Lake A44 in 2019.

Lake - year	Electro-seconds	Slimy Sculpin	juvenile *Salvelinus*	Ninespine Stickleback	juvenile Burbot	Round Whitefish
Lake D1 - 2018	2460	27	7	0	1	0
Lake 8 - 2018	5869	100	11	0	0	0
Mammoth - 2018	6309	69	3	9	0	3
Mammoth - 2019	3138	36	0	7	0	0
A44 - 2019	2158	35	1	2	1	0
Total	19,934	267	22	18	2	3
mean CPUE		13.4	1.1	0.9	0.1	0.2

Summary

Of the six fish species in the study area, only Lake Trout and Slimy Sculpin can be captured in sufficient numbers to be feasible sentinel species. Lake Trout would be captured by gill netting and Slimy Sculpin by backpack electrofishing.

4 Lake Trout Study Design

The Lake Trout study design is informed by three cycles of EEM fish surveys using Lake Trout as a sentinel species at the Meadowbank Mine, baseline investigations at the Whale Tail site, and a fish-out of the north basin of Whale Tail Lake, which is immediately upstream from Mammoth lake, conducted in 2018. The Study Design Report recommended a lethal study with sufficient power ($\alpha=\beta=0.1$) to evaluate the effect indicators total body weight at length and liver weight at total body weight, using the pooled data for all fish (mature and immature males and females). It was not proposed to address the effect indicator gonad size at total body length because of the small proportion of Lake Trout populations that consists of mature individuals that would spawn in the current year. Using pooled data for immature and mature males and females was considered appropriate because the reproductive endpoints were not being examined. It should be noted that the data are pooled when the total body weight versus length effect indicator is examined in non-lethal studies.

Examination of the age-related effect indicators (total body weight at age and age distribution) was not recommended for multiple reasons. First, error is inherent in fish aging and old Lake Trout from cold, ultra-oligotrophic lakes like those in the study area are difficult to age, which makes data interpretation of age-related endpoints difficult (Munkittrick et al, 2000). Second, with long-lived species, the growth-related effect indicator (total body weight at age) does not reflect recent conditions (Munkittrick et al, 2000). Third, the age distribution of Lake Trout in the Cycle 3 Meadowbank EEM violated one (with log transformed data) or both (with raw data) of the assumptions of normality and homogeneity of variance among lakes. In light of those concerns, it was felt that killing approximately 75% more Lake Trout to address total body weight at age, would not be justified.

In the following sections Agnico reviews the results and study design recommendations of the previous three Meadowbank EEM studies that used Lake Trout as a sentinel species. In addition, the numbers of mature Lake Trout in Mammoth Lake, the exposed area for the Whale Tail EEM study, are estimated to assess the potential to obtain adequate sample sizes to examine reproductive endpoints.

4.1 Review of Completed EEM Studies at the Meadowbank Mine Using Lake Trout as the Sentinel Species

As described in the Study Design Report, Lake Trout has been used as a sentinel fish species in three successive EEM cycles at the Meadowbank Mine. The Meadowbank Mine experience is considered relevant for the following reasons:

- the Meadowbank site is geographically close to Whale Tail Pit, with similar climate;
- the lakes that have been sampled in the Meadowbank EEM studies are ultra-oligotrophic, as are the Whale Tail Pit exposed and reference lakes; and
- Lake Trout is the most abundant species in gill net catches in the exposure and reference lakes in the Meadowbank studies, as they are in Mammoth Lake and the proposed Whale Tail reference lakes.

4.1.1 Meadowbank Cycle 1

The Meadowbank Cycle 1 study design (Azimuth, 2010) for Lake Trout proposed a ‘non-lethal’ study that would examine 100 individuals from the exposed lake and each of the two reference lakes. One of the reasons for proposing a non-lethal study was that a fish-out at the Meadowbank site had determined that only 9% of the Lake Trout population that was susceptible to gill nets were adult fish that would spawn in the current year. Therefore, it was felt that too many Lake Trout would have to be killed in order to acquire a sufficient sample size of fish that would spawn in the current year and allow the evaluation of reproductive endpoints (Azimuth, 2010). The parameters to be measured from each Lake Trout were:

- fork length;
- total weight;
- age (pelvic fin ray); and
- deformities, erosions, lesions, tumours (DELT), and parasites.

Incidental Lake Trout mortalities were to be processed as they would be for a lethal survey. The study design was approved by Environment Canada.

During the field work, the mortality rate of Lake Trout was higher than expected and, as anticipated, few mature individuals that would spawn in the current year were captured (Azimuth, 2012). Fish sampling was discontinued, following consultation with Environment Canada, when preliminary analysis determined that enough Lake Trout had been sampled to achieve the targeted power for the body weight versus length (condition) effect indicator (Azimuth, 2012).

Statistical analyses were conducted for the following:

- weight at length;
- weight at age;
- length at age;
- length distribution; and
- age distribution.

The analyses were performed on pooled data for both sexes, which is the standard approach for a non-lethal study design. The interpretive report (Azimuth, 2012) recommended that for the next EEM cycle a non-lethal study of Lake Trout be conducted that would address the total weight versus length, total weight versus age and length versus age endpoints. It was estimated that the required sample sizes would be 70 to 80 Lake Trout per lake. Age of non-lethally sampled Lake Trout was to be determined from fin-rays, as it was in Cycle 1, although it was acknowledged that those ages were inaccurate; a correction factor was applied in Cycle 1, based on the relationship between fin-ray ages and otolith ages, which were both determined for lethally sampled fish. There was no suggestion that addressing reproductive endpoints should be attempted or that the sexes would be analyzed separately, presumably because, based on the earlier fish-out and the Cycle 1 results, it was expected that sufficient numbers of mature, current-year spawners could not be captured without an unreasonable amount of effort and killing unacceptably large numbers of Lake Trout.

4.1.2 Meadowbank Cycle 2

The Cycle 2 study design report (C. Portt and Associates, and Kilgour & Associates Ltd., 2014) proposed, as an alternative to the study design recommended in the Cycle 1 interpretive report (Azimuth, 2012), a non-lethal study of Lake Trout (*Salvelinus namaycush*) captured by gill netting in one exposure area and two reference areas, assessing the weight versus length relationship (condition) but not age-related endpoints, with a target sample size of 25 fish per area. The rationale for excluding the age-related endpoints was that it is necessary to kill Lake Trout in order to acquire otoliths, which are the most accurate aging structure, and there is error inherent in aging long-lived Lake Trout, even when otoliths are used. Furthermore, the Technical Guidance Document recommends caution when interpreting age-related endpoints in the EEM decision-making process, due to the acknowledged inherent error (Environment Canada, 2012). Assessing reproductive endpoints was not proposed because, based on the previously discussed fish-out data and the results of Cycle 1, it was not considered reasonably feasible to collect a sufficient number of Lake Trout that would spawn in the current year, nor was it considered acceptable to kill the required number of Lake Trout to accomplish that.

Following the review of the study design and discussions with Environment Canada, it was agreed that age-related relationships would be examined using age determinations based on pectoral fin rays collected from released Lake Trout and that the target sample size would be 60 fish per site, in order to have sufficient statistical power to address age-related endpoints. For Lake Trout that died, liver weight, gonad weight and reproductive status would be determined and both otoliths and fin-rays would be collected for age determinations. These data were to be included in the Cycle 2 assessment. The data for both sexes and mature and immature fish would be pooled for the analyses.

A total of 292 Lake Trout were captured in gill nets in Cycle 2. The observed mortality rate was 37%, with additional fish likely to have died after their release due to handling and removal of a pectoral fin ray (C. Portt and Associates and Kilgour & Associates, 2015). Combining the data for all three lakes, of the 104 fish that were examined internally, 6 females and 21 males were mature and would have spawned in the current year. This was considered confirmation that it would not be reasonably feasible to collect enough Lake Trout that spawn in a given year to conduct meaningful analyses of reproductive endpoints. Furthermore, if a study was undertaken to characterize gonad weight and fecundity with minimum numbers (i.e. 20 males and 20 females per lake) it would result in unacceptable numbers of juvenile and non-spawning adults being killed.

The Cycle 2 interpretive report (C. Portt and Associates and Kilgour & Associates, 2015) reiterated that, at Meadowbank, fish surveys using Lake Trout as a sentinel species are limited to examining relationships based on length, weight, liver weight and age (i.e. reproductive endpoints could not be examined). Power analysis based on the results of the Cycle 2 study indicated that a sample size of less than 20 Lake Trout per site would be adequate to detect the critical effect sizes for the total weight versus length and liver weight versus total weight EEM effect indicators, and the liver weight versus length and length versus age supporting endpoints with α and β both equal to 0.1. More than twice as many fish per site would be required to achieve this power for the weight versus age relationship. It was therefore recommended that future EEM studies be lethal studies with a target sample size of 20 Lake Trout per lake that examined the total weight versus length, liver weight versus total weight, liver weight versus length and length versus age relationships. Implicit in this approach was that data for both sexes and for mature and immature fish would be pooled for the statistical analyses.

In the comments and recommendations provided in the review of the Cycle 2 interpretive report, the Technical Advisory Panel, consisting of representative from the Nunavut Water Board, Crown-Indigenous Relations and Northern Affairs Canada, and Environment and Climate Change Canada stated "*A fish study is required for the next EEM biological field work. TAP members are supportive of a lethal study on 20 Lake Trout.*" This review accompanied a letter to Agnico (Stephane Robert) from ECCC (Suzanne Forbrich) dated January 20, 2017.

4.1.3 Meadowbank Cycle 3

As recommended in the Cycle 2 interpretive report, and supported by the Technical Advisory Panel (TAP) in their review of that report, the Meadowbank Cycle 3 EEM study design report (C. Portt and Associates and Kilgour and Associates, 2017) proposed a lethal study with a target sample size of 20

Lake Trout from the exposed lake and each of two reference lakes. ANCOVA would be used to investigate the following relationships:

- total weight versus length;
- liver weight versus total weight;
- liver weight versus length; and
- length versus age.

Reproductive endpoints would not be examined because Agnico and ECCC agreed that it was not feasible to capture enough Lake Trout that would spawn in the current year. It was recognized that the sample size of 20 individuals would not achieve the desired power for comparisons involving fish weight versus age, and therefore the study design did not propose to examine that effect indicator. Implicit in this approach was that data for both sexes and for mature and immature fish would be pooled for the statistical analyses. In their comments and recommendations provided in the review of the Meadowbank Cycle 3 study design report, which accompanied a letter to Marie-Pier Marcil (Agnico) from Suzanne Forbrich (ECC), dated April 10, 2017, the Technical Advisory Panel (TAP), consisting of representatives from the Nunavut Water Board, Crown-Indigenous Relations and Northern Affairs Canada, and Environment and Climate Change Canada stated "*the proposed design of 20 lethal Lake Trout is supported provided that power analyses continue to indicate that it is suitable*."

In total, 70 Lake Trout were sampled in Meadowbank Cycle 3 (C. Portt and Associates and Kilgour and Associates, 2018). Of those, based on egg size and GSI, a total of four females, one from the exposed area, one from one of the reference lakes, and two from the second reference lake, would have spawned in the current year. Based on GSI and the condition of the testes, 16 males, two and four from the two reference lakes and ten from the exposure lake, would have spawned in the current year. This was interpreted as confirming, once again, that it would not be feasible to collect a sufficient number of Lake Trout that would spawn in the current year to examine reproductive endpoints; if such as study was attempted an unacceptable number of Lake Trout would be killed.

The review comments and recommendations of the Technical Advisory Panel on "Environmental Effects Monitoring: Cycle 3, Meadowbank Mine Interpretive Report" that accompanied a letter to Marie-Pier Marcil (Agnico) from ECCC (A. McLandress), dated November 25, 2019, notes that neither Cycle 3 nor the previous two cycles include an assessment of the effect indicator for reproduction and asks "*what steps could you take in the next study to ensure that fish reproduction is assessed in accordance with MDMER?*" Based on the results of the three previous studies at the Meadowbank Mine, no such steps are apparent to Agnico at this time.

4.2 Feasibility of Examining Reproductive Endpoints at Whale Tail Pit

As described in the preceding section, the principal factor limiting the execution of a traditional EEM adult fish survey that examines reproductive endpoints when using Lake Trout as the sentinel species in this region is the inability to capture sufficient numbers of mature individuals that will spawn in any given year. To explore this issue further, the data collected during the Cycle 2 and Cycle 3 EEM studies at the Meadowbank site were used to determine the size at maturity for male and female Lake Trout and to calculate the proportion of mature fish that spawn in a given year. Those proportions were applied to the numbers of Lake Trout in the size ranges for mature males and mature females captured in the fish-out of the north basin of Whale Tail Lake (the North Basin) to estimate the numbers of mature males and females in that basin that would and would not spawn in a given year. The North Basin, which is immediately upstream from Mammoth Lake, was isolated and drained in order to operate the Whale Tail Pit. Mammoth Lake is 150 ha in area and the North Basin is 70 ha in area, so the estimated numbers in the North Basin were multiplied by 2.14 (150/70) to estimate the numbers in Mammoth Lake.

4.2.1 Female Lake Trout Size at Maturity and Proportion of Mature Females That Spawns Each Year

A plot of gonadosomatic index (GSI; gonad weight as a percent of total weight), versus fork length for all lethally sampled female Lake Trout from Meadowbank EEM Cycle 2 and Cycle 3 (all lakes combined) reveals three groups (**Figure 2**). The first group consists of 34 females shorter than 475 mm, all of which have a GSI of less than 1.0%. Based on the weight and appearance of the ovaries of those females, an example of which is shown in Photograph 1, Agnico consider them to be immature. The second group consists of eight females ranging from 502 mm to 1010 mm in length, with GSIs ranging from 9.2% to 17.6%. Based on the weight and appearance of the ovaries of those females, an example of which is shown in Photograph 2, Agnico consider them to be mature females that will spawn in the current year. The third group consists of 27 females longer than 475 mm with GSIs ranging from 0.6% to 2.7%. Based on the weight and appearance of the ovaries of those females, an example of which is shown in Photograph 3, Agnico consider them to be mature females that will not spawn in the current year. Based on Figure 2, 475 mm is the approximate length of maturity for female Lake Trout. Based on their GSI, 23% of female Lake Trout (8 of 35) greater than 475 mm in length would have spawned in the current year (**Figure 2**). This suggests that in this region, on average, mature female Lake Trout spawn, every fourth year.

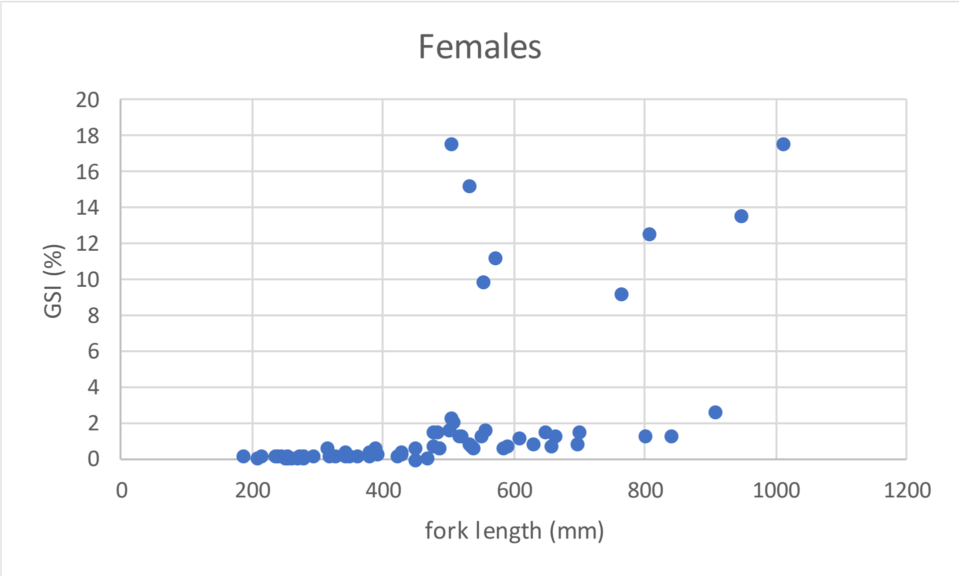
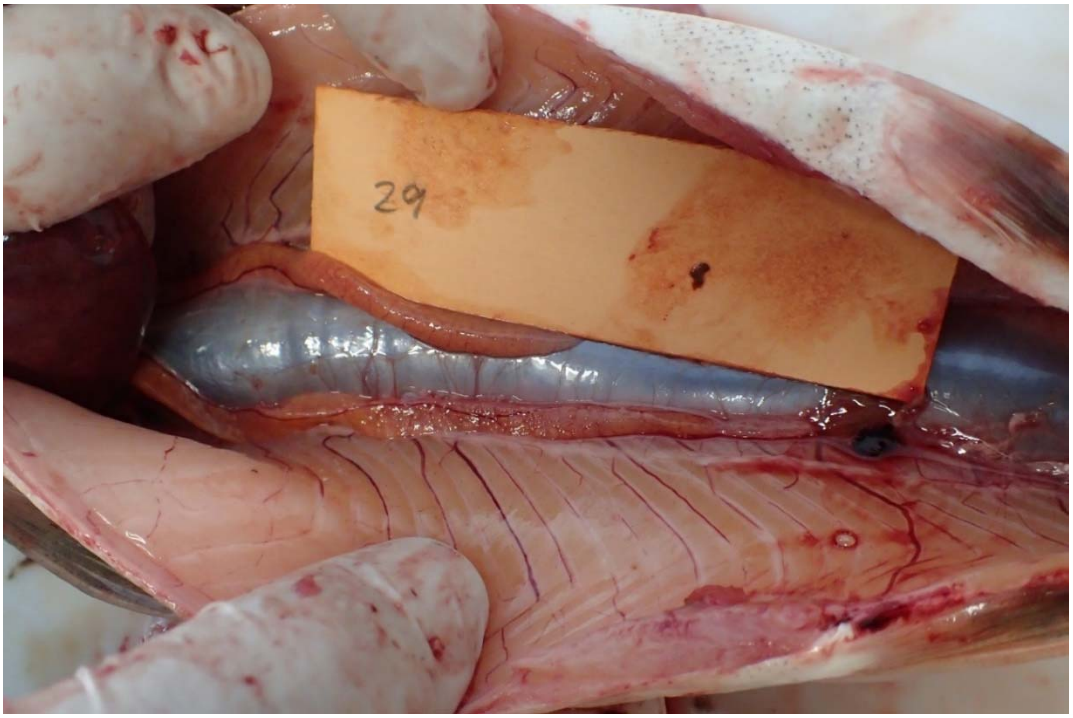
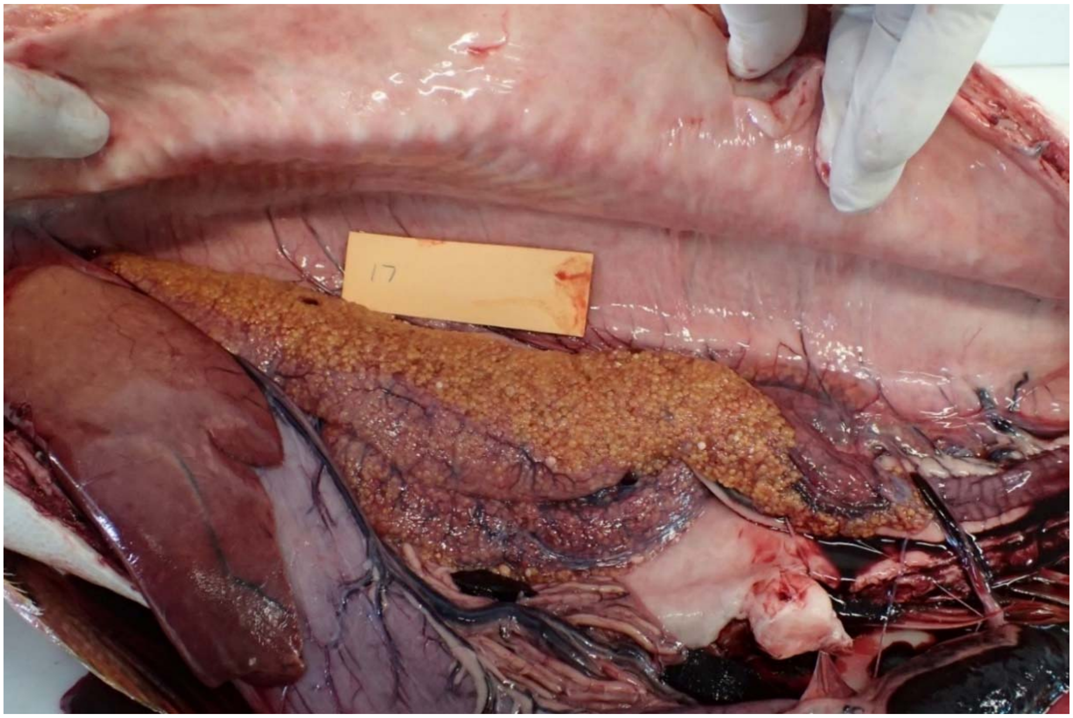


Figure 2. GSI (ovary weight/body weight *100) versus fork length for all female Lake Trout sampled at exposure or reference areas during EEM Cycle 2 and Cycle 3 at the Meadowbank Mine.



Photograph 1. Ovaries of a female Lake Trout, considered to be immature, that would not spawn in the current year, captured during the Meadowbank Cycle 3 EEM fish study. Fork length = 426 mm; GSI = 0.3.

Photograph 2. Ovaries of a mature female Lake Trout that would spawn in the current year, captured during the Meadowbank Cycle 3 EEM fish study. Fork length = 553 mm; GS I= 9.9%.



Photograph 3. Ovaries of a mature female Lake Trout that would not spawn in the current year, captured during the Meadowbank Cycle 3 EEM fish study. Fork length = 905 mm; GSI = 2.7%.

4.2.2 Male Lake Trout Size at Maturity and Proportion of Mature Males That Spawns Each Year

Unlike immature females, whose sex can usually be determined visually by observing small ova directly or when a portion of the ovary is pressed between two microscope slides once the ovaries are large enough to remove and weigh, immature males cannot be definitively identified visually. It is thought, therefore, that most of the immature Lake Trout for which gonad weights were determined but sex was recorded as unknown during Meadowbank Cycle 2 and Cycle 3 were immature males. A plot of GSI versus fork length for males and individuals of unknown sex in the Meadowbank Cycle 2 and Cycle 3 samples (Figure 3) indicates that male Lake Trout mature at a fork length of approximately 400 mm. Of the 49 males longer than 400 mm that were sampled, 42 (86%) had a GSI of 1.4 or higher and, based on GSI and testes appearance, would have spawned in the current year. The remaining 14% of males longer than 400 mm had a GSI of 0.2% or less and, based on GSI and testes appearance, would not have spawned in the current year.

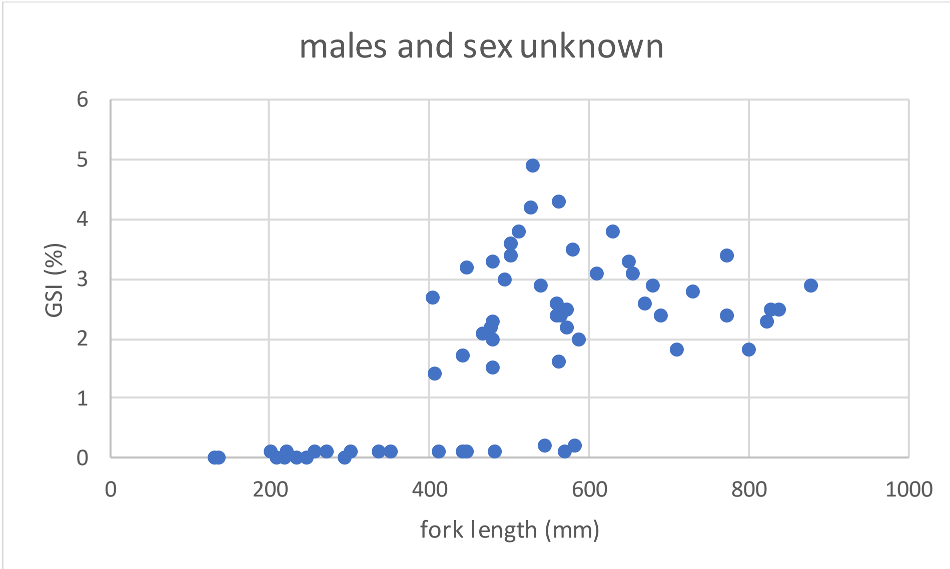


Figure 3. GSI (gonad weight/body weight *100) versus fork length for all male Lake Trout and Lake Trout for which sex could not be determined, sampled at exposure or reference areas during EEM Cycle 2 and Cycle 3 at the Meadowbank Mine.

4.2.3 Estimated Number of Mature Lake Trout in Whale Tail North Basin and in Mammoth Lake that Would Spawn in Any Given Year

Abundance and size distribution data from the fish-out of the north basin of Whale Tail Lake, together with the estimated length at maturity and proportion of mature fish that would spawn each year in the Meadowbank Cycle 2 and Cycle 3 EEM samples (refer to Sections 4.2.1 and 4.2.3), were used to estimate the number of mature male and female Lake Trout present in the north basin of Whale Tail Lake (the North Basin) and in Mammoth Lake, and the number that would spawn in any given year (Table 4).

The North Basin was isolated with a dike in July 2018 and a fish-out was conducted in August and September of 2018. A total of 1,288 Lake Trout were captured and, based on removal method estimates, this was nearly all of the Lake Trout present that were large enough to be susceptible to the gill nets that were used in the fish-out (Agnico Eagle Ltd., 2019); it was estimated that, at most, approximately 104 large-bodied fish, comprised of all four large-bodied species present, remained in the lake. Fork length was determined for all but seven of the Lake Trout captured. For estimation purposes, it was assumed that the Lake Trout that were measured (n=1281) represented all the Lake Trout susceptible to the gill nets that were present in the North Basin; no correction factor was applied to account for Lake Trout that were not captured or were captured but not measured. Based on the sizes at maturity determined from the Meadowbank EEM data, 400 mm for males and 475 mm for females, most of the Lake Trout in the North Basin were immature (Figure 4).

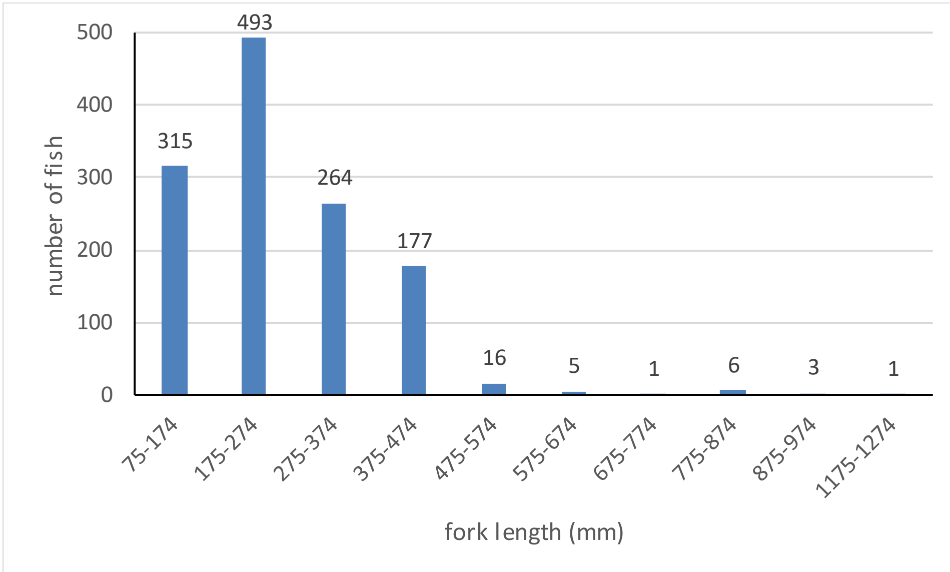


Figure 4. Number of Lake Trout, by 100 mm size classes, captured and measured during the fish-out of the north basin of Whale Tail Lake (total n=1281).

The total number of mature females and the number of mature females that would spawn in a given year in the North Basin were estimated by multiplying the number of Lake Trout (both male and female) longer than 475 mm captured in the North Basin fish-out (32) by the proportions of the Lake Trout longer than 475 mm in the Meadowbank Cycle 2 and Cycle 3 EEM studies that were mature females that would (11%) or would not (36%) have spawned in the year of capture (Table 4). It is estimated that the North Basin contained 3 females that would have spawned in the current year and 11 mature females that would not have spawned in the current year (Table 4). Extrapolating based on area, it is estimated that Mammoth Lake would contain 32 mature females, 7 of which would spawn in any given year. Based on those estimates, the total number of female Lake Trout in Mammoth Lake that would spawn in the current year is about one third of the recommended minimum sample size of 20 that would be required for an adult fish study that examines effects indicators for each of the sexes separately. In other words, based on those estimates, it is not possible to conduct a study that examines reproductive endpoints for female Lake Trout in Mammoth Lake. Attempting such a study would decimate the spawning population and still result in statistical power that was grossly insufficient.

Table 4. Number of mature males, mature males that would spawn in the current year, and females among fish longer than 400 mm, based on the Meadowbank Cycle 2 and Cycle 3 EEM data, and the estimated numbers of the same in the North Basin of Whale Tail Lake and in Mammoth Lake, and the same calculations for mature females, mature females that would spawn in the current year, and males among fish longer than 475 mm. Green-shaded cells contain data from the Cycle 2 and Cycle 3 Meadowbank EEM Studies. Red-shaded cells contain the number of fish in the size range that were captured in the Whale Tail Lake north basin fish-out. Gold-shaded cells contain estimates of the numbers of Lake Trout matching the criteria present in the north basin of Whale Tail Lake or Mammoth Lake.

Estimate of the number of mature females							
Numbers and proportions from Meadowbank Cycle 2 and Cycle 3 EEM							
		number of fish			percent of total number of fish		
		would spawn in current year	would not spawn in current year	total	would spawn in current year	would not spawn in current year	total
FL >475 mm	females	8	27	35	11	36	46
	males			41			54
	Total			76			100
Estimated number in north basin of Whale Tail Lake							
FL >475 mm	females	3	11	15			
	males			17			
	Total			32			
Estimated number in Mammoth Lake							
FL >475 mm	females	7	24	32			
	males			37			
	Total			69			
Estimate of the number of mature males							
Numbers and proportions from Meadowbank Cycle 2 and Cycle 3 EEM							
FL >400 mm	females			41			46
	males	42	7	49	47	8	54
	Total			90			100
Estimated numbers in north basin of Whale Tail Lake							
FL >400 mm	females			42			
	males	43	7	51			
	Total			93			
Estimated numbers in Mammoth Lake							
FL >400 mm	females			91			
	males	93	16	109			
	Total			199			

The number of mature males in the North Basin and the number that would spawn in a given year were estimated by multiplying the number of Lake Trout longer than 400 mm (male and female) captured (93) by the proportions of the Lake Trout longer than 400 mm in the Meadowbank Cycle 2 and Cycle 3 EEM studies that were males that would (47%) or would not (8%) have spawned in the year of capture (Table 5). The number of females in the greater than 400 mm size group was estimated by multiplying the number of Lake Trout longer than 400 mm captured (93) by the proportions of the Lake Trout longer than 400 mm in the Meadowbank Cycle 2 and Cycle 3 EEM studies that were females (46%). It is estimated that Mammoth Lake would contain 109 mature males, 93 of which would spawn in any given year. Based on this estimate, there are enough male Lake Trout in Mammoth Lake that would spawn in the current year to achieve adequate power for the examination of reproductive endpoint; a sample size of 20 males that would spawn in the current year would be 22% of the current year's male spawners.

4.2.4 Estimated Number of Fish That Must be Sampled to Acquire a Sample of 20 Mature Males that Would Spawn in the Current Year

The calculations in Section 4.2.3 indicate that Mammoth Lake does not contain enough mature females that would spawn in the current year to conduct a study that examines gonad size and fecundity, but that enough males are present to examine gonad size. When Lake Trout are in pre-spawning condition (i.e. gametes cannot be readily expelled), it is not possible to reliably distinguish between males and females or between fish that will spawn in the current year and those that will not. Therefore, it is necessary to kill and internally examine all the fish in the appropriate size range that are captured until the necessary number of the target group have been sampled. For Lake Trout longer than 400 mm, the proportion of males that will spawn in the current year and the proportion of total females are 42% and 41% respectively (Table 5). Assuming equal catchability of males and females, approximately 20 females would be sampled in order to sample 20 males that would spawn in the current year. It is estimated that four (4) of those 20 females would have spawned in the current year (Table 5). In other words, in order to conduct a study that examines the gonad weight at total weight relationship for males only, it is estimated that approximately half of the females in Mammoth Lake that would spawn that year would be killed. As gill nets are not perfectly size-selective, additional fish shorter than 400 mm would also be captured and killed. Agnico is of the opinion that this is unacceptable, both from a population sustainability standpoint and from the standpoint of confounding future EEM fish studies at Whale Tail Pit, which are intended to determine if effects are occurring due to the discharge of effluent.

Table 5. Numbers and proportions of Lake Trout longer than 400 mm that were female and males that would and would not spawn in the current year, based on the Meadowbank Cycle 2 and Cycle 3 data, and the estimated numbers that would be sampled in order to obtain a sample of 20 mature males that would spawn in the current year.

Numbers and proportions for fish > 400 mm, from Meadowbank Cycle 2 and Cycle 3 EEM						
	number of fish			percent of total number of fish		
	would spawn in current year	would not spawn in current year	total	would spawn in current year	would not spawn in current year	total
females	8	33	41	9	37	46
males	42	7	49	47	8	54
Total			90			100
Estimated numbers of fish >400 mm sampled in order to sample 20 mature males that would spawn in the current year						
	would spawn in current year	would not spawn in current year	total			
females	4	16	20			
males	20	3	23			
Total			43			

4.3 Sample Size Required to Address Age-Related Effect Indicators

The MDMER considers two age-related effect indicators: age, or age-frequency distribution, and total body weight at age. It is estimated, based on the Cycle 2 and Cycle 3 EEM data from Meadowbank, that a sample size of 36 – 42 Lake Trout per lake would be required to achieve the desired power to detect a 25% difference in body weight at age. Those sample size estimates are based on power analysis of pooled data for both immature and mature individuals of both sexes but, in the absence of sex-specific data, it is assumed that similar numbers will be required if comparisons were made for individuals of each sex. If only mature individuals that will spawn in the current year are to be included in the analyses, then the same ratios that were used in Section 4.2.4 to estimate the total number of Lake Trout that would have to be sampled can be applied. To acquire a sample of 36 males that would spawn in the current year, it is estimated that a total sample of 77 Lake Trout longer than 400 mm, 42 males and 35 females, would be sampled. It would not be possible to acquire a sample of 36 females that would spawn in the current year, but theoretically, a total sample of 342 Lake Trout longer than 475 mm, 158 females and 185 males, would be required.

Power analysis on ages of Lake Trout sampled in Cycle 3 at Meadowbank indicates that the required sample size per lake to detect at 25% difference in mean age with $\alpha=\beta=0.1$ is 143. Once again, the estimate is based on pooled data for both immature and mature individuals of both sexes.

Apart from the sample size requirements, Lake Trout from the region have attributes which are not desirable in a sentinel species. Munkittrick et al (2000) recommend against using species that are difficult to age as sentinel species because the uncertainty in aging will make data interpretation difficult. The EEM Technical Guidance document (Environment Canada, 2012) recognizes the uncertainty in accurately determining ages, and cautions "*Problems associated with determining the age of some species of fish should be discussed and reviewed before effects on weight-at-age and age are used to choose a path through the EEM program.*" Lake Trout in Arctic regions are in the category of fish that are difficult to age. Campana *et al* (2008) reported that otolith growth in the oldest Arctic Lake Trout which they examined was so low as to be unresolvable under conventional examination with a dissecting microscope.

Lake Trout in the region are also long-lived. The oldest Lake Trout captured in the Meadowbank EEM studies thus far was estimated to be 48 years old and 13% of the Lake Trout aged using otoliths for the Meadowbank EEM studies were estimated to be older than 30 years. The EEM Technical Guidance document (Section 3.3, page 3-6) implies that species that live greater than 30 years should not be used as sentinel species.

Given the problems associated with determining the age (and therefore weight-at-age) of slow-growing, long-lived Lake Trout in low productivity northern lakes, such as the lakes that are the subject of this study, C. Portt and Associates and Kilgour & Associates (2014) suggested that neither weight-at-age nor age distribution are appropriate for choosing a path through the EEM program due to their unreliability. For this reason, they recommended not sampling the additional Lake Trout required to address age-related endpoints in the Meadowbank Cycle 3 EEM and the TAP agreed. The basis for this recommendation is considered equally valid for the Whale Tail Pit EEM study.

4.4 Sample Sizes Required to Examine the Total Body Weight at Length and Liver Weight at Total Body Weight Effect Indicators

There is a very strong correlation between total body weight and length, even when data from both males and females and mature and immature fish are pooled. With no outliers removed, the r^2 of the reduced ANCOVA of log of total body weight versus log of length relationship for Lake Trout was 0.994 in Cycle 1 and Cycle 3 and 0.996 in Cycle 2 (Table 6). Power analysis indicated that the required sample size to detect a 10% difference in total body weight at length ranged from 16 (Cycle 2) to 21 (Cycle 1 and Cycle 3).

There is also a very strong relationship between liver weight and total body weight, even when data from both males and females and mature and immature fish are pooled. With no outliers removed, the r^2 of the reduced ANCOVA of log of liver weight versus log of total body weight relationship was 0.976 in Cycle 2 and 0.974 in Cycle 3 (Table 6; liver weight was not determined in Cycle 1). Power analysis indicated that the required sample size to detect a 25% difference in liver weight at total body weight was 16, based on either the Cycle 2 or the Cycle 3 data.

Based on those results, it is not necessary to separate the data from males and females or mature and immature individuals in order to detect differences in the total body weight versus length or the liver weight versus total body weight relationships that are equal to the critical effect sizes with relatively small sample sizes. It should be borne in mind that the data from males and females and mature and immature individuals are pooled in analyses of data from non-lethal surveys.

Table 6. MDMER effect indicators, critical effect sizes, the r^2 and estimated sample size to detect the critical effect size with α=β=0.1 based on reduced ANCOVA or, for age, from ANOVA, from Meadowbank EEM studies. Analyses were conducted using pooled data from both mature and immature individuals of both sexes.

Effect indicator	Critical effect size	Meadowbank Cycle 1		Meadowbank Cycle 2		Meadowbank Cycle 3	
		r^2	N	r^2	N	r^2	N
Total body weight at length	±10%	0.994	21	0.996	16	0.994	21
Liver weight at total body weight	±25%	na	na	0.976	16	0.974	16
Total body weight at age	±25%	na	na	0.943	42	0.936	36
Age	±25%	na	na	na	na	na	143
Gonad weight at total length	±25%	na	na	na	na	na	na

4.5 Lake Trout Study Design

4.5.1 Overall Approach and Sample Size

Based initially on the results of a fish-out at the Meadowbank Mine, and subsequently on the results of each successive EEM fish survey using Lake Trout as a sentinel species at the Meadowbank mine, it was concluded that it was not feasible to collect a sufficient number of adult Lake Trout that would spawn in the current year to address reproductive effects indicators. Each of the Cycle 1, Cycle 2, and Cycle 3 study designs explicitly stated that reproductive endpoints could not be addressed. Each of those study designs was approved by Environment Canada and the Technical Advisory Panel.

In their comments and recommendations provided in the review of the Meadowbank Cycle 2 interpretive report (appended to a letter to Stephane Robert (Agnico) from Suzanne Forbrich (ECCC), January 20, 2017), the Technical Advisory Panel (TAP), consisting of representatives from the Nunavut Water Board, Crown-Indigenous Relations and Northern Affairs Canada, and Environment and Climate Change Canada stated "*A fish study is required for the next EEM biological field work. TAP members are supportive of a lethal study on 20 Lake Trout.*" In their comments and recommendations provided in the review of the Meadowbank Cycle 3 study design report (appended to a letter to Marie-Pier Marcil (Agnico) from Suzanne Forbrich (ECCC), April 10, 2017), the Technical Advisory Panel (TAP), consisting of representatives from the Nunavut Water Board, Crown-Indigenous Relations and Northern Affairs Canada, and Environment and Climate Change Canada stated "*the proposed design of 20 lethal Lake Trout is supported provided that power analyses continue to indicate that it is suitable*."

It is estimated that Mammoth Lake supports approximately seven mature female Lake Trout that would spawn in a given year, which is less than half the number that would be required to obtain adequate power to examine even the strongest relationships (total weight at length, liver weight at total weight; Table 6). A study of mature males that would spawn in the current year would require that approximately 20% of the males that would spawn in the current year be sampled and would result in approximately 50% of the females that would spawn in the current year being killed as 'by-catch' (Table 5). Agnico is of the opinion that this is unacceptable, both from a population sustainability standpoint and from the standpoint of confounding future EEM fish studies at Whale Tail Pit. An EEM fish study is intended to determine if the discharge of mine effluent is affecting fish populations. EEM is iterative, with subsequent cycles intended to confirm or refute that effects are or are not occurring due to effluent discharge. Conducting a fish survey that could have a significant effect on the fish population is not consistent with this objective.

Considering the information reviewed in Sections 4.1 through 4.4 and the EEM studies conducted at Meadowbank using Lake Trout as a sentinel species, it is recommended that the 1st Biological Study for Whale Tail Pit include a lethal study of Lake Trout with a target sample size of 25 individuals from the portion of Mammoth Lake where the effluent concentration at the bottom and surface exceeds 1% after two weeks of effluent discharge (**Figure 6**) in Mammoth Lake and from reference lakes D1 and Lake 8 (**Figure 1**). It is recognized that this is not an ideal study; It will not address reproductive endpoint and will not have the desired power to assess differences in age distribution or total body weight versus age. This sample size, however, is feasible and should not cause serious harm to the Lake Trout populations in the study lakes.

4.5.2 Collections

4.5.2.1 Timing

Lake trout sampling for the Whale Tail Pit EEM Cycle 1 will be conducted in the second half of August 2020.

4.5.2.2 Gear

Index gill nets comprised of six panels of stretched mesh (sizes 126, 102, 76, 51, 38, and 25 mm) will be used as the primary means of fish capture for this study. Each panel of gill net is 1.8 m (6 feet) deep by 22.7 m (25 yards) long, so that the length of a six-panel gang is 136.4 m (150 yards). This is the gear that was used in EEM Cycle 2 and EEM Cycle 3 at the Meadowbank Mine, as well as in fish-outs at Meadowbank and Whale Tail.

4.5.2.3 Net Deployment and Retrieval

Gill nets will be set, targeting Lake Trout, within each of the three sampling areas (Figure 1), with the specific locations determined based on local habitat conditions and, in the exposure area, the extent of the 1% plume. The geographic coordinates of each end of each net will be recorded, as will depth and the date and time of deployment and retrieval. Set duration will be determined in the field based on local conditions, with the objective of capturing 25 Lake Trout and minimizing the mortality of additional Lake Trout and incidental catch. The number of individuals of each species captured in each net will be recorded.

4.5.2.4 Supporting Environmental Variables

Specific conductance (µS/cm), pH, dissolved oxygen (mg/L and % saturation) and temperature (°C) will be determined in the field within the Exposure and Reference Areas in conjunction with the benthic invertebrate collections. In reference lakes, the water quality monitoring for the benthic invertebrate sampling will also serve as the sampling for the fish study, as there is no reason to expect significant spatial variation. In Mammoth Lake, temperature and conductivity profiles will be conducted at the gill net locations to confirm effluent presence and absence of stratification.

4.5.3 Lake Trout Measurements

The following information will be determined for each Lake Trout that is part of the lethal sample:

- fork length in millimetres
- total weight in grams
- presence of external deformities, lesions, tumours, or parasites.
- liver weight in grams
- sex, gonad condition and gonad weight in grams
- mean egg weight for mature females that will spawn in the current year
- presence of internal or external deformities, lesions, tumours, or parasites.

Otoliths will be collected and placed in envelopes labeled with the sampling area, date, species, and specimen number. Otoliths will be mounted whole on a glass slide, ground to the core on one side, flipped to adhere the core area to the glass, and then ground to a thin section on the other side. Age will be estimated based on the number of annuli counted using transmitted light and a stereo microscope. As a QA/QC measure, annuli will be counted by a second person for at least 10% of the otoliths.

4.5.4 Statistical Analysis

Data assessment and interpretation will be conducted following the guidelines presented in Environment Canada (2012).

4.5.4.1 Initial Data QA/QC

Data will be entered into an Excel© spreadsheet. The entered data will be compared with the original data sheets, and any data entry errors that are identified will be corrected. Scatterplots of length versus weight will be prepared. If aberrant values are identified, the original data sheets will be re-checked to ensure that these are not due to transcription errors. Any transcription errors found will be corrected. If clearly aberrant values for length or weight occur in the original data, these will be eliminated from the dataset.

4.5.4.2 Calculated Indices

Condition (K) will be calculated using the formula:

$$K = \frac{100 \bullet \text{weight}}{\text{length}^3}$$

Gonado-somatic index (GSI) will be calculated using the formula:

$$GSI = \frac{100 \bullet \text{gonad weight}}{\text{total weight}}$$

Hepato-somatic index (HSI) will be calculated using the formula:

$$HSI = \frac{100 \bullet \text{liver weight}}{\text{total weight}}$$

4.5.4.3 Summary Statistics and Comparisons of Size

Summary statistics (sample size, mean, median, minimum, maximum, standard deviation, standard error) will be generated for length, weight, condition, HSI and GSI by lake, sex, and maturity, and for all lake trout from each lake pooled.

4.5.4.4 Comparisons of Size and Age

Skewness and kurtosis will be determined for both raw and $\log_{10}$ transformed length, weight, and age at each area and divided by their respective standard errors. A value greater than two will be taken to indicate that a distribution deviates significantly from normal. As normality is an assumption of ANOVA, if either the raw or the transformed data have values of skewness or kurtosis divided by their respective standard errors that are less than two at all areas then the data will be analyzed using ANOVA. Otherwise, the two-sample Kolmogorov-Smirnov (K-S) test, which is recommended for comparing length-frequency distributions between areas in non-lethal surveys (Environment Canada, 2007), will be used to compare length and weight distributions between pair of areas.

4.5.4.5 ANCOVA Analyses

ANCOVA will be used to determine if significant differences occur in the following relationships between the exposure area in Mammoth lake and the two reference lakes:

- total weight versus length
- total weight versus age
- length versus age
- liver weight versus total weight
- liver weight versus length
- gonad weight versus total weight, if more than 5 individuals of one sex that will spawn in the current year are collected from more than 1 site
- egg weight versus total weight, if more than 5 females that will spawn in the current year are collected from more than 1 site

Except for the reproductive endpoints, these comparisons will use pooled data from both sexes and mature and immature individuals.

Using log-transformed values, ANCOVA will first be used to test for significant differences (P>.0.05) in slopes between the two reference areas. If none exist, then ANCOVA will be used to test for significant differences (P>0.05) in intercepts between the two reference areas. In cases where the interaction term accounts for < 2% of the total variation in the response variable, the reduced model will be considered appropriate and used to assess significance, as per Barrett *et al*. (2010). If there are no significant differences in either slopes or intercepts between the reference areas, the reference areas data will be pooled for comparisons to the exposure area. If there area significant differences between the reference areas then the exposure area and each of the reference areas will be included in the ANCOVA and pair-wise comparisons will be used to determine if there area significant differences (P>0.1) between the exposure area and each of the reference areas.

Residuals from each ANCOVA will be examined for normality and outliers. Observations producing large Studentized residuals (i.e., > 4) will be removed from the data set, and the analyses repeated and any changes in conclusions considered. This process will be continued until no additional outliers are identified.

The percent difference in least-square means between Mammoth Lake and the reference lakes will be calculated as:

$$\%\,\text{Difference} = \frac{\overline{x}_{\text{exp osure}} - \overline{x}_{\text{reference}}}{\overline{x}_{\text{reference}}}$$

When log transformed data are analyzed, the least-mean square values used will be antilogs of the calculated values.

4.5.4.6 Power Analysis

Post-hoc power analysis will be conducted to determine the ability of the EEM First Biological to detect the specified critical effect size for each of the parameters examined.

5 SLIMY SCULPIN STUDY DESIGN

5.1 BACKGROUND INFORMATION

As described in Section 3.2, Slimy Sculpin CPUE from previous sampling indicates that it would be possible to capture enough individuals to conduct an EEM fish survey (Table 3) in Mammoth Lake and three potential reference lakes. Lake A44 is not proposed as a reference lake for the Lake Trout study because of its small size (and presumably small Lake Trout population) but could serve as an alternate reference lake for Slimy Sculpin, if necessary.

As described in the study design report (C. Portt and Associates and Kilgour & Associates Ltd., 2019), a study by University of Waterloo is currently underway using Slimy Sculpin to examine the effects of flooding at Whale Tail Pit. Slimy Sculpin were collected in 2018 from Mammoth Lake, Lake 8, and other lakes now subject to flooding, and in 2019 from Mammoth Lake and Lake A44. In 2020, the University of Waterloo study would like to acquire a sample of approximately 30 Slimy Sculpin from Mammoth Lake and other lakes in the area to examine the weight versus length relationship (condition), length at age, and mercury concentrations; those fish will be frozen and taken to the University of Waterloo where lengths and weights will be determined and otoliths will be removed and used to estimate age; the whole fish (minus otoliths) samples will then be frozen and archived for future stable isotope and metals analyses, as has been done with the samples from previous years (Professor Heidi Swanson, University of Waterloo. Personal communication with C. Portt, July 5, 2019). To be consistent with previous years, the first 30 individuals captured should be the University of Waterloo samples. It is proposed that, in order to make maximum use of the Slimy Sculpin captured, these fish be weighed and measured in the field and used in a 'non-lethal' study of Slimy Sculpin conducted for this Cycle 1 EEM.

In their review of the proposed study design (refer to Sections 1 and 2) ECCC requested that a Slimy Sculpin study be designed that evaluates all of the EEM endpoints, including reproductive endpoints, but stated "*If there are concerns about the potential impacts of sampling on small fish populations, non-lethal sampling may be warranted*." Agnico does have concerns about the potential impacts of sampling on the Slimy Sculpin populations. Agnico also has concerns about the feasibility of collecting adequate numbers of sexually mature Slimy Sculpin that will spawn in the following spring and thus be suitable for assessing reproductive endpoints.

5.2 Slimy Sculpin Size at Maturity and CPUE

No data are available regarding the age or size at maturity of Slimy Sculpin in the study area, nor is it known if mature adults spawn each year.

At three sites on the Rainy River, Ontario, female Slimy Sculpin less that 55 mm long were immature and most of those greater than 55 mm long were mature. At those sites, all males longer than 50 mm were mature, with both mature and immature males present in the 45 – 49 mm length range (C. Portt and Associates and Stantec Consulting Ltd., 2007; C. Portt, unpublished data). Golder (2018) reported 48 mm as the length at which Slimy Sculpin from Lac du Sauvage in the North West Territories had a 50% probability of being sexually mature (both sexes combined). Bond et al (2015) reported the mean length at which 50% of Slimy Sculpin were mature (both sexes combined) to range from 51 mm to 57 mm in two tributaries to Lake Superior, Ontario.

Owens and Noguchi (1998) reported that the minimum size of gravid female Slimy Sculpin was 55 mm at one sampling location in Lake Ontario and 63 mm at two others; immature females up to 70 mm in length were encountered. Owens and Noguchi (1998) also reported that some females ≥ 75 mm long contained only small eggs and hypothesized that these were mature females that had spawned in the previous year but would not spawn in the current year.

Van Vliet (1964) examined several slimy sculpin populations in northern Saskatchewan and reported that sexual maturity appeared to be related more to size than to age. Based on the mean lengths in Van Vliet (1964), it appears that the slimy sculpin in the Cree River, the site where Slimy Sculpin grew most slowly, matured at about 70 mm. Some of the females considered to be immature by Van Vliet were more than 80 mm long. The presence of 'immature' fish among the largest individuals suggests that, as postulated by Owens and Noguchi (1998), only a portion of the mature female slimy sculpin spawn each year.

The length-frequency distributions of slimy sculpin captured in Mammoth Lake and potential reference lakes in 2018 and 2019 are presented in Figure 5. Individuals 50 mm or longer accounted for an average of 17% of the total catch (range = 8% - 36%) and individuals 55 mm or longer accounted for an average of 5% of the catch (range = 2% - 8%). The catch-per-1000-electroseconds for all Slimy Sculpin and those 50 mm or longer and those 55 mm or longer is presented in Table 7 for Mammoth Lake and potential reference lakes.

Arciszewski et al (2010) recommend a sample size of 100 Slimy Sculpin for a non-lethal survey and state that electrofishing effort of a maximum of 12,000 electro-seconds should be considered as adequate to try and capture the target number of Slimy Sculpin at each site for EEM studies in northern lakes. Based on the CPUE from 2018 and 2019 (Table 7), with 12,000 electroseconds of effort it should be feasible to capture 100 Slimy Sculpin, the number recommended by Arciszewski et al (2010) for a non-lethal survey, in all of the lakes that have been examined. The recommended minimum sample size for a standard EEM survey that would examine reproductive endpoints is 20 mature males and 20 mature females that will spawn in current year. Based on the CPUEs presented in Table 7, 12,000 electroseconds of effort would capture, on average, about half the required number of mature fish if the length at maturity was 50 mm and about one sixth of the required number of mature fish if the length at maturity was 55 mm. This suggests that conducting a standard EEM that examines reproductive endpoints will not be feasible.

Arciszewski et al (2010) suggest that removing the number of mature adult Slimy Sculpin required for a standard EEM survey may not be sustainable in northern lakes with low productivity. They recommend that ten mature males and ten mature females be lethally sampled to examine liver and gonad weights, as well as age.

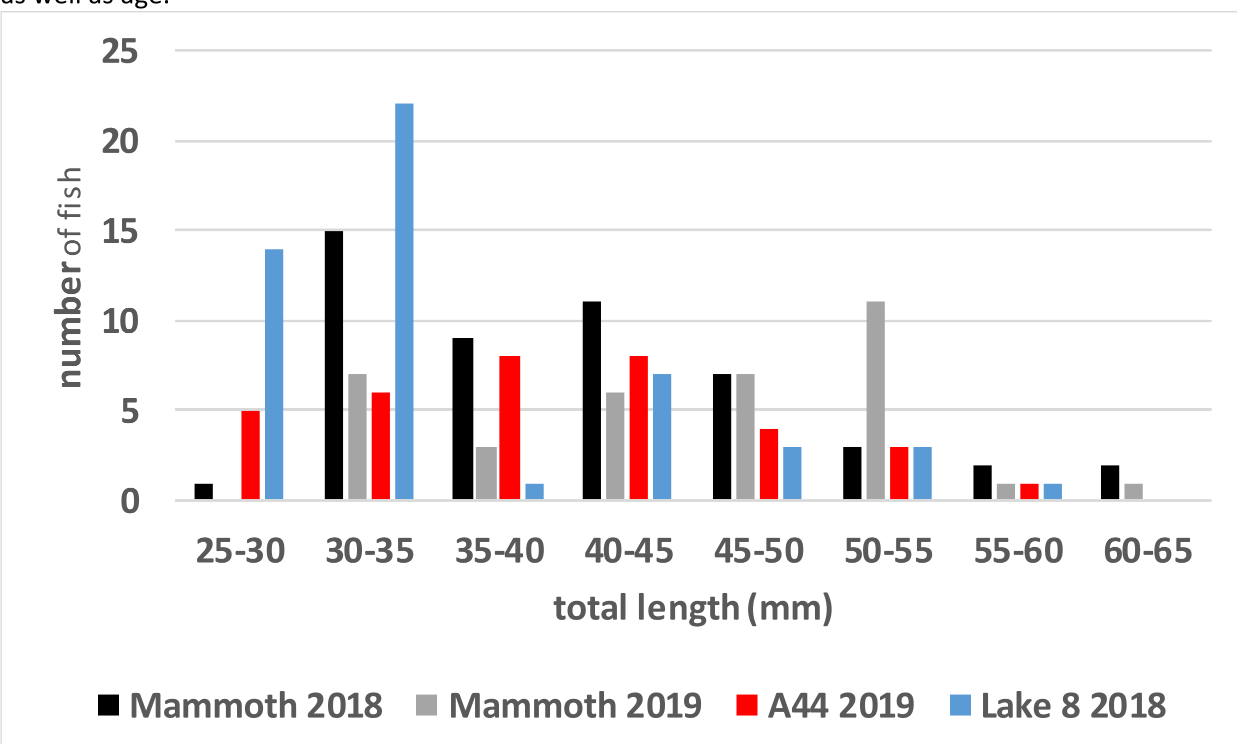


Figure 5. Length-frequency of slimy sculpin captured in Mammoth Lake in 2018 and 2019, A44 in 2019 and Lake 8 in 2018. (Source: Jared Ellenor, M.Sc. Candidate, University of Waterloo).

Table 7. Catch per 1000 electroseconds of Slimy Sculpin in 2018 and 2019, by lake, year, and length category.

	catch per 1000 electroseconds		
Lake - year	all lengths	≥ 50 mm	≥ 55 mm
Mammoth - 2018	10.9	1.5	0.9
Mammoth - 2019	11.5	4.1	0.6
D1 - 2018	11.0	na[1]	na[1]
Lake 8 - 2018	17.0	1.3	0.3
A44 - 2019	16.2	1.9	0.5
Mean	13.4	2.2	0.6

1 – Length data are not available

5.3 Timing of Slimy Sculpin Collections

Arciszewski et al (2010) state that the best sampling time for slimy sculpin in northern lakes would be late August or even into September. This increases the chance of capturing YOY sculpin and, in a lethal survey, provides more meaningful data on mature fish gonad sizes.

5.4 Slimy Sculpin Study Design

5.4.1 Overall Approach and Sample Size

It is proposed that a 'non-lethal study' be conducted using slimy sculpin as the sentinel species, with Mammoth Lake as the exposed area and two of lakes D1, 8 or A44 as reference areas, (**Figure 1**) with a target sample size of 100 individuals per lake. Lengths and weights will be determined for all Slimy Sculpins captured. The first 30 Slimy Sculpin from each site will be weighed and measured and then used for the University of Waterloo study. The ten largest males and ten largest females will be lethally sampled and preserved in 10% Formalin, so that gonad and liver weights can be determined in the laboratory. The remainder of the fish will be released near the point of capture.

5.4.2 Collections

5.4.2.1 Timing

Slimy Sculpin sampling will be conducted in the second half of August 2020.

5.4.2.2 Gear and effort

Slimy Sculpin will be collected by backpack electrofishing along the shorelines of Mammoth Lake where the effluent concentration exceeds 1% after two weeks of effluent discharge (**Figure 6**) and from two reference lakes. A maximum of 12,000 electroseconds of effort will be applied per lake. Sampling in Mammoth Lake will be conducted within the area where the effluent concentration at the bottom is predicted to be 1% or more after two weeks of discharge (**Figure 6**), based on the plume modelling study (Golder, 2020). The exact sampling locations will be selected in the field, based on habitat characteristics. Experience collecting sculpin in area lakes has shown that CPUE is lowest in areas with boulder or multi-layered cobble substrate and highest where there are patches of cobble or gravel amongst areas of finer substrate. This is consistent with the observations of Gray et al (2005, cited in Arciszewski et al, 2010). It is not known if the CPUEs are representative of actual abundance, catchability, or both. The electrofisher settings, the time when electrofishing begins and ends, and the number of electroseconds that elapse will be recorded at each location where electrofishing occurs. The coordinates where electrofishing starts and ends and the distance electrofished will be determined using a handheld GPS. CPUE will be calculated for each lake.

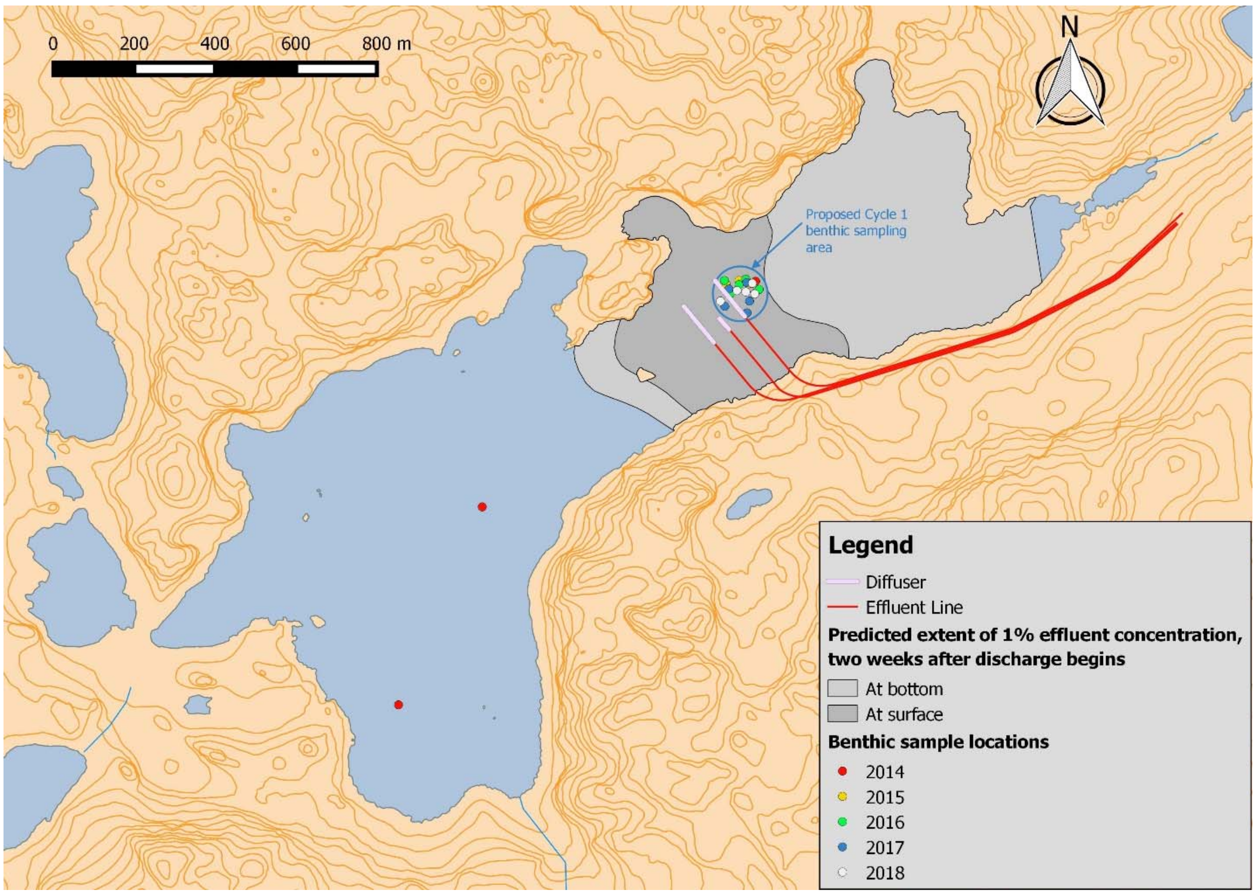


Figure 6. Extent of the 1% effluent plume two weeks after effluent discharge begins. The proposed benthic invertebrate sampling area, and the locations where benthic invertebrate samples were collected in previous years, are also shown.

5.4.3 Fish processing in the field

Each captured slimy sculpin will be measured to the nearest mm using a standard fish measuring board, weighed to the nearest 0.01 g using an electronic balance. The first 30 individuals captured will not be processed further for the EEM study; they will be frozen for use in the University of Waterloo study. The ten largest males and ten largest females in the remainder of the sample will be lethally sampled if they are longer than 45 mm. The remainder of the fish will be released near their point of capture.

The otoliths will be removed from the lethally sampled fish and stored dry for subsequent aging in the laboratory. Those fish will then be preserved in 10% buffered formalin for subsequent processing.

5.4.4 Processing of lethally sampled fish

The lethally sampled fish will be remeasured to the nearest mm and reweighed to the nearest 0.01 g in the laboratory. An examination of the external and internal condition of each fish will be conducted to determine the presence of abnormalities, lesions, tumours and parasites. If possible, the weight of parasites present will be determined (± 0.001g). Livers will be extracted and weighed (± 0.0001g). Gonads will be removed and weighed (± 0.001g) and sex and maturity will be determined. Ovaries will not be well developed in late August and therefore fecundity and egg weight will not be determined. As noted by Arciszewski et al (2010), sampling of pre-spawning female Slimy Sculpin is not possible in Arctic lakes because the lakes are ice-covered.

5.4.5 Aging

The number of annuli on otoliths will be counted under a dissecting microscope. Each annulus will be interpreted to indicate one year of growth. Ten percent of the otoliths will be aged a second time, blind, by a second investigator.

5.4.6 Statistical Analysis

Data assessment and interpretation will be conducted following the guidance presented in Environment Canada (2012).

5.4.6.1 Initial Data QA/QC

Data will be entered into an Excel© spreadsheet. The entered data will be compared with the original data sheets, and any data entry errors that are identified will be corrected. Scatterplots of length versus weight will be prepared. If aberrant values are identified, the original data sheets will be re-checked to ensure that these are not due to transcription errors. Any transcription errors found will be corrected. If clearly aberrant values for length or weight occur in the original data, these will be eliminated from the dataset.

5.4.6.2 Calculated Indices

Condition (K) will be calculated using the formula:

$$K = \frac{100 \bullet \text{weight}}{\text{length}^3}$$

For the lethally sampled individuals, gonado-somatic index (GSI) will be calculated using the formula:

$$GSI = \frac{100 \bullet \text{gonad weight}}{\text{total weight}}$$

For the lethally sampled individuals hepato-somatic index (HSI) will be calculated using the formula:

$$HSI = \frac{100 \bullet \text{liver weight}}{\text{total weight}}$$

5.4.6.3 Summary Statistics and Comparisons of Size

Summary statistics (sample size, mean, median, minimum, maximum, standard deviation, standard error) will be generated for length, weight, and condition for all Slimy Sculpin from each lake. Those same summary statistics will be generated for length, weight, condition, liver weight, HIS, gonad weight and GSI by lake, sex and maturity for the lethally sampled individuals from each lake.

5.4.6.4 Comparisons of Size and Age

Skewness and kurtosis will be determined for both raw and $\log_{10}$ transformed length and weight at each area and divided by their respective standard errors. A value greater than two will be taken to indicate that a distribution deviates significantly from normal. As normality is an assumption of ANOVA, if either the raw or the transformed data have values of skewness or kurtosis divided by their respective standard errors that are less than two at all areas then the data will be analyzed using ANOVA. Otherwise, the Kruskall-Wallace test, which is recommended for comparing length-frequency distributions between areas in non-lethal surveys (Environment Canada, 2007), will be used to compare length and weight distributions between pair of areas.

If the first age class is clearly defined by length-frequency, the length of fish in that age class class will be compared between sites using the same methods as for overall length.

5.4.6.5 Weight Versus Length Relationship

ANCOVA will be used to determine if significant differences occur in the total weight versus length relationship between the exposure area in Mammoth lake and the two reference lakes:

Using log-transformed values, ANCOVA will first be used to test for significant differences (P>0.5) in slopes between the two reference areas. If none exist, then ANCOVA will be used to test for significant differences (P>.0.1) in intercepts between the two reference areas. In cases where the interaction term accounts for < 2% of the total variation in the response variable, the reduced model will be considered appropriate and used to assess significance, as per Barrett *et al*. (2010). If there are no significant differences in either slopes or intercepts between the reference areas, the reference areas data will be pooled for comparisons to the exposure area. If there are significant differences between the reference areas then the exposure area and each of the reference areas will be included in the ANCOVA and pair-wise comparisons will be used to determine if there are significant differences (P>0.1) between the exposure area and each of the reference areas.

Residuals from each ANCOVA will be examined for normality and outliers. Observations producing large Studentized residuals (i.e., > 4) will be removed from the data set, and the analyses repeated and any changes in conclusions considered. This process will be continued until no additional outliers are identified.

The percent difference in least-square means between Mammoth Lake and the reference lakes will be calculated as:

$$\%\,\text{Difference} = \frac{\overline{x}_{\text{exp osure}} - \overline{x}_{\text{reference}}}{\overline{x}_{\text{reference}}}$$

5.4.6.6 Length and Age at Maturity

The sex, maturity and GSI data for the lethally sampled individuals from each lake will be compared to length and age, to estimate the size and age of maturity for male and female Slimy Sculpin. This information will inform future EEM studies at Whale Tail Pit.

5.4.6.7 Power Analysis

Post-hoc power analysis will be conducted to determine the ability of the EEM First Biological to detect the specified critical effect size for each of the parameters examined.

6 REFERENCES

Agnico Eagle Meadowbank Mine. 2019. Whale Tail Lake Fishout Report Under DFO Fisheries Act Authorization 16-HCAA-00370. 16 p + appendices.

Azimuth. 2010. Environmental Effects Monitoring (EEM): Cycle 1 Study Design, Meadowbank Division, Nunavut. Report prepared by Azimuth Consulting Group Partnership, Vancouver, BC for Environment Canada, Edmonton, AB on behalf of Agnico-Eagle Mines Ltd., Baker Lake, NU. December, 2010.

Azimuth. 2012. Environmental Effects Monitoring (EEM): Cycle 1 Interpretative Report. Meadowbank Division, Nunavut. Report prepared by Azimuth Consulting Group Partnership, Vancouver, BC for Environment Canada, Edmonton, AB on behalf of Agnico-Eagle Mines Ltd., Baker Lake, NU. June, 2012.

Barrett, T.A., M.A. Tingley, K.R. Munkittrick, and R.B. Lowell. 2010. Environmental Effects Monitoring Data: Dealing with Heterogeneous Regression Slopes in Analysis of Covariance. Environmental Monitoring and Assessment, 166:279-291.

Bond, M.J., N.E. Jones and T.J. Haxton. Growth and life history patterns of a small-bodied stream fish, Cottus Cognatus, in hydropeaking and natural rivers of northern Ontario. River Res. Applic. 32: 721-733.

Campana, S.E., J.M. Casselman and C.M. Jones. 2008. Bomb radiocarbon chronologies in the Arctic, with implications for the age validation of lake trout (Salvelinus namaycush) and other Arctic species. Can. J. Fish. Aquat. Sci. 65: 733-743.

C. Portt and Associates. 2018. Whale Tail Pit 2014 - 2016 Fish and Fish Habitat Field Investigations: Agnico Eagle Mines Ltd.- Meadowbank Division. Prepared for Agnico-Eagle Mines Ltd., Regional Office - 93, Rue Arseneault, suite 202, Val-d'Or, Québec, J9P 0E9. xi. + 157 p.

C. Portt and Associates. 2019. Whale Tail Expansion Project 2019 Fish and Fish Habitat Field Investigations: Agnico Eagle Mines Ltd.- Meadowbank Division. Prepared for Agnico-Eagle Mines Ltd.: Meadowbank Division. Regional Office - 11600 Rue Louis-Bisson, Mirabel, J7N 1G9. iv + 52 p.

C. Portt and Associates and Kilgour & Associates Ltd. 2014. Environmental Effects Monitoring: Agnico-Eagle Mines Ltd.- Meadowbank Division Cycle 2 Study Design. Prepared for Agnico-Eagle Mines Ltd., Regional Office - 93, Rue Arseneault, suite 202, Val-d'Or, Québec, J9P 0E9. 55 p. + 4 appendices.

C. Portt and Associates and Kilgour & Associates Ltd. 2015. Environmental Effects Monitoring: Cycle 2 Meadowbank Mine Interpretive Report. Prepared for Agnico-Eagle Mines Ltd., Regional Office - 93, Rue Arseneault, suite 202, Val-d'Or, Québec, J9P 0E9. 73 p. + 6 appendices.

C. Portt and Associates and Kilgour & Associates Ltd. 2017. Environmental Effects Monitoring: Agnico-Eagle Mines Ltd.- Meadowbank Division Cycle 3 Study Design. Prepared for Agnico-Eagle Mines Ltd., Regional Office - 93, Rue Arseneault, suite 202, Val-d'Or, Québec, J9P 0E9. 62 p. + 5 appendices.

C. Portt and Associates and Kilgour & Associates Ltd. 2018. Environmental Effects Monitoring: Cycle 3 Meadowbank Mine Interpretive Report. Prepared for Agnico-Eagle Mines Ltd., Regional Office - 93, Rue Arseneault, suite 202, Val-d'Or, Québec, J9P 0E9. 86 p. + 6 appendices.

C. Portt and Associates and Kilgour & Associates Ltd. 2019. Environmental Effects Monitoring: Agnico-Eagle Mines Ltd.- Whale Tail Pit Cycle 1 Biological Study Design. Prepared for Agnico-Eagle Mines Ltd., Regional Office - 93, Rue Arseneault, suite 202, Val-d'Or, Québec, J9P 0E9. 67 p. + 3 appendices.

C. Portt and Associates and Stantec Consulting Ltd., 2007. Cycle 4 EEM Interpretive Report. Abitibi-Consolidated Company of Canada Fort Frances Division. 81 p. + appendices.

Environment Canada. 2012. Metal mining technical guidance for environmental effects monitoring. http://www.ec.gc.ca/Publications/default.asp?lang=En&xml=D175537B-24E3-46E8-9BB4-C3B0D0DA806D.

Golder Associates Ltd. 2016. Fish health baseline report for the Jay Project. Report prepared for Dominion Diamond Mines. iii + 64 p.

Gray, M.A., Munkittrick, K.R., Palace, V., and Baron, C. 2005b. Assessment of slimy sculpin (Cottus cognatus) collected from East Island, Lac de Gras, NWT. Prepared for Diavik Diamond Mines Inc. 30 p.

Golder. 2020. Predicted 1% water treatment plant effluent dilution location in Mammoth Lake. Prepared for Agnico-Eagle Mines Ltd., Regional Office - 93, Rue Arseneault, suite 202, Val-d'Or, Québec, J9P 0E9. April 2, 2020. 8 p. + 1 appendix.

Munkittrick, K.R., M.E. McMaster, G.J. Van Der Kraak C. Portt, W.N. Gibbons, A. Farwell, and. M Gray. 2000. Development of methods for effects-driven cumulative effects assessment using fish populations: Moose River Project. Published by the Society of Environmental Toxicology and Chemistry (SETAC). 256 p.

Owens, R.W. and G. E. Noguchi. 1998. Intra-lake variation in maturity, fecundity, and spawning of Slimy Sculpins (Cottus cognatus) in southern Lake Ontario. J. Great Lakes Res. 24: 383-391.

Van Vliet, W.H. 1964. An ecological study of Cottus cognatus Richardson in Northern Saskatchewan. M.A. Thesis. University of Saskatchewan. viii +148 pp plus appendices.

AGNICO EAGLE

APPENDIX B

Predicted 1% Water Treatment Plant Effluent Dilution Location in Mammoth Lake

TECHNICAL MEMORANDUM

DATE 2 April 2020 **Project No.** 19136093-443-TM-Rev0

TO Robin Allard
Agnico Eagle Mines Limited

CC Marie-Pier Marcil (Agnico Eagle), and Jen Range (Golder)

FROM Alison Snow, Steve Mitchell, and John Faithful **EMAIL alison_snow@golder.com**

PREDICTED 1% WATER TEATMENT PLANT EFFLUENT DILUTION LOCATION IN MAMMOTH LAKE

1.0 INTRODUCTION

Agnico Eagle Mines Limited - Meadowbank Division (Agnico Eagle) is currently operating the Whale Tail satellite deposit on the Amaruq exploration property, which is approximately 50 km north of the Meadowbank Mine, in the Kivalliq region of Nunavut. The Approved Project supports mining of ore from one open pit (i.e., Whale Tail Pit) and its processing over a three- to four-year mine life. As part of the mine development, treated effluent from the water treatment plants (WTP) will be released through submerged diffusers into Mammoth Lake during operations.

According to Metal and Diamond Mining Effluent Regulations (MDMER, Schedule 5, s.9 (a, b), Government of Canada 2019), fish population and benthic invertebrate community studies may be required in Mammoth Lake. A fish population study may be required if: "(a) the highest concentration of effluent in the exposure area, during a period in which there are deposits, is greater than 1% at any location that is 250 m from a point at which the effluent enters the area from a final discharge point" and a benthic invertebrate community study may be required if "(b) the highest concentration of effluent in the exposure area, during a period in which there are deposits, is greater than 1% at any location that is 100 m from a point at which the effluent enters the area from a final discharge point".

The purposes of this technical memorandum are to:

- Present the predicted 1% WTP effluent dilution locations in Mammoth Lake
- Determine if benthic invertebrate community and/or fish population studies need to be conducted in accordance with Schedule 5, s.9 (a, b) of MDMER

2.0 METHODS

The 1% WTP effluent dilution locations in Mammoth Lake were determined using a three-dimensional hydrodynamic and water quality model of Mammoth Lake (Mammoth Lake model). The Mammoth Lake model accounted for recorded water transfers in 2019 and the simultaneous discharge of effluent from multiple diffusers. In addition to the effluent discharge from the WTP, the Mammoth Lake model also accounted for inflows to Mammoth Lake from direct precipitation, natural watershed runoff, Whale Tail Lake (North Basin) natural inflow, Whale Tail Lake (South Basin) diversion and pumping, quarry 1 discharge, and outflows from Mammoth Lake from evaporation and downstream discharge to Lake A15 (Appendix A).

Effluent discharge from the WTP to Mammoth Lake occurred from June 2019 to November 2019 and was predicted to occur over a 19-month period from June 2020 to December 2021. The 1% WTP effluent dilution locations in Mammoth Lake were determined for each month when discharge was predicted to occur. The 1% WTP effluent dilution locations in Mammoth Lake were determined by:

- Adding a conservative generic parameter to the effluent discharge from the WTP to Mammoth Lake at a constant concentration of 100 mg/L
- Adding conservative generic parameters to inflows from natural watershed runoff, Whale Tail Lake (North Basin), and Whale Tail Lake (South Basin) overflow at a constant concentration of 0 mg/L
- Setting the initial generic parameter concentration in Mammoth Lake to 0 mg/L
- Running the Mammoth Lake model for operations
- Identifying the location where the effluent concentration in Mammoth Lake is 1 mg/L (i.e., the 1% WTP effluent discharge location) on a concentration contour map of Mammoth Lake.

To determine if benthic invertebrate community and/or fish population studies need to be conducted, the distance from the submerged diffusers to the 1% WTP effluent dilution locations was measured on concentration contour maps of the surface (i.e., top 1 m of the lake) and bottom (i.e., bottom 1 m of the lake) of Mammoth Lake.

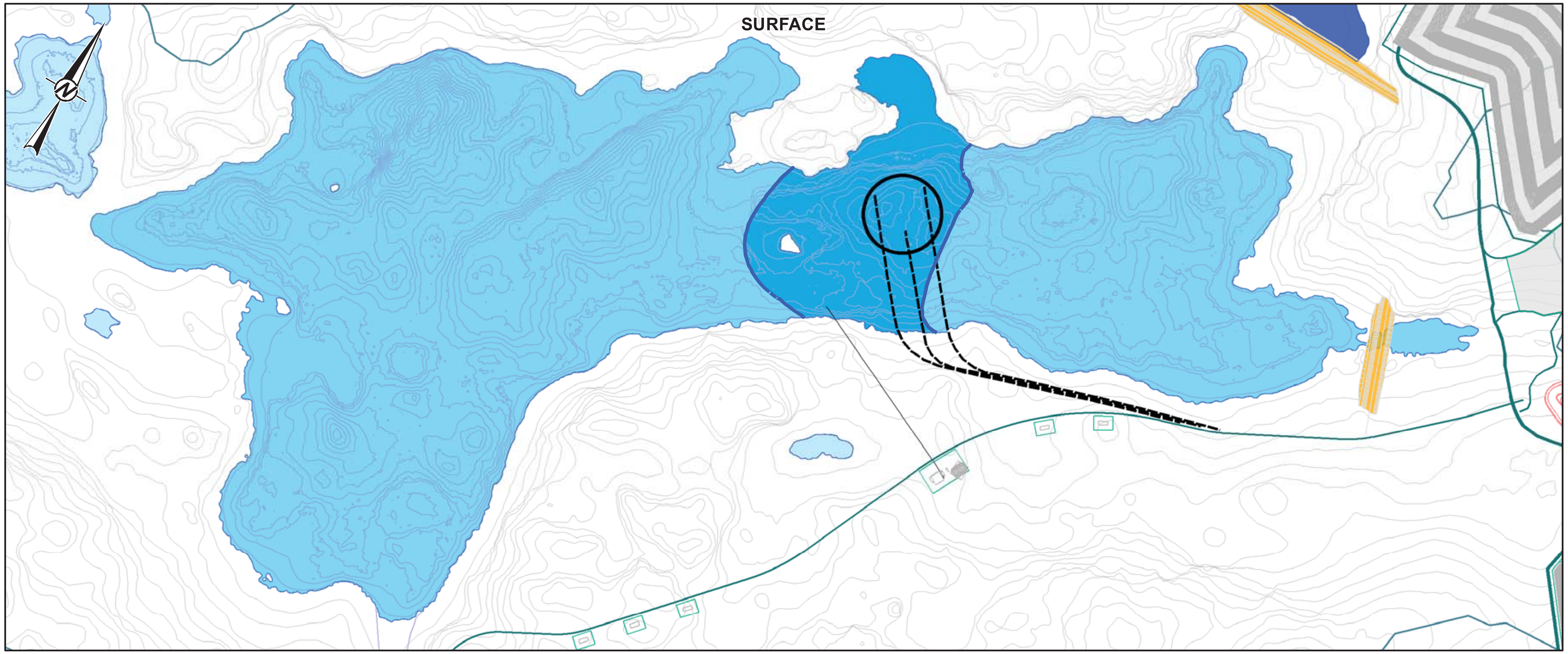
3.0 RESULTS

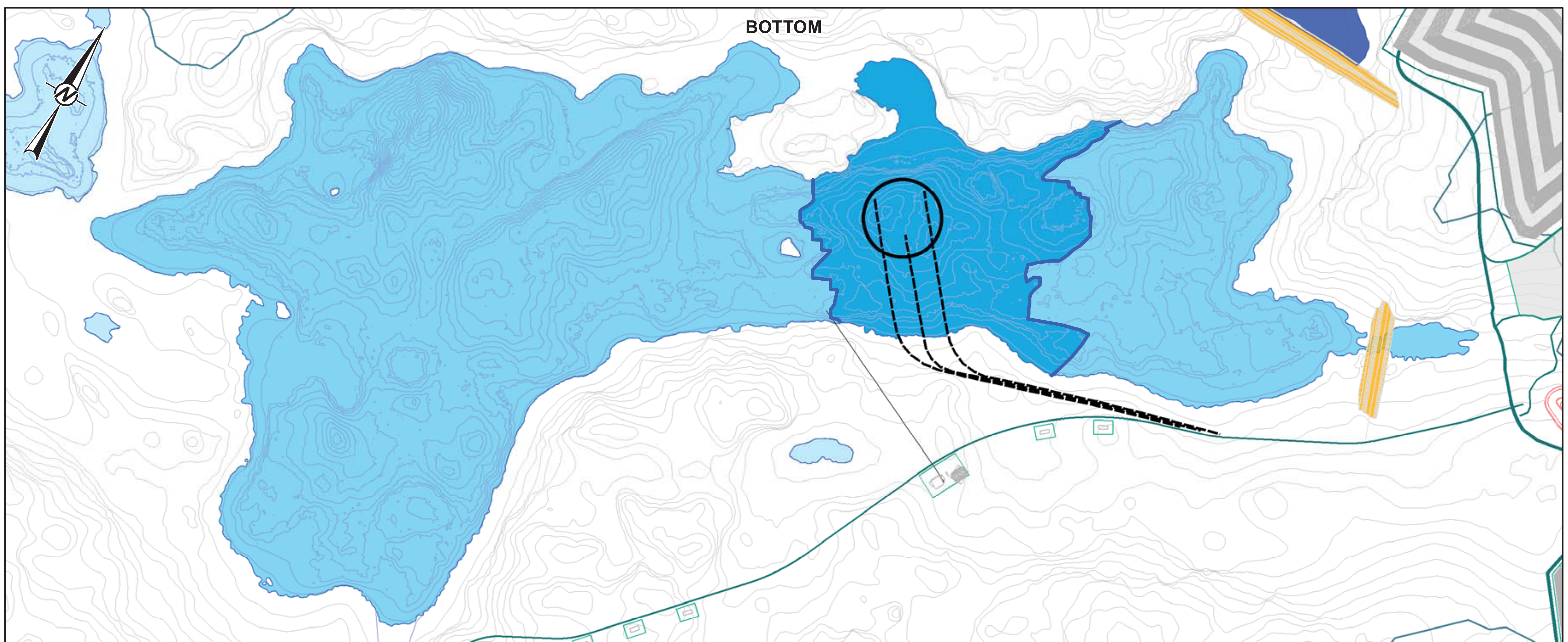
Approximately one and two weeks after the start of effluent discharge from the WTP, the 1% WTP effluent dilution location is greater than 80 m and greater than 170 m from the final point of effluent discharge (Table 1 and Figures 1 and 2). Approximately three and four weeks after the start of effluent discharge from the WTP, the 1% WTP effluent dilution location is greater than 400 m and greater than 1,300 m from the final point of effluent discharge (Table 1 and Figures 3 and 4). After the first 4 weeks of effluent discharge from the WTP (i.e., after July 7, 2019), the predicted 1% WTP effluent dilution location extends beyond the outlet of Mammoth Lake. The 1% WTP effluent dilution location is expected to extend beyond the outlet of Mammoth Lake during the period of effluent discharge from the WTP, as well as for a period of time (i.e., years) after effluent discharge from the WTP has ended. As a result, benthic invertebrate community and fish population studies will need to be conducted in Mammoth Lake in accordance with Schedule 5, s.9 (a, b) of MDMER.

Table 1: 1% Water Treatment Plant Effluent Dilution Locations in Mammoth Lake

Start Date of WTP Effluent Discharge	Time Since WTP Effluent Discharge Started	Approximate Distance from Diffusers (m)	
		Surface (min – max)[(a)]	Bottom (min – max)[(b)]
10 June 2019	One week	80 – 280	180 – 300
	Two weeks	170 – 280	340 – 780
	Three weeks	440 – 560	430 – 720
	Four weeks	1,340 – 1,440	1,300 – 1,400

(a) The surface represents the top 1 m of the lake.
(b) The bottom represents the bottom 1 m of the lake.
m = metres.





LEGEND

- 1% WATER TREATMENT PLANT EFFLUENT DILUTION LINE
- GREATER THAN 1%
- LESS THAN 1%

SURFACE = THE TOP 1 m OF THE LAKE

BOTTOM = THE BOTTOM 1 m OF THE LAKE

0 200 400

1:10,000 METRES

REFERENCE(S)

1. INFRASTRUCTURE OBTAINED FROM AGNICO EAGLE MINES LIMITED FROM AMQ_2025Q4V7.DWG
2. WATERCOURSE AND WATERBODY DATA OBTAINED FROM PHOTOSAT

DATUM: NAD 83 CSRS PROJECTION: UTM ZONE 14

CLIENT

AGNICO EAGLE

AGNICO EAGLE MINES LIMITED:
MEADOWBANK DIVISION

PROJECT

WHALE TAIL PIT - APPROVED PROJECT

TITLE

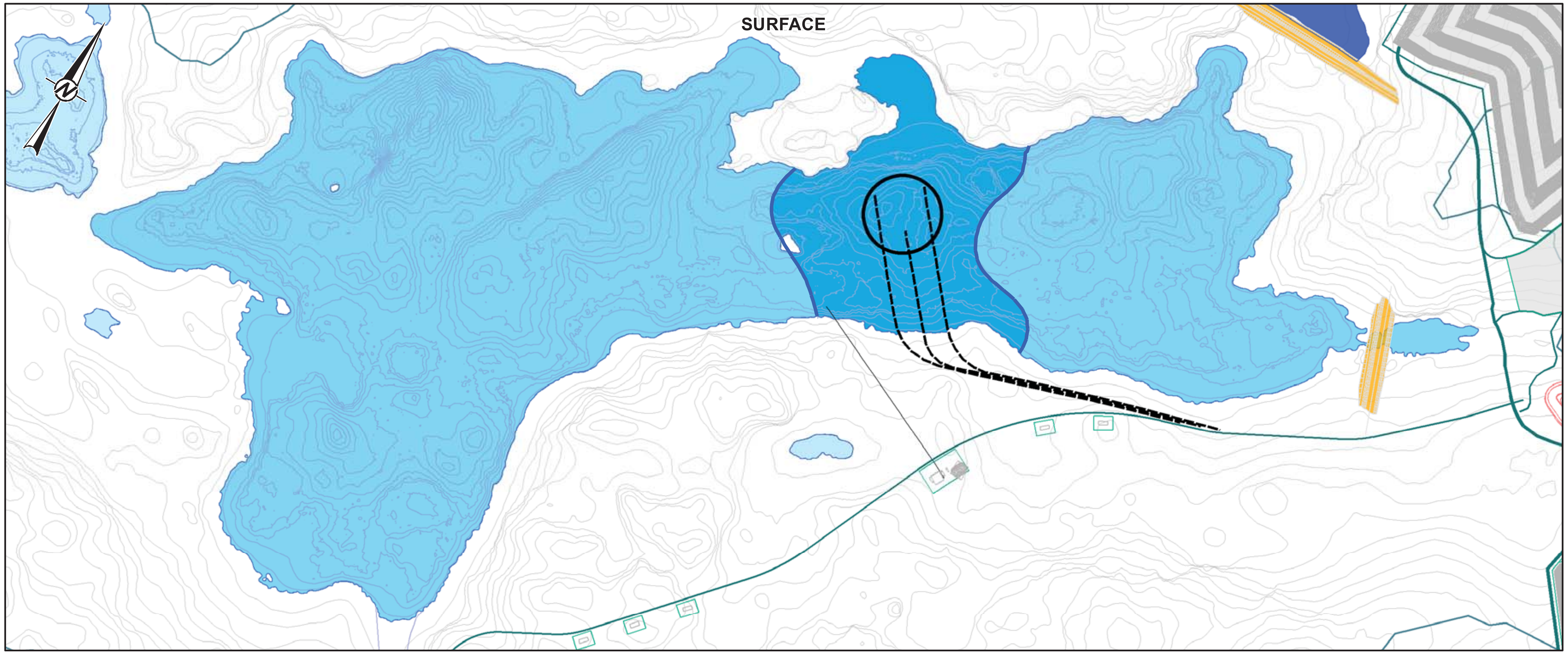
1% WATER TREATMENT PLANT EFFLUENT DILUTION LINE ONE WEEK AFTER DISCHARGE STARTED

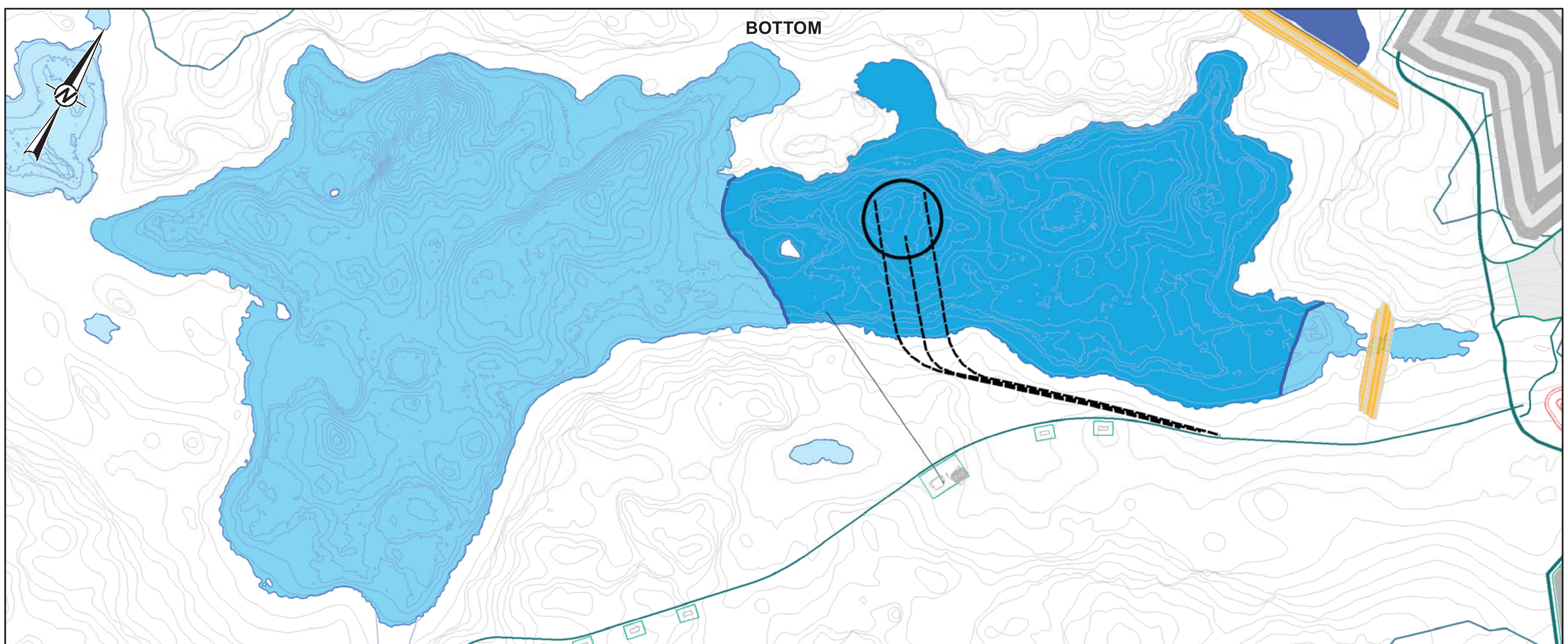
CONSULTANT

GOLDER

YYYY-MM-DD	2020-04-01
DESIGNED	AS
PREPARED	CO/PS
REVIEWED	AS
APPROVED	JF

PROJECT NO.	CONTROL	REV.	FIGURE
19136093	1000/1010	0	1





LEGEND

- 1% WATER TREATMENT PLANT EFFLUENT DILUTION LINE
- GREATER THAN 1%
- LESS THAN 1%

SURFACE = THE TOP 1 m OF THE LAKE

BOTTOM = THE BOTTOM 1 m OF THE LAKE

0 200 400

1:10,000 METRES

REFERENCE(S)

1. INFRASTRUCTURE OBTAINED FROM AGNICO EAGLE MINES LIMITED FROM AMQ_2025Q4V7.DWG
2. WATERCOURSE AND WATERBODY DATA OBTAINED FROM PHOTOSAT

DATUM: NAD 83 CSRS PROJECTION: UTM ZONE 14

CLIENT

AGNICO EAGLE

AGNICO EAGLE MINES LIMITED: MEADOWBANK DIVISION

PROJECT

WHALE TAIL PIT - APPROVED PROJECT

TITLE

1% WATER TREATMENT PLANT EFFLUENT DILUTION LINE TWO WEEKS AFTER DISCHARGE STARTED

CONSULTANT

GOLDER

YYYY-MM-DD	2020-04-01
DESIGNED	AS
PREPARED	CO
REVIEWED	AS
APPROVED	JF

PROJECT NO.	CONTROL	REV.	FIGURE
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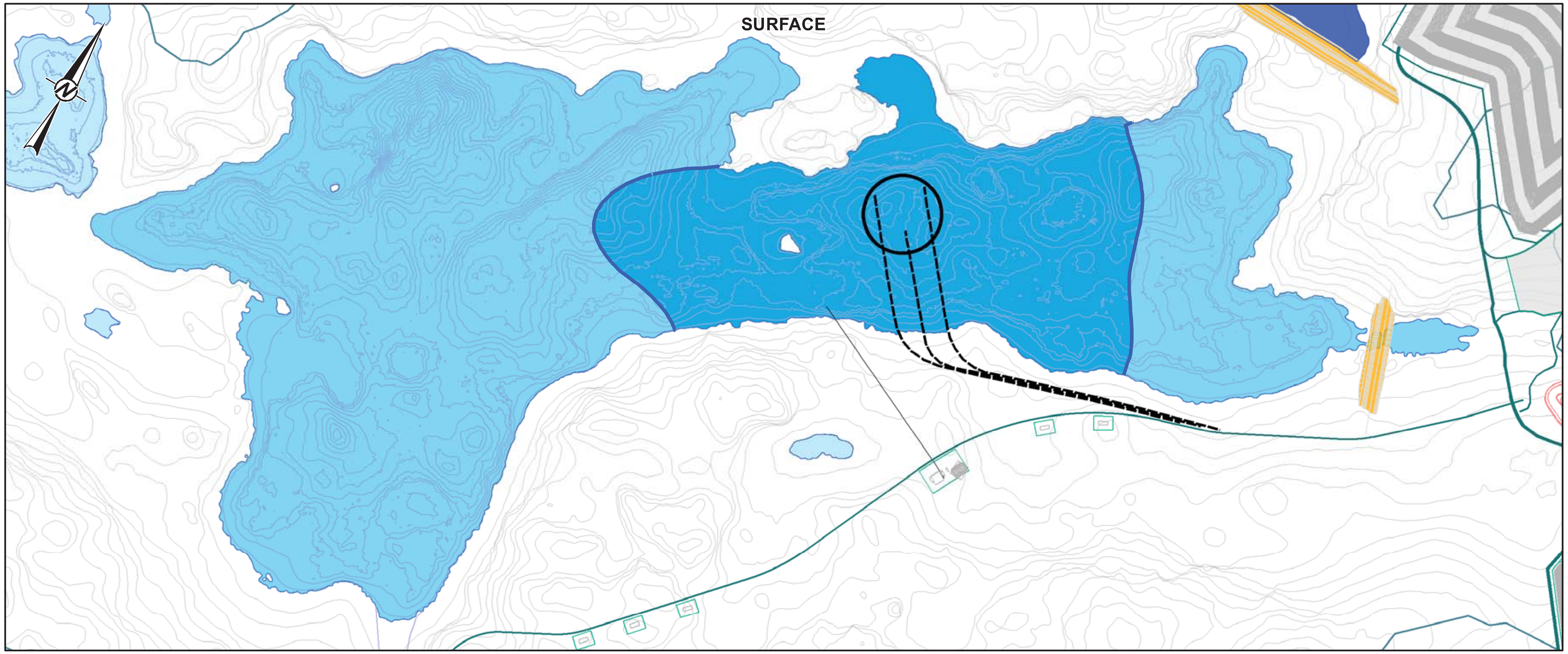
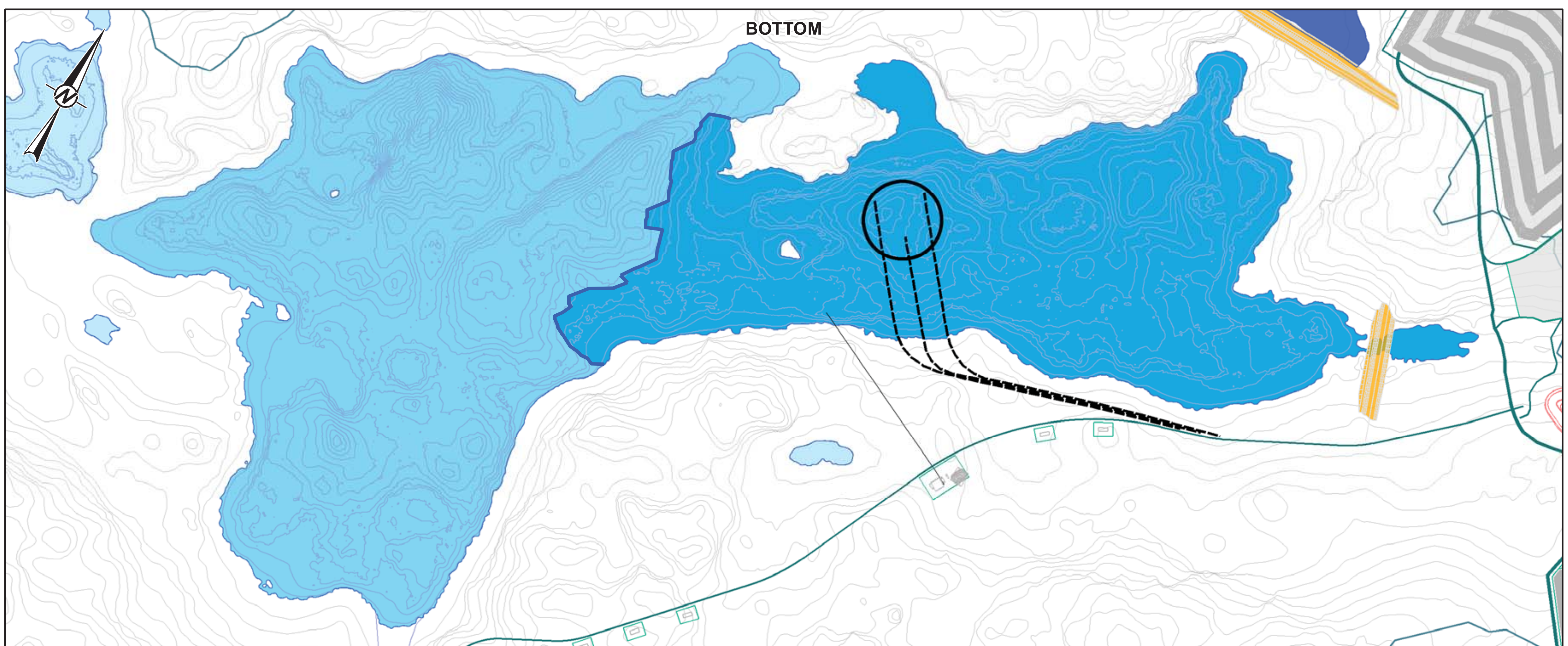




LEGEND

- 1% WATER TREATMENT PLANT EFFLUENT DILUTION LINE
- GREATER THAN 1%
- LESS THAN 1%

SURFACE = THE TOP 1 m OF THE LAKE

BOTTOM = THE BOTTOM 1 m OF THE LAKE

0 200 400

1:10,000 METRES

REFERENCE(S)

1. INFRASTRUCTURE OBTAINED FROM AGNICO EAGLE MINES LIMITED FROM AMQ_2025Q4V7.DWG
2. WATERCOURSE AND WATERBODY DATA OBTAINED FROM PHOTOSAT

DATUM: NAD 83 CSRS PROJECTION: UTM ZONE 14

CLIENT

AGNICO EAGLE

AGNICO EAGLE MINES LIMITED:
MEADOWBANK DIVISION

PROJECT

WHALE TAIL PIT - APPROVED PROJECT

TITLE

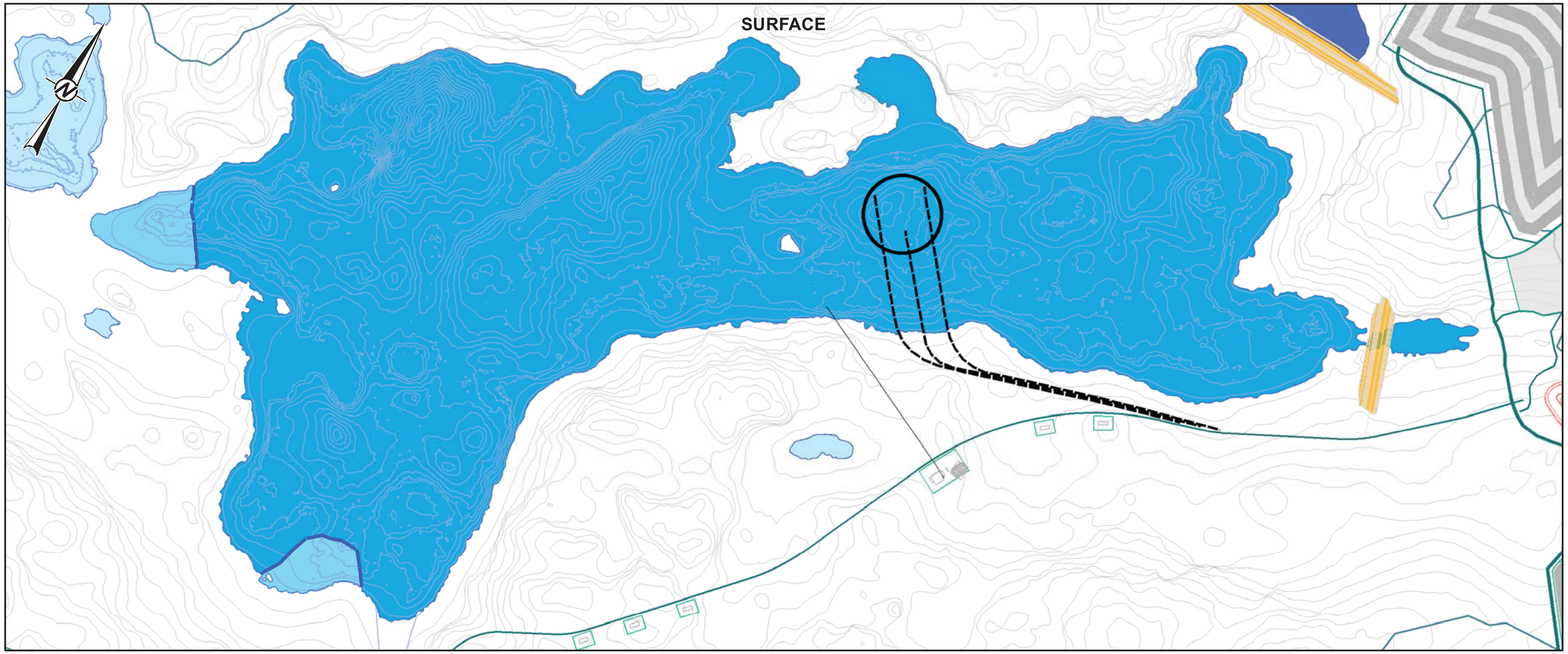
1% WATER TREATMENT PLANT EFFLUENT DILUTION LINE THREE WEEKS AFTER DISCHARGE STARTED

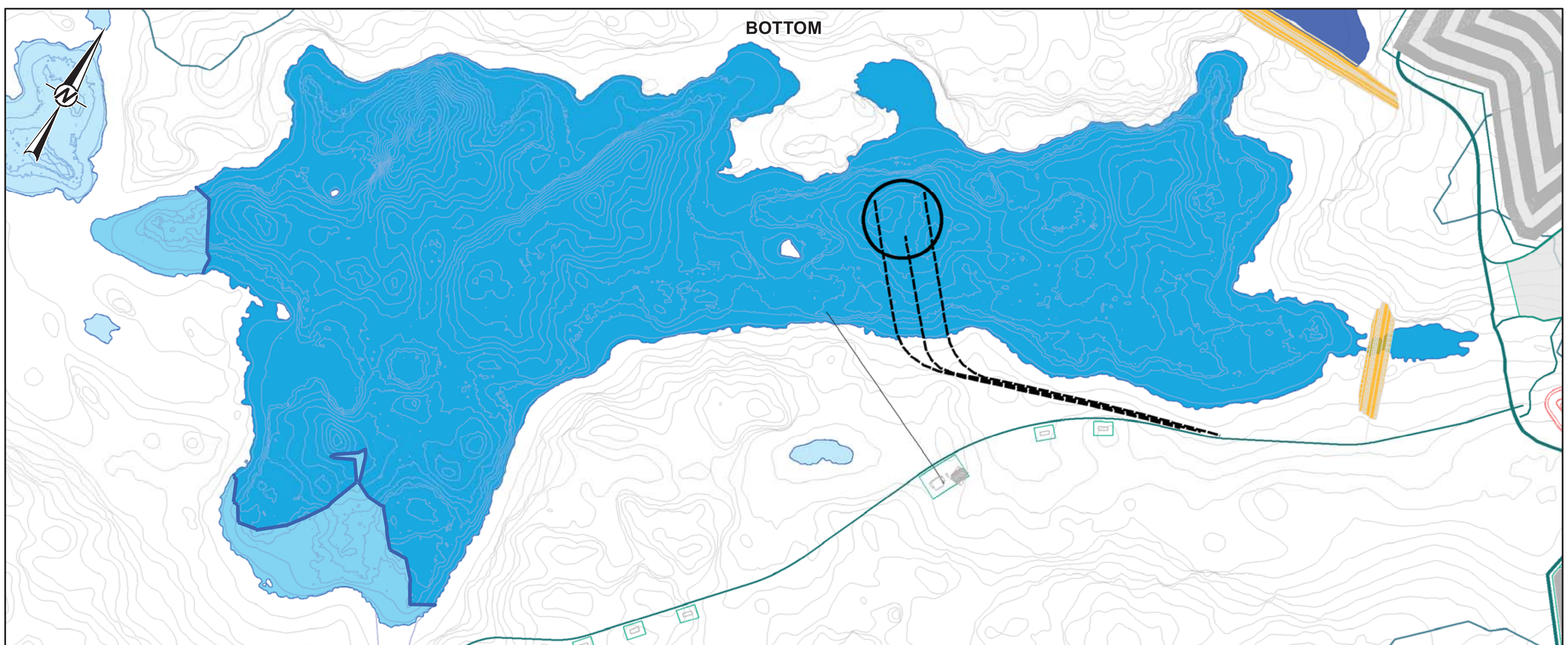
CONSULTANT

GOLDER

YYYY-MM-DD	2020-04-01
DESIGNED	AS
PREPARED	CO/PS
REVIEWED	AS
APPROVED	JF

PROJECT NO.	CONTROL	REV.	FIGURE
19136093	1000/1010	0	3





LEGEND

1% WATER TREATMENT PLANT EFFLUENT DILUTION LINE

GREATER THAN 1%

LESS THAN 1%

SURFACE = THE TOP 1 m OF THE LAKE

BOTTOM = THE BOTTOM 1 m OF THE LAKE

0 200 400

1:10,000 METRES

REFERENCE(S)

1. INFRASTRUCTURE OBTAINED FROM AGNICO EAGLE MINES LIMITED FROM AMQ_2025Q4V7.DWG
2. WATERCOURSE AND WATERBODY DATA OBTAINED FROM PHOTOSAT

DATUM: NAD 83 CSRS PROJECTION: UTM ZONE 14

CLIENT

AGNICO EAGLE

AGNICO EAGLE MINES LIMITED:
MEADOWBANK DIVISION

PROJECT

WHALE TAIL PIT - APPROVED PROJECT

TITLE

1% WATER TREATMENT PLANT EFFLUENT DILUTION LINE
FOUR WEEKS AFTER DISCHARGE STARTED

CONSULTANT

GOLDER

YYYY-MM-DD	2020-04-01
DESIGNED	AS
PREPARED	CO/PS
REVIEWED	AS
APPROVED	JF

PROJECT NO.	CONTROL	REV.	FIGURE
19136093	1000/1010	0	4

IF THIS MEASUREMENT DOES NOT MATCH WHAT IS SHOWN, THE SHEET SIZE HAS BEEN MODIFIED FROM: ANSI B

25mm

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4.0 CLOSURE

We trust that the above meets your requirements. If you have any questions, please contact the undersigned.

Steve Mitchell, BSc
Water Quality Modeller

Alison Snow, MASc
Water Quality Modeller

John Faithful, BSc (Hons)
Principal, Senior Water Quality Specialist

SM/AS/JF/jr

https://golderassociates.sharepoint.com/sites/120433/project files/5 technical work/effluent_dilution_tm/19136039-443-tm_mamlake_1%dilution-rev0.docx

REFERENCES

Government of Canada. 2019. Metal and Diamond Mining Effluent Regulations SOR/2002-222. [accessed 4 December 2019]. https://laws-lois.justice.gc.ca/PDF/SOR-2002-222.pdf

APPENDIX A

Mammoth Lake Water Balance

Table A1: Mammoth Lake Water Balance[(a)]

Year	Month	Inflows							Outflows		
		Natural Watershed Runoff (m^3)	Direct Precipitation (m^3)	SWTL Diversion (m^3)	Natural Inflow from NWTL (m^3)	SWTL Pumping (m^3)	Quarry 1 Discharge (m^3)	WTP Effluent Discharge (m^3)	Evaporation (m^3)	Emulsion (m^3)	Downstream Discharge (m^3)
2018	July	65,851	63,338	0	1,161	0	0	0	142,430	90	8,312
2018	Aug	93,143	70,651	0	145,869	0	0	0	143,868	90	216,188
2018	Sept	193,361	69,777	0	315,734	0	0	0	55,691	87	595,286
2018	Oct	0	0	0	0	0	0	0	0	90	0
2018	Nov	0	0	0	0	0	0	0	0	87	0
2018	Dec	0	0	0	0	0	0	0	0	90	0
2019	Jan	0	0	0	0	0	0	0	0	90	0
2019	Feb	0	0	0	0	0	0	0	0	81	0
2019	March	0	0	0	0	0	0	0	0	90	0
2019	April	0	0	0	0	0	0	0	0	87	0
2019	May	0	0	0	0	0	0	0	0	90	0
2019	June	659,368	54,763	0	0	0	0	339,975	12,530	87	1,040,870
2019	July	65,851	63,338	0	0	0	0	537,997	142,430	90	524,579
2019	August	93,143	70,651	426,939	0	0	46,443	782,130	143,868	90	1,299,683
2019	Sept	193,361	69,777	1,170,098	0	0	238,312	865,282	55,691	87	2,481,052
2019	Oct	0	0	102,725	0	332,239	93,824	621,860	0	90	1,150,559
2019	Nov	0	0	0	0	930,731	0	77,661	0	87	983,320
2019	Dec	0	0	0	0	438,244	0	0	0	90	438,192
2020	Jan	0	0	0	0	0	0	0	0	90	0

Year	Month	Inflows							Outflows		
		Natural Watershed Runoff (m^3)	Direct Precipitation (m^3)	SWTL Diversion (m^3)	Natural Inflow from NWTL (m^3)	SWTL Pumping (m^3)	Quarry 1 Discharge (m^3)	WTP Effluent Discharge (m^3)	Evaporation (m^3)	Emulsion (m^3)	Downstream Discharge (m^3)
2020	Feb	0	0	0	0	0	0	0	0	84	0
2020	March	0	0	2,761	0	0	0	0	0	90	2,460
2020	April	0	0	229,243	0	0	0	0	0	87	229,156
2020	May	0	0	232,829	0	0	0	0	0	90	232,739
2020	June	659,512	54,763	2,211,550	0	0	0	451,925	12,530	87	3,364,931
2020	July	65,851	63,338	418,763	0	0	0	362,796	142,430	90	768,430
2020	August	93,143	70,651	500,966	0	0	0	393,404	143,868	90	914,205
2020	Sept	193,361	69,777	735,646	0	0	0	381,050	55,691	87	1,324,056
2020	Oct	0	0	98,580	0	0	0	154,469	0	90	252,959
2020	Nov	0	0	95,400	0	0	0	150,911	0	87	246,224
2020	Dec	0	0	98,580	0	0	0	154,183	0	90	252,673
2021	Jan	0	0	98,580	0	0	0	139,755	0	90	238,246
2021	Feb	0	0	89,040	0	0	0	127,833	0	81	216,792
2021	March	0	0	98,580	0	0	0	141,484	0	90	239,974
2021	April	0	0	95,400	0	0	0	135,973	0	87	231,286
2021	May	0	0	98,580	0	0	0	141,587	0	90	240,078
2021	June	659,368	54,763	1,969,491	0	0	0	701,203	12,530	87	3,372,302
2021	July	65,851	63,338	418,556	0	0	0	350,986	142,430	90	756,117
2021	August	93,143	70,651	500,966	0	0	0	374,394	143,868	90	895,195

Year	Month	Inflows							Outflows		
		Natural Watershed Runoff (m^3)	Direct Precipitation (m^3)	SWTL Diversion (m^3)	Natural Inflow from NWTL (m^3)	SWTL Pumping (m^3)	Quarry 1 Discharge (m^3)	WTP Effluent Discharge (m^3)	Evaporation (m^3)	Emulsion (m^3)	Downstream Discharge (m^3)
2021	Sept	193,361	69,777	735,646	0	0	0	365,584	55,691	87	1,308,589
2021	Oct	0	0	98,580	0	0	0	145,167	0	90	243,657
2021	Nov	0	0	95,400	0	0	0	139,369	0	87	234,682
2021	Dec	0	0	98,580	0	0	0	146,720	0	90	245,210

(a) The water balance for Mammoth Lake is based on modelling completed on 13 March 2020.

SWTL = South Whale Tail Lake; NWTL = North Whale Tail Lake; WTP = water treatment plant; m^3 = cubic meters.

AGNICO EAGLE

APPENDIX C

Examination of the ability to detect differences among areas by three sampling designs

Examination of the ability to detect differences among areas by three sampling designs

C. Portt and Associates and Kilgour & Associates

Sample sizes for EEM benthic invertebrate designs are constructed around the premise that differences between reference and exposure areas that exceed about two times the among-station standard deviation (i.e., ± 2 σ_A) are important to detect. Anything that results in an increase in within-area variance will not change the reported power of the study but will change the magnitude of the true effect that can be detected. Further, a study that minimizes the within-station variance will generally have greater likelihood of detecting effects equal to 2 σ_A than a study that has a higher within-station variance. In the Guidance Document, Environment Canada recommends that the number of within-station sub-samples be the number that ensures that means of effect variables will be estimated with a precision (D) of 0.2, or to within ± 20% of the true value. A better precision, or smaller D would reduce within-station variance, while a poorer precision and larger D would increase within-station variance. Not meeting Environment Canada's precision guideline will increase the within-station variance and would have some impact on the ability of a sampling program to detect differences of ± 2 σ_A. However, it is not so much precision (relative to the station means), but the magnitude of the within-station variance relative to the among-station variance that affects the likelihood of a sampling program to detect differences of ± 2 σ_A (Kilgour and Rosaasen, 2008).

The statistical power of a nested design with sub-samples within stations, and stations within areas, is not handled by any of the statistical power analysis packages we are familiar with (e.g., SYSTAT, PASS2020, GPower 3.1, piface). Agnico therefore explored the statistical power of 2-grab and 3-grab survey designs through a simulation exercise in Excel. The exercise used a synthetic variable (say density) for which the following conditions applied:

- The reference and exposure areas were assigned the same among-station variance (σ_A^2).
- The reference areas had the same mean, but the exposure area had a mean that was truly two standard deviations (+2 σ_A) from the mean of the reference areas.
- The within-station standard deviation (σ_W) was varied in the calculations from 0.1 to 1.0 x σ_A to explore the influence of the ratio of within- to among-station noise (i.e., σ_W:σ_A).

The following design variations were compared in their ability to reject the null hypothesis that there was 'no difference' between reference and exposure area means:

- Design 1: Conventional EEM design with 2 Areas, 5 stations per area, and 3 grabs per station (i.e., 2-Area, 3-Grab);
- Design 2: Proposed Whale Tail Pit design with 3 Areas (2 reference, 1 exposure), 5 stations per area, and 2 grabs per station (i.e., 3-Area, 2-Grab); and,
- Design 3: Modified Whale Tail Pit design with 3 Areas (2 reference, 1 exposure), 5 stations per area, but with 3 grabs per station (i.e., 3-Area, 3-Grab).

In Excel, station means were determined from normal populations with the defined Area means and standard deviations. Sub-samples were subsequently drawn for each station given the sampled station mean and assigned among-station standard deviations. Data from sub-samples were then 'pooled', and analysis of variance was used to test for a difference between reference and exposure areas. For the 3-Area designs (i.e., Designs 2 and 3), linear orthogonal contrasts were used to test for the difference between reference and exposure areas. For the simpler Design 1, a simple one-way ANOVA (or t test) was carried out. From theses ANOVA's, F ratios with p values < 0.1 were used to 'reject' the null hypothesis. These analyses were 'run' 1000 times per design. The fraction of the 1000 runs that produced a significant difference was used as the estimate of power, or the likelihood of rejecting the null hypothesis of 'no difference'.

The range of within- and among-station variances in this simulation is similar to what was observed by Azimuth in the preparation of the study design for the first EEM study Meadowbank Mine. The data presented in Table 1 shows that the standard deviation of log of density within stations (i.e., σ_W) is about 0.27 while the standard deviation of station means (i.e., σ_A) is about 0.09, or a ratio of about 3:1 ($\sigma_{W:}\sigma_A$). For family richness σ_A is about 0.76, while σ_W is about 0.73, for a ratio of about 1:1 ($\sigma_{W:}\sigma_A$). The variance ratios explored in the analysis (see Figure 1) therefore covered those that have been described for density and richness in for lakes in the Meadowbank study area.

Table 1. Within-station standard deviations (SD) for abundance and richness, from the 1st Cycle EEM program for the Meadowbank Mine.

Variable	Replicate Station	Mean	Within Station SD
Log of Density	1	1.42	0.24
	2	1.49	0.46
	3	1.38	0.10
	4	1.27	0.28
	Among station SD	0.09	
	Average within station SD		0.27
Richness	1	4.20	0.28
	2	3.60	0.58
	3	2.75	0.83
	4	2.50	1.33
	Among station SD	0.73	
	Average within station SD		0.76

Table Note: Copied from C. Portt and Associates and Kilgour & Associates Ltd. (2014), study design for the 2nd EEM program for the Meadowbank Mine. SD = standard deviation.

The results of the simulation are presented in Figure 1. The design proposed in this current Cycle 1 program for the Whale Tail Pit (i.e., Design 2 with 3-Areas, 2-Grabs), has better power than a conventional EEM design (2-Area, 3-Grab) across the entire range of within-station variances explored (Figure 1).

When the among-station noise is less than the among-area noise (i.e., when $\sigma_W:\sigma_A$ is ≤1:1, such as for family richness in Table 1) the power of Designs 2 and 3 are about equal (Figure 1). When the among-station noise is greater than the among-area noise (i.e., when $\sigma_W:\sigma_A$ > 1:1 such as for Density in Table 1) Design 3 the ability to detect differences diminishes for all Designs. When the $\sigma_W:\sigma_A$ is 3:1, Design 3 (with 3 grabs) modestly outperforms Whale Tail Pit's Design 2 (with 2 grabs), with the difference in detection likelihood being about 8%.

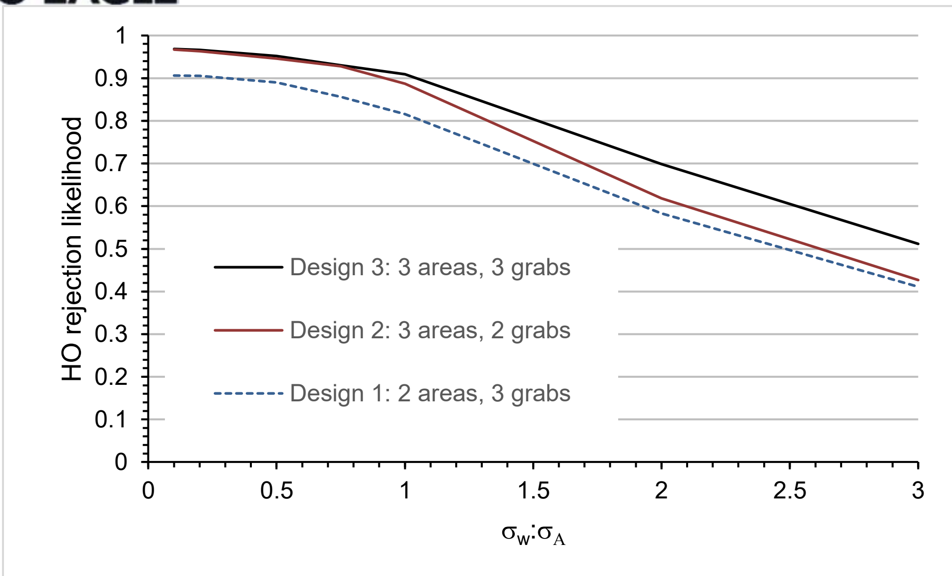

Figure 1. Power curve for CREMP and EEM design options

Agnico, through the CREMP, has been implementing an even more powerful design, collecting 2-grab samples for four baseline years (2015-2018) and in 2019 from each of 5 stations from the Mammoth Lake exposure area. Baseline data have also been collected from the Whale Tail Pit reference lakes. Longer-term reference data sets are available for Innug and Pipedream lakes, which can also serve as reference lakes. So, not only is the proposed 2 Reference Area, 1 Exposure Area, 2-grab surveys proposed by Agnico Eagle for the Whale Tail Pit Cycle 1 EEM approximately equivalent in terms of statistical power to the 3-grab survey recommended by Environment Canada, but the ability to incorporate Before-After-Control-Impact comparisons makes the proposed program with 2-grabs far superior to changing to a 3-grab design.

References

Kilgour, B.W. and A. Rosaasen. 2008. The influence of subsampling on the ability to detect effects in surveys of benthic macroinvertebrates. K. Liber, D.M. Janz and L.E. Burridge (eds). Proceedings of the 35th Annual Aquatic Toxicity Workshop: October 5 to 8, 2008, Saskatoon, Saskatchewan.