



AGNICO EAGLE

AGNICO EAGLE MINES: MEADOWBANK DIVISION

**FISH HABITAT OFFSETTING PLAN:
PHASER LAKE**

NOVEMBER, 2016

TABLE OF CONTENTS

EXECUTIVE SUMMARY IV

SECTION 1 • INTRODUCTION.....5

1.1 Goal.....5

1.2 Phaser Lake Water Quality5

SECTION 2 • HABITAT EVALUATION PROCEDURE.....8

2.1 HEP Model8

2.1.1 Habitat Type Area (HT₁₋₁₀)9

2.1.2 Habitat Suitability Index (HSI_{sp,nu,fo,ow})9

2.1.3 Life Function Weight10

2.1.4 Species Weight.....10

2.1.5 Access Factor11

2.1.6 Habitat Co-factor.....12

SECTION 3 • HABITAT LOSSES13

3.1.1 HU Calculation.....13

SECTION 4 • HABITAT GAINS18

4.1 Re-flooding of Dewatered Phaser Lake, with Access for Arctic Char18

4.1.1 Description.....18

4.1.2 HU Calculation.....21

4.2 Research Funding24

4.3 Unmapped area.....24

4.4 Timeline, Design and Construction of the Offsetting Measures25

4.5 Monitoring.....25

SECTION 5 • DISCUSSION AND CONCLUSIONS26

5.1 Summary26

5.2 Allotment of Gains26

REFERENCES.....27

LIST OF FIGURES

Figure 3-1. Footprint of mining activities in the Vault Lake Area.15

Figure 3-2. Baseline substrate and depth zones for the Vault Lake Area.....16

Figure 3-3. Baseline habitat types for the Vault Lake Area.17

Figure 4-1. Substrate and depth zones for Phaser Lake following re-flooding.22

Figure 4-2. Habitat types for Phaser Lake following re-flooding.....23

LIST OF TABLES

Table 1-1. Water quality in Phaser Lake; September and October, 2013.6

Table 2-1. Physical characteristics of the habitat types proposed for the Phaser Lake HEP. Note that habitat type 10 is applied to all non-backfilled pit areas, independent of depth and substrate characteristics.9

Table 2-2. HSI values for the Meadowbank region fish species (sp=spawning, nu=nursery, fo=foraging, ow=overwintering). *Habitat type 10 is applied to all non-backfilled pit areas.10

Table 2-5. Access factor theoretically applied to each species for habitat loss and gain calculations, based on presence/absence (or anticipated presence/absence, for offsetting projects).11

Table 2-6. Access factor values used for each species in Phaser Lake for habitat loss and gain calculations.12

Table 2-7. Habitat co-factor for various pre- and post-compensation scenarios, according to Minns, 2012.12

Table 3-1. Baseline, or lost habitat type areas (ha) and habitat units (HUs) for Phaser Lake. *HT 10 is applied only to pit areas in offsetting calculations.14

Table 4-1. Mine-related features of Phaser Lake and details of the changes and assumptions used to determine habitat type for each area, following re-flooding.20

Table 4-2. Habitat units (HUs) gained through re-flooding in Phaser Lake. *HT 10 refers to non-backfilled pit habitat.21

Table 4-5. Total area of each mine feature in Phaser Lake and area for which habitat units could not be mapped.25

LIST OF APPENDICES

Appendix A – HU Summary, Subtotals and Totals by Species

Appendix B – Summary of Phaser Lake eDNA Study

EXECUTIVE SUMMARY

This offsetting plan is presented as an addendum to Meadowbank's No-Net-Loss plan (NNLP; AEM, 2012). It characterizes the anticipated serious harm to fish that would be associated with the development of Phaser Pit and BB Phaser Pit, and identifies Agnico Eagle's proposed offsetting measures.

Although the 2012 NNLP quantified serious harm to fish associated with the dewatering of Phaser Lake as a result of development of the Phaser Pit, Agnico Eagle did not apply to DFO for a Fisheries Authorization for Phaser Lake at that time. Since then, development of an additional pit area in Phaser Lake (BB Phaser Pit) has been proposed, and changes to Fisheries Act legislation have occurred. Following discussions with DFO throughout 2015 and 2016, this addendum presents the updated habitat offsetting calculations for Phaser Lake.

Losses and gains in fish habitat were quantified using the Habitat Evaluation Procedure (HEP) approach applied in the 2012 NNLP, with a few adjustments to certain parameters, based on DFO feedback. Development of both Phaser and BB Phaser pits will require dewatering of Phaser Lake, following a fish-out program, resulting in losses to fish habitat. Baseline habitat units for Phaser Lake were calculated to be 9.49 HUs. These are the losses to fish habitat that will occur due to dewatering Phaser Lake if no offsets are implemented. Onsite offsets are planned to include re-flooding of the de-watered Phaser Lake following habitat improvement measures such as substrate changes, partial backfilling of Phaser Pit, and access enhancements for Arctic char. A total of 14.53 HUs are gained through these measures. In addition to onsite offsetting measures, Agnico Eagle also proposes to provide a portion of the offsetting costs for research funding (10% on top of estimated costs of constructed offsetting), with a planned focus on aquatic research such as eDNA and/or remote fish tracking to confirm habitat usage. Assuming this project is equivalent to 10% of the onsite habitat gains, the overall gains: losses ratio for the Phaser Lake project is 1.68:1.

Re-flooding of Phaser Lake will occur in the relatively near term (estimated to re-fill naturally by summer of 2027) and fish introduction will be allowed once monitoring indicates the lake is hydraulically and chemically stable and suitable for aquatic biota.

SECTION 1 • INTRODUCTION

In 2012, Agnico Eagle developed a revised No-Net-Loss Plan (NNLP) for the Meadowbank site to account for habitat alterations, disruptions or destructions (HADD) that were planned to occur in Second Portage Lake, Third Portage Lake, Vault Lake and Phaser Lake. Based on this plan, DFO authorizations (NU-03-0191.3 and NU-03-0191.4) were issued under Paragraph 35(2)(b) of the Fisheries Act for works in Second Portage Lake, Third Portage Lake and Vault Lake.

In July 2014, Agnico Eagle applied for a DFO authorization for Phaser Lake. However, since 2012, changes have been made to the footprint of planned development in Phaser Lake, and to Fisheries Act legislation. This offsetting plan is therefore presented as an addendum to Agnico Eagle's 2012 No-Net-Loss-Plan, and aims to characterize the residual harm to fish and fish habitat that will occur throughout the mine development and operational phase of pits in Phaser Lake, and the offsetting measures that will be implemented.

Phaser Lake is located to the north of the main minesite area at Meadowbank. It is adjacent to Vault Lake, where dewatering has already occurred and Vault pit development is underway. Phaser Lake is relatively small and isolated, with a surface area of 27 ha and a maximum depth of 4-5 m. For further descriptions of the Meadowbank site, ecological setting, fish species, their habitat preferences, and history of the NNLP at Meadowbank, refer to the 2012 NNLP (AEM, 2012).

1.1 GOAL

The main goal of this plan is to characterize the residual serious harm to fish that will occur as a result of mining activities in Phaser Lake at the Meadowbank mine, and to select and quantify offsetting measures. This plan supports Agnico Eagle's application to DFO for an authorization for works in Phaser Lake under Paragraph 35(2)(b) of the Fisheries Act.

Offsetting (at the time, "compensation") options were previously proposed for losses associated with Phaser Pit (and other areas) in the 2012 NNLP after researching techniques and projects implemented at other northern mines, holding workshops and site visits with the local Hunter's and Trapper's Organization, Kivalliq Inuit Association and the DFO Habitat and Science & Research Departments, and reviewing the literature for information on effectiveness of compensation techniques in the north.

1.2 PHASER LAKE WATER QUALITY

Water quality in Phaser Lake was analyzed on three dates in September and October, 2013. Parameters included total and dissolved metals, cyanide, hardness, alkalinity, ammonia, sulfate, nitrate and nitrite. Results of these analyses are provided in Table 1-1. As with other lakes in the area, Phaser Lake is considered to be ultraoligotrophic, and the majority of parameters were below limits of detection.

Table 1-1. Water quality in Phaser Lake; September and October, 2013.

Parameter	Units	10/09/2013	23/09/2013	02/10/2013
Alkalinity	mg CaCO ₃ /L	49	49	45
Ammonia nitrogen (NH ₃ -NH ₄)	mg N/L	0.08	0.32	<0.01
TDS	mg/L	36	36	36
CN total	mg/L	0.007	<0.005	<0.005
CN Free	mg/L	<1	<1	<1
pH (field)		6.83	7.12	7.57
Conductivity (field)		37	56.2	60.1
Turbidity (field)	NTU	0.7	0.68	0.56
Chloride	mg/L	1.2	0.8	1.1
Fluoride	mg/L	0.15	0.03	0.12
Hardness	mg CaCO ₃ /L	26	19	23
Nitrate	mg/L	0.03	0.07	0.15
Nitrite	mg/L	<0.01	<0.01	<0.01
Sulphate	mg/L	3.9	8.3	4.2
Dissolved Aluminium (Al)	mg/L	<0.006	<0.006	<0.006
Dissolved Arsenic (As)	mg/L	<0.0005	<0.0005	0.0006
Dissolved Barium (Ba)	mg/L	0.002	0.0019	0.0026
Dissolved Cadmium (Cd)	mg/L	<0.00002	<0.00002	<0.00002
Dissolved Copper (Cu)	mg/L	0.0006	<0.0005	<0.0005
Dissolved Iron (Fe)	mg/L	<0.01	<0.01	<0.01
Dissolved Lead (Pb)	mg/L	<0.0003	<0.0003	<0.0003
Dissolved Manganese (Mn)	mg/L	<0.0005	<0.0005	<0.0005
Dissolved Mercury (Hg)	mg/L	<0.0001	<0.0001	<0.0001
Dissolved Molybdenum (Mo)	mg/L	<0.0005	<0.0005	<0.0005
Dissolved Nickel (Ni)	mg/L	<0.0005	<0.0005	<0.0005
Dissolved Selenium (Se)	mg/L	<0.001	<0.001	<0.001
Dissolved Silver (Ag)	mg/L	<0.001	<0.001	<0.0001
Dissolved Thallium (Tl)	mg/L	<0.005	<0.005	
Dissolved Zinc (Zn)	mg/L	<0.001	<0.001	
Aluminium (Al)	mg/L	0.023	<0.006	<0.006
Antimony (Sb)	mg/L	<0.0001	<0.0001	<0.0001
Arsenic (As)	mg/L	<0.0005	<0.0005	0.0022
Boron (B)	mg/L	<0.01	<0.01	<0.01
Barium (Ba)	mg/L	0.0024	0.0019	0.0029
Beryllium (Be)	mg/L	<0.0005	<0.0005	<0.0005
Cadmium (Cd)	mg/L	<0.00002	<0.00002	<0.00002
Copper (Cu)	mg/L	<0.0005	<0.0005	<0.0005
Chromium (Cr)	mg/L	<0.0006	<0.0006	<0.0006

AGNICO EAGLE: MEADOWBANK DIVISION

Cobalt (Co)	mg/L	<0.0005	<0.0005	<0.0005
Iron (Fe)	mg/L	<0.01	<0.01	0.04
Lithium (Li)	mg/L	<0.005	<0.005	<0.005
Manganese (Mn)	mg/L	<0.0005	<0.0005	0.0006
Mercury (Hg)	mg/L	<0.00001	<0.00001	<0.00001
Molybdenum (Mo)	mg/L	<0.0005	<0.0005	<0.0005
Nickel (Ni)	mg/L	<0.0005	<0.0005	0.0005
Lead (Pb)	mg/L	<0.0003	<0.0003	<0.0003
Selenium (Se)	mg/L	<0.001	<0.001	<0.001
Tin (Sn)	mg/L	<0.001	<0.001	<0.001
Strontium (Sr)	mg/L	0.026	0.027	0.032
Titanium (Ti)	mg/L	<0.01	<0.01	<0.01
Thallium (Tl)	mg/L	<0.005	<0.005	<0.005
Uranium (U)	mg/L	<0.001	<0.001	<0.001
Vanadium (V)	mg/L	<0.0005	<0.0005	<0.0005
Zinc (Zn)	mg/L	<0.001	<0.001	0.001

SECTION 2 • HABITAT EVALUATION PROCEDURE

The habitat evaluation procedure (HEP) that was used to quantify habitat losses and offsets for Phaser Lake in this report is nearly identical to the procedure used for the 2012>NNL assessment, with adjustments to a number of parameters. Losses and gains in fish habitat were quantified using the Habitat Evaluation Procedure (HEP) approach applied in the 2012>NNL, with a few adjustments to certain model parameters based on DFO feedback during the Nunavut Impact Review Board (NIRB) review process, which began in 2014, and subsequent discussions with DFO during the authorization stage of the project which continued to the end of 2016. A summary of the HEP is provided below, and further rationale is available in the 2012 document.

The HEP involves the multiplication of each affected area (in hectares) by a habitat suitability index (HSI) and series of weights in order to derive a value in habitat units (HUs) that describes both the quality and quantity of habitat. In the first stage of the habitat evaluation, pre-construction (natural, or baseline) habitat units are calculated for all areas where residual harm to fish habitat will occur. The offsets for this loss to fish habitat are then described, and quantified using the same procedure.

The net HUs will depend on the habitat types (10 groups, by substrate and depth) that are lost and gained, and the suitability of each habitat type for each fish species. Suitability of each habitat type is ranked between 0-1 for each life stage of each fish species (spawning, nursery, foraging, overwintering).

For each minesite feature (e.g. pits, dikes, roads) where losses or gains are expected to occur, HUs are calculated by multiplying the area of each habitat type in that feature by the HSI allotted to each life function of each fish species, multiplied by the species weight and life function weight, and summed. This subtotal is then multiplied by an access factor, which represents the accessibility of the area to each species (or their estimated presence/absence), and a habitat co-factor which describes changes in hydrological, thermal or chemical water quality. The HEP model is described in further detail below, and an example calculation is provided in Appendix A of the 2012>NNL.

2.1 HEP MODEL

The HEP model used here can be described, for each fish species (spp 1-n) as:

$$HU_{spp\ 1-n} = \sum_{HT\ 1-10} \left(\sum_{sp,nu,fo,ow} (HT_{1-10} \times HSI_{sp,nu,fo,ow} \times \text{life function weight} \times \text{species weight}) \right) \times \text{access factor} \times \text{habitat co-factor}$$

Where HT_{1-10} = area (ha) of habitat types 1 through 10

$HSI_{sp,nu,fo,ow}$ = habitat suitability index for each life function:

sp = spawning use

nu = nursery use

fo = foraging use

ow = overwintering use

2.1.1 Habitat Type Area (HT₁₋₁₀)

The foundation of the HEP is the delineation of “habitat types” – the method by which habitat areas are grouped, and thereby mapped. The Meadowbank HEP model for Phaser Lake uses 10 habitat types, which are based on various combinations of substrate and depth. Habitat types 1-9 are applied to natural habitat for various combinations of substrate type and depth zone. Habitat type 10 is included in the HEP in recognition of potentially reduced habitat quality in deep pit areas. It is proposed that in cases where pits are planned to be backfilled to depths occurring naturally in surrounding lakes, habitat types 1-9 may be applied for the pit area. Substrate and depth zones associated with each habitat type are shown in Table 2-1.

Note that habitat types for pit areas have been changed from the 2012 NNLP, which identified type 10 for use in pit areas for which stratification was expected to occur, and type 11 for pit areas with complete mixing and suitable water quality for aquatic biota.

Table 2-1. Physical characteristics of the habitat types proposed for the Phaser Lake HEP. Note that habitat type 10 is applied to all non-backfilled pit areas, independent of depth and substrate characteristics.

Habitat Type	Depth Zone	Substrate
1	0-2 m	Fine
2	0-2 m	Mixed
3	0-2 m	Coarse
4	2-4 m	Fine
5	2-4 m	Mixed
6	2-4 m	Coarse
7	>4 m	Fine
8	>4 m	Mixed
9	>4 m	Coarse
10*	mixolimnion	Coarse

*Habitat type 10 is applied to all non-backfilled pit areas independent of depth, and carries a habitat value of 0. Substrate in pits is assumed to be coarse. Although the current water quality model indicates full mixing of the Phaser Pits, a chemocline may develop and is therefore conservatively assumed. While the mixolimnion may provide suitable pelagic fish habitat, the value of this habitat is conservatively assumed to be 0 in this habitat model (see Section 2.1.2). However, habitat monitoring after re-flooding will confirm the value of habitat type 10.

In order to calculate the extents of each habitat type, depth zones and substrate were mapped for the entire Meadowbank site, for baseline and post-closure scenarios. Maps for Phaser Lake were updated during this assessment to include BB Phaser Pit. The area (in ha) for each habitat type was then determined by overlaying depth and substrate layers. All habitat type area calculations and mapping were completed by Dougan and Associates, and methods are described in further detail in the 2012 NNLP.

2.1.2 Habitat Suitability Index (HSI_{sp,nu,fo,ow})

The habitat suitability term represents the relative quality of each habitat type for each life function of each fish species present in the region. In the case of this HEP, the life functions spawning, nursery, foraging and overwintering were considered. Habitat suitability for each life function is indicated through a ranking of 0, 0.25, 0.5, 0.75 or 1. HSIs for all fish species

and habitat types used in this HEP are shown in Table 2-2, and their derivation is further described in AEM (2012).

Note that the habitat suitability for habitat type 10 (non-backfilled pit areas) has been adjusted to 0 following recent discussions with DFO. This is in recognition of the unknown value of pit areas within lakes as fish habitat.

Table 2-2. HSI values for the Meadowbank region fish species (sp=spawning, nu=nursery, fo=foraging, ow=overwintering). *Habitat type 10 is applied to all non-backfilled pit areas.

Habitat Type	Depth	Substrate	Arctic Char				Lake Trout				Round Whitefish			
			SP	NU	FO	OW	SP	NU	FO	OW	SP	NU	FO	OW
1	<2 m	Fines	0	0.25	0.25	0	0	0.25	0.25	0	0	0.25	0.75	0
2	<2 m	Mixed	0	0.25	0.25	0	0	0.5	0.5	0	0	0.75	0.5	0
3	<2 m	Coarse	0	0.5	0.5	0	0	1	0.75	0	0	0.75	0.5	0
4	2-4 m	Fines	0	0.5	0.5	0.75	0	0.5	0.5	0.75	0	0.25	1	0.75
5	2-4 m	Mixed	0.5	0.75	0.75	0.75	0.5	0.75	0.75	0.75	0.5	0.75	0.75	0.75
6	2-4 m	Coarse	1	1	1	0.75	1	1	1	0.75	1	1	0.75	0.75
7	>4 m	Fines	0	0.25	0.5	1	0	0.25	0.5	1	0	0.25	1	1
8	>4 m	Mixed	0.5	0.5	0.75	1	0.5	0.5	0.75	1	0.25	0.25	0.5	1
9	>4 m	Coarse	1	0.5	1	1	1	0.5	1	1	0.75	0.5	0.5	1
10*	mixolimnion	Coarse	0	0	0	0	0	0	0	0	0	0	0	0

Habitat Type	Depth	Substrate	Burbot				Slimy Sculpin				Ninespine Stickleback			
			SP	NU	FO	OW	SP	NU	FO	OW	SP	NU	FO	OW
1	<2 m	Fines	0	0.25	0.25	0	0	0	0.25	0	1	1	1	0
2	<2 m	Mixed	0	0.75	0.5	0	0.25	0.25	0.5	0	0.5	0.5	0.75	0
3	<2 m	Coarse	0	1	0.5	0	1	1	1	0	0	0.25	0.75	0
4	2-4 m	Fines	0	0.25	0.25	0.75	0	0	0.25	0.75	0	0	0.5	0.75
5	2-4 m	Mixed	1	0.5	0.75	0.75	0.25	0.25	0.5	0.75	0	0	0.25	0.75
6	2-4 m	Coarse	0.75	0.5	1	0.75	0.75	0.75	1	0.75	0	0	0.25	0.75
7	>4 m	Fines	0	0	0.25	1	0	0	0	1	0	0	0	1
8	>4 m	Mixed	1	0	0.75	1	0	0	0.25	1	0	0	0	1
9	>4 m	Coarse	0.75	0.25	1	1	0.5	0.5	0.5	1	0	0	0	1
10	mixolimnion	Coarse	0	0	0	0	0	0	0	0	0	0	0	0

2.1.3 Life Function Weight

This HEP values all life functions equally, with a weight of 0.25 each assigned for spawning, nursery, foraging and overwintering.

2.1.4 Species Weight

In the 2012 NNLP, Agnico Eagle used a fishery value and an estimated biomass value to derive species weights for eight species present regionally. However, as recommended in DFO's "Review of Habitat Evaluation Procedure (HEP) Input Parameters and Model Results for the Meadowbank Gold Mine Project" (Canadian Science Advisory Secretariat, Science Response 2016/038) and as discussed with DFO by conference call on September 30, 2016, the HEP has been amended to include equal species weights for this assessment.

Further, the list of species has been reduced to those six identified or assumed to be present in the project lake and predicted to have improved habitat as a result of proposed offsetting measures (lake trout, Arctic char, round whitefish, burbot, ninespine stickleback, and slimy sculpin).

2.1.5 Access Factor

In a workshop conducted in February, 2012 (The Basic Concepts of No Net Loss Accounting - February, 2012) Dr. Charles K. Minns suggested the use of an access factor when fish assemblages are expected to change in the offsetting scenario. According to this concept, the access factor is 1 for any species present in the habitat area, and 0 for any species not present. Each species receives an access factor in both the loss and gain calculations. Therefore, the opening of access to a habitat area for a specie (that did not have access pre-construction), results in an increase of habitat units. Similarly, the loss of access results in a loss of habitat units. These gains or losses may be complete (affect all species, e.g. conversion to a tailings storage facility), or partial (only some species are affected). Note that presence or absence of a species in loss calculations is based on surveys in the affected habitat area, whereas presence or absence in the offsetting scenario is anticipated (to be confirmed after access is altered as part of compensation monitoring – see Section 4.6).

Table 2-3. Access factor theoretically applied to each species for habitat loss and gain calculations, based on presence/absence (or anticipated presence/absence, for offsetting projects).

Scenario	Access Factor	
	Losses	Gains
Species Present	1	1
Species Not Present	0	0

For Phaser Lake, access factors applied are shown in Table 2-6, based on noted presence/absence of each species in the 2016 fishout. This lake was found to contain populations of lake trout, burbot and round whitefish. As described previously, few small-bodied species (slimy sculpin and ninespine stickleback) have been caught in fish surveys to date, however they were conservatively assumed to be present (access factor of 1) as they have commonly been found in stomach contents in area lakes. The rationale for use of an access factor of 1 for gains for Arctic char is further described in Section 4. In the 2012 NNLP, burbot, ninespine stickleback, and slimy sculpin were excluded from habitat calculations in Phaser Lake (access factor of 0 was applied for loss and gain calculations). They were added into calculations here.

Table 2-4. Access factor values used for each species in Phaser Lake for habitat loss and gain calculations.

Species	Access Factor	
	Losses	Gains
Arctic char	0	1
Lake trout	1	1
Round whitefish	1	1
Burbot	1	1
Slimy sculpin	1	1
Ninespine stickleback	1	1

2.1.6 Habitat Co-factor

The habitat co-factor represents any changes to non-mapped habitat quality (thermal, hydrological, biological or chemical regimes) that will occur in the fish habitat in question as a result of impacts or offsetting. The use of this factor is suggested by Dr. Ken Minns, and his suggested values as presented in a workshop for DFO in February, 2012 (see Section 2.1.5), are shown in Table 2-7.

Table 2-5. Habitat co-factor for various pre- and post-compensation scenarios, according to Minns, 2012.

Change in regime	Description	Baseline conditions factor	Post-closure factor
Degradation (expected)	Thermal, hydrologic, chemical and/or biological regime shifts away from preferred state for fish habitat	1	> 0 and < 1
No change	-	1	1
Enhancement (anticipated or proposed)	Thermal, hydrologic, chemical and/or biological regime expected to shift towards preferred state for fish habitat	> 0 and < 1	1

The habitat co-factor is an appropriate weighting to apply when degradation is expected to occur, or remediation of non-pristine lakes is proposed as offsetting. When there is no change in habitat quality pre- and post-mining, the weighting is 1 for both loss and gain calculations (as applied in this plan). This factor is therefore not an integral part of any offsetting calculation that does not affect water quality. Since fish will not be allowed to populate Phaser Lake until monitoring indicates water quality is suitable for aquatic biota and meets conditions of the Type A Water License, no habitat co-factor is applied in this assessment.

SECTION 3 • HABITAT LOSSES

Following the authorization by DFO, Phaser Lake was dewatered in the summer 2016 to allow development of Phaser and BB Phaser Pits. This section presents the calculation of habitat units for the baseline scenario in Phaser Lake, which represents the losses to fish habitat that will occur if no offsets are implemented. Impacts to habitat in Phaser Lake were quantified in the 2012 NNLP as a component of the Vault Lake Area. Since complete dewatering of Phaser Lake was planned at that time, there are no changes to the total impacted area (ha lost). However, a number of changes to the habitat model, as described above, have resulted in an adjustment to the baseline HUs in Phaser Lake.

It should also be noted that as discussed in the 2012 NNLP, and similar to calculations for Vault Lake, resolution differences between substrate maps and base maps for Phaser Lake were found to produce an un-mapped zone of 2.23 ha over several pockets of the lake, for which HUs could not be calculated (losses or gains). Impacts of this unmapped area on habitat calculations are further described in Section 4.

The footprint of planned mining activities for Phaser Lake is shown in Figure 3-1, including the location of pits, roads and dikes.

3.1.1 HU Calculation

Substrate zones (fines, mixed, coarse) for Phaser Lake under baseline conditions are shown in Figure 3-2a. The majority of substrate is fine grained, with coarse and mixed-grain substrate typically occurring around the shoreline.

The depth zones in Phaser Lake considered for the baseline scenario are shown in Figure 3-2b. Baseline depths are shallow, compared to the Main Minesite Area lakes (Second Portage and Third Portage Lakes). Phaser Lake reaches a maximum depth of 4-5 m.

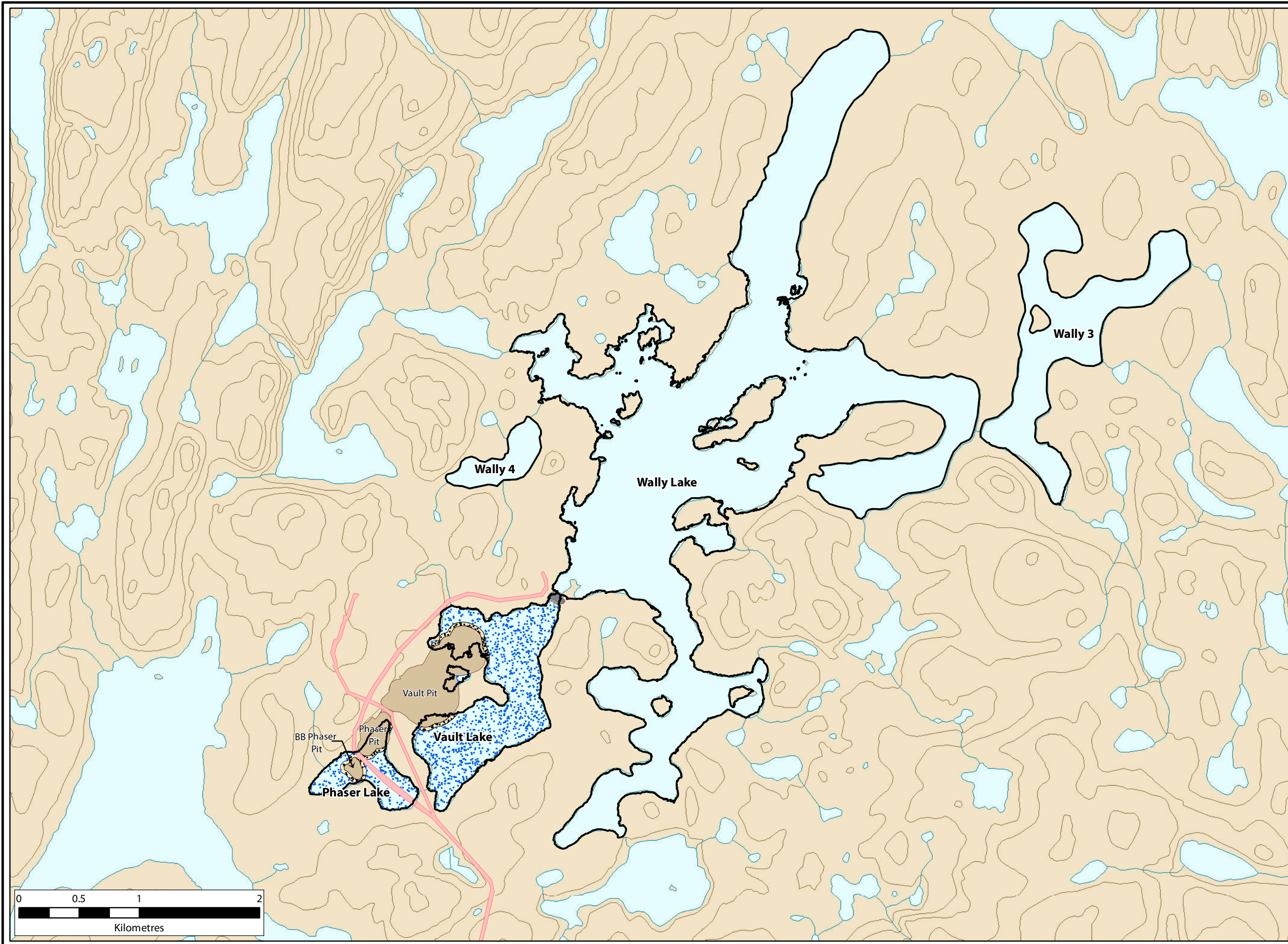
The extents of habitat types 1-9 were calculated by overlaying substrate and depth maps. Habitat types for Phaser Lake are shown in Figure 3-3. This area consists of a relatively mixed proportion of habitat types.

A summary of the total habitat type areas and habitat units, calculated as described in Section 2, are shown in Table 3-1. The HU subtotal and total by species and minesite feature are provided in Appendix A.

Table 3-1. Baseline, or lost habitat type areas (ha) and habitat units (HUs) for Phaser Lake. *HT 10 is applied only to pit areas in offsetting calculations.

Habitat Type	Area (ha)	HUs
1	0.86	0.19
2	5.44	1.42
3	16.19	5.73
4	1.03	0.31
5	1.72	0.81
6	1.21	0.73
7	0.52	0.16
8	0.20	0.08
9	0.11	0.06
10*	-	-
Total	27.29	9.49

Impacted aquatic habitat for Phaser Lake totals 27.29 hectares, or 9.49 HUs. This includes 2.23 ha of Phaser Lake that could not be mapped (see Section 4.3).



- Legend**
- Study Lakes
 - Dike
 - Dike Base
 - Roads
 - Pit
 - Pit Cap
 - Lake Basin

**Features
Vault Lake Area**



77 Wyndham Street South • Guelph ON N1E 5R3
 T 519.822.1609 • F 519.822.5389 • www.dougan.ca

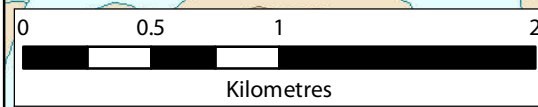
PROJECT: DA11-062-06

CLIENT: Agnico-Eagle Mines Ltd., Meadowbank Div.

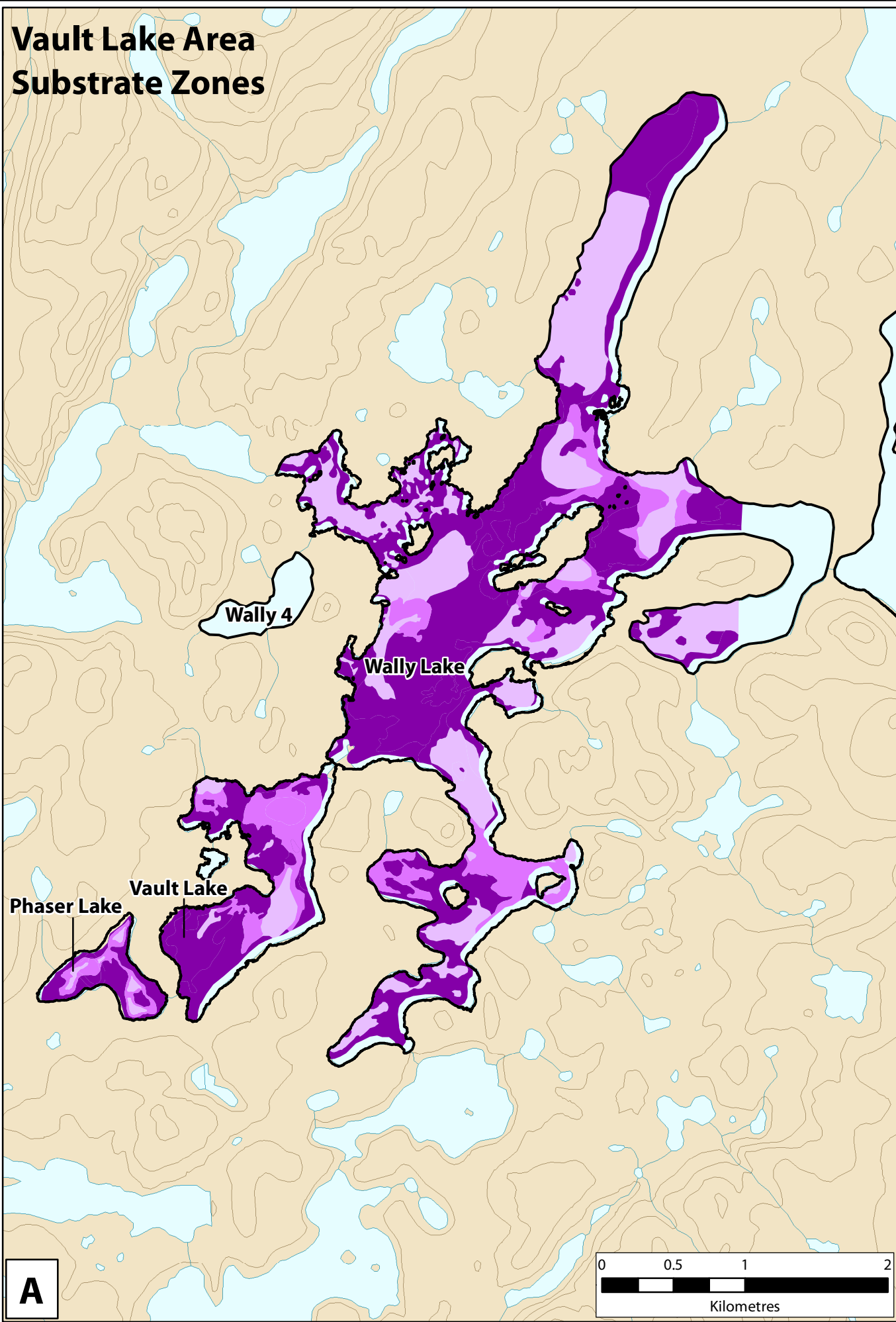
	DATE: JUNE 2015
	SCALE: 1:29,531
	DRAWN BY: LC
	CHECKED BY:

FIGURE: **3-1**

The information displayed on this map has been compiled from various sources. While every effort has been made to accurately depict the information, this map should not be relied on as being a precise indicator of locations, features, or roads, nor as a guide to navigation. MNR data provided by Queen's Printer of Ontario. Use of the data in any derivative product does not constitute an endorsement by the MNR or the Ontario Government of such products.

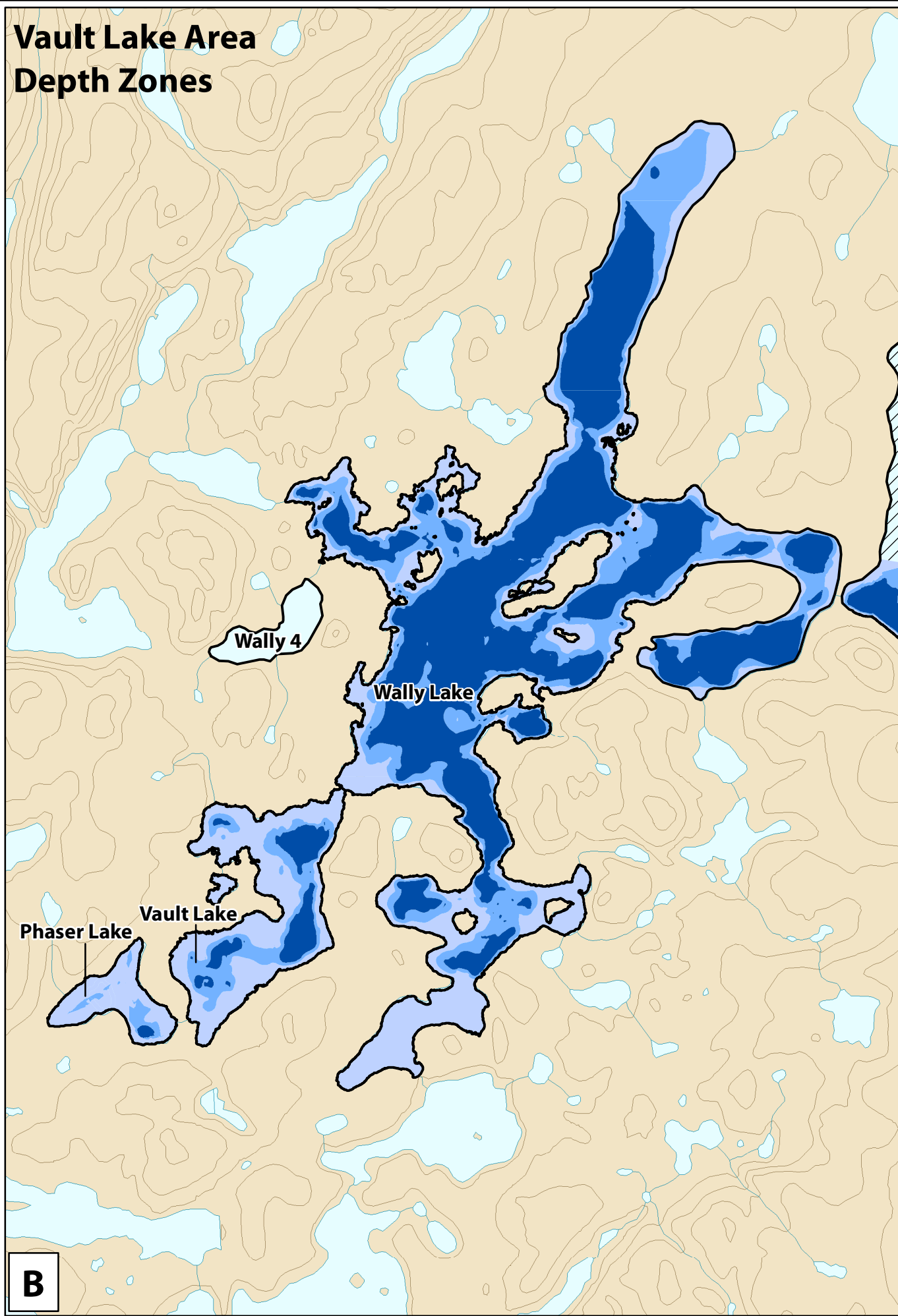


Vault Lake Area Substrate Zones



A

Vault Lake Area Depth Zones



B

Legend

- Study Lakes
- Substrate Zone**
 - Fines
 - Mixed
 - Coarse
- Depth Zone**
 - <2m
 - 2-4m
 - >4m
 - N/A

**Substrate and Depth Zones
Pre-Construction**



77 Wyndham Street South • Guelph ON N1E 5R3
T 519.822.1609 • F 519.822.5389 • www.dougan.ca

PROJECT: DA11-062-02

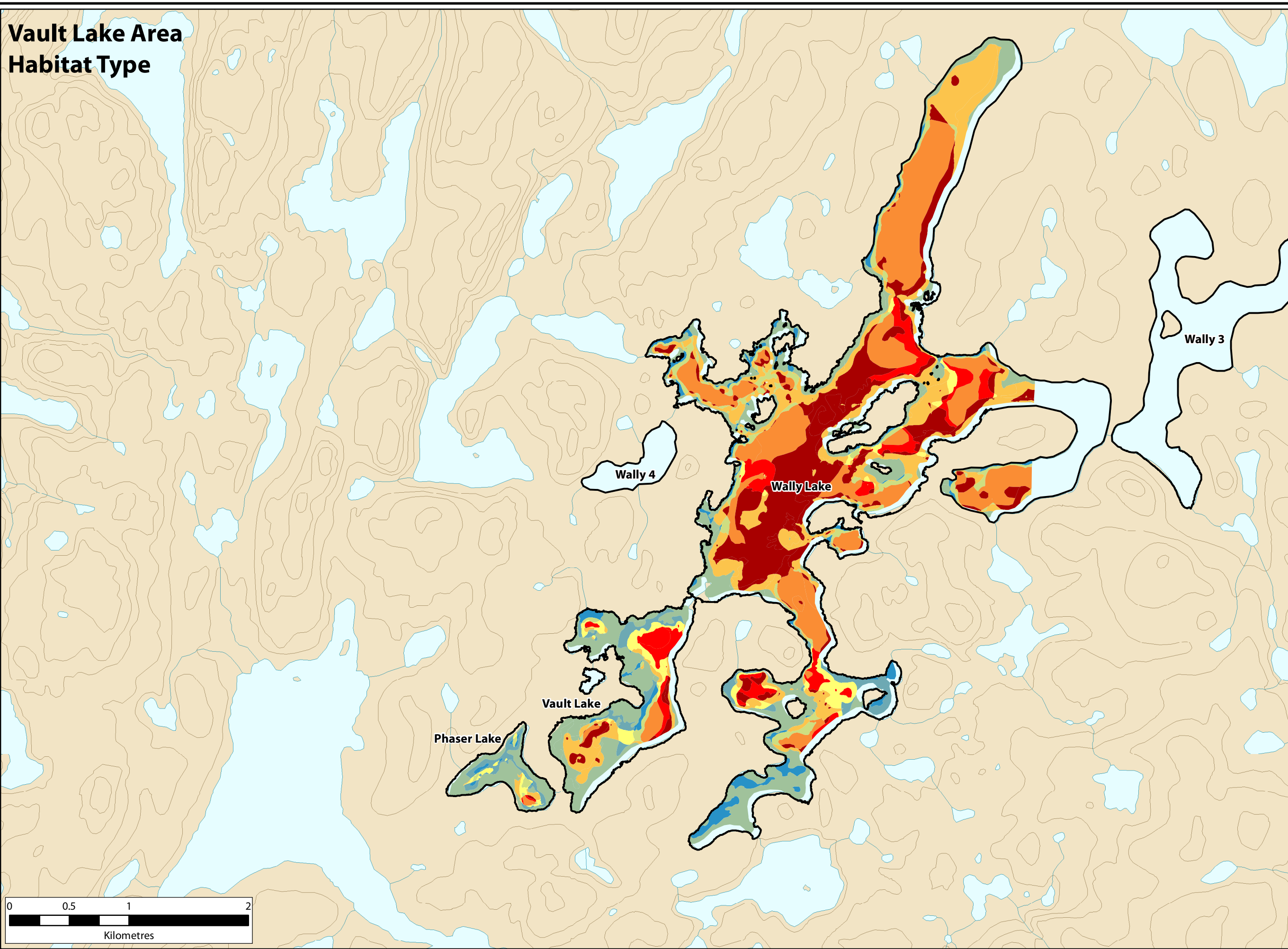
CLIENT: Agnico-Eagle Mines Ltd., Meadowbank Div.

	DATE: FEBRUARY 2016
	SCALE: 1:35,000
	DRAWN BY: LC
	CHECKED BY:

FIGURE: **3-2**

The information displayed on this map has been compiled from various sources. While every effort has been made to accurately depict the information, this map should not be relied on as being a precise indicator of locations, features, or roads, nor as a guide to navigation. MNR data provided by Queen's Printer of Ontario. Use of the data in any derivative product does not constitute an endorsement by the MNR or the Ontario Government of such products.

Vault Lake Area Habitat Type



Legend

Study Lakes

Habitat Type

- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9

Habitat Type	Depth Zone	Substrate
1	<2 m	Fines
2	<2 m	Mixed
3	<2 m	Coarse
4	2-4 m	Fines
5	2-4 m	Mixed
6	2-4 m	Coarse
7	>4 m	Fines
8	>4 m	Mixed
9	>4 m	Coarse

Habitat Types Pre-Construction



77 Wyndham Street South • Guelph ON N1E 5R3
T 519.822.1609 • F 519.822.5389 • www.dougan.ca

PROJECT: DA11-062-02

CLIENT: Agnico-Eagle Mines Ltd., Meadowbank Div.

DATE: FEBRUARY 2016

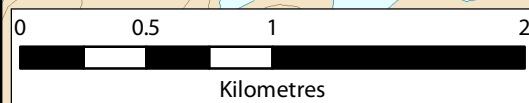
SCALE: 1:30,000

DRAWN BY: LC

CHECKED BY:

FIGURE:
3-3

The information displayed on this map has been compiled from various sources. While every effort has been made to accurately depict the information, this map should not be relied on as being a precise indicator of locations, features, or roads, nor as a guide to navigation. MNR data provided by Queen's Printer of Ontario. Use of the data in any derivative product does not constitute an endorsement by the MNR or the Ontario Government of such products.



SECTION 4 • HABITAT GAINS

Habitat gains to offset losses in Phaser Lake will be obtained from re-flooding of the dewatered lake following the construction of habitat improvement features such as boulder gardens, reefs and shoals. The addition of Arctic char to Phaser Lake through access improvements also contributes to habitat unit gains. In addition, funding for research is proposed as a complementary measure, amounting to 10% on top of the construction costs associated with other habitat offsetting. The details of each offsetting feature for Phaser Lake and the calculation of gained habitat units are described below.

4.1 RE-FLOODING OF DEWATERED PHASER LAKE, WITH ACCESS FOR ARCTIC CHAR

4.1.1 Description

In accordance with DFO authorizations NU-03-0191.3 and NU-03-0191.4, the major fish habitat offsetting or compensation measures currently authorized for the Meadowbank site focus on the re-flooding of dewatered basins and associated pits following mining activities (see 2012 NNLP, Section 2.2.1). In order to recover the greatest number of HUs and ultimately re-establish a natural fish population, considerations for improving fish habitat have been incorporated into the natural basin and pit designs (e.g. boulder gardens, backfilling of deep pits). Following discussions with DFO (January, 2016), AEM has agreed to adjust habitat suitability indices and remove the non-backfilled pit areas in Phaser Lake from offsetting calculations. However, the restoration of the remainder of the natural Phaser Lake basin, as well as backfilled pit areas will provide fish habitat following re-flooding.

After mining, Vault Pit will connect Vault and Phaser Lakes. Once these lakes are reflooded, hydraulically and chemically stable, water quality is considered suitable for aquatic biota and meets conditions of the Type A Water License, the Vault Dike will be removed, allowing fish from Wally Lake access to Vault and Phaser Lakes.

Alterations to fish habitat in Vault and Phaser Lakes will result from construction of pits, pit caps, roads and dikes (as seen in Figure 3-1). Both lakes will be expanded as a result of land-to-lake conversion in the Vault and Phaser pits. Backfilling the Phaser pit to a depth that is within the range of local natural lakes (est. 20 – 40 m maximum) will maximize habitat value, and provide deep water habitat which is lacking in Phaser Lake naturally. Further habitat improvements in these lakes will be made through development of shoals and areas of mixed substrate from temporary haul roads that will be re-contoured as necessary. A description of how each mine-related feature of Phaser Lake is converted to fish habitat following re-flooding is provided in Table 4-1.

In addition to these habitat alterations, the creation of access for Arctic char to Phaser Lake will occur. This species was not identified in baseline studies in Phaser Lakes, and the absence of Arctic char was confirmed during the Phaser Lake fishout. It was predicted that the absence of Arctic char is due to historical isolation and the lack of deep-water habitat, which is generally recognized as niche habitat required by this species when landlocked. Pit

development in Phaser and Vault Lakes will provide a significant quantity (approximately 47 ha) of this deep-water habitat.

Table 4-1. Mine-related features of Phaser Lake and details of the changes and assumptions used to determine habitat type for each area, following re-flooding.

Feature Name	Description of Feature	Description of Habitat
Basin	Area in Phaser Lake that is not covered by other features	Placement of coarse material for temporary roads will result in mixed substrate throughout basin area at depths based on pre-construction contours
BB Phaser Pit	Smaller pit entirely within Phaser Lake	Pit will not be backfilled; assumed to provide no habitat value (HT 10)
BB Phaser Pit Cap	Cap around outside of Baby Phaser Pit (assumed 30 m width)	Cap area provides coarse substrate shoal habitat; no change in depth
Phaser Pit – In-water portion	Portion of Phaser Pit that overlays Phaser Lake	Pit backfilled to an estimated 20-40 m to provide increased habitat suitability; all substrate is coarse (HT 9)
Phaser Pit - Land-to-Lake	Portion of Phaser Pit that overlays land	Pit backfilled to an estimated 20-40 m to provide increased habitat suitability; all substrate is coarse (HT 9)
Phaser Pit Cap	Cap around outside of Phaser Pit (assumed 30 m width)	Cap area provides coarse substrate shoal habitat; no change in depth
Roads	Haul road to run north-south across Phaser Lake	Simulated coarse substrate reef habitat at pre-construction depth

4.1.2 HU Calculation

Substrate zones (fines, mixed, coarse) for Phaser Lake following re-flooding, as determined by the method described in Section 2.1, and incorporating the changes detailed in Table 4-1, above, are shown in Figure 4-1a. Changes to substrate occur through pit and road development, creating areas of coarse and mixed sediment in previously fine-grained basins.

The extents of the depth zones for Phaser Lake following re-flooding, calculated as described in Section 2.1, are shown in Figure 4-1b. The partially backfilled Phaser Pit will provide enhanced overwintering habitat, which is not abundant in this area.

The extent of habitat types 1-10, as described in Section 2.1, were calculated by overlaying substrate and depth maps. Habitat types for Phaser Lake are shown in Figure 4-2. Habitat type 10 is assigned to non-backfilled pit areas independent of estimated depth or substrate characteristics.

In addition, the access factor for Arctic char increases from 0 in the pre-construction scenario, to 1 in the post-construction scenario.

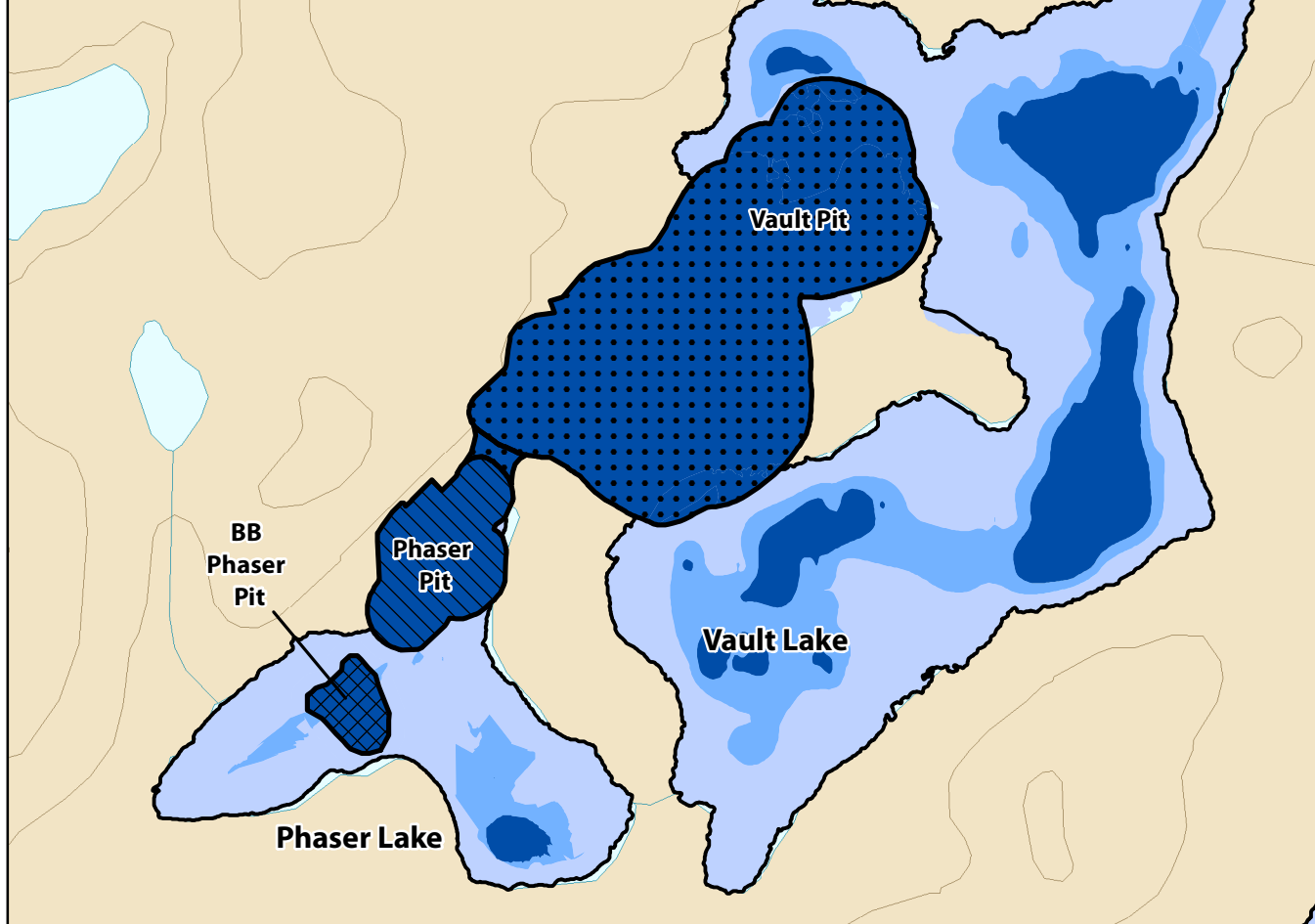
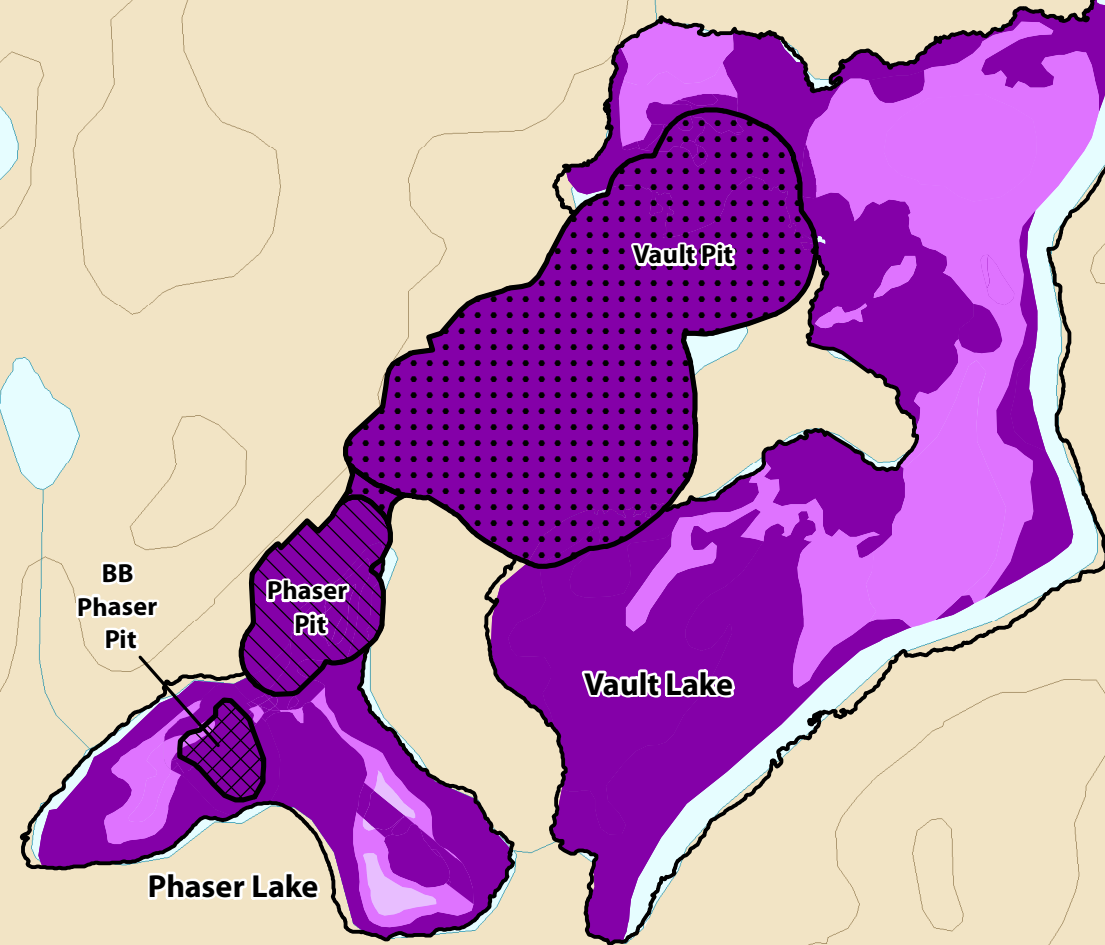
A summary of the total habitat type areas and habitat units, calculated as described in Section 2 are shown in Table 4-2. The HU subtotal and total by species and minesite feature are provided in Appendix A.

Table 4-2. Habitat units (HUs) gained through re-flooding in Phaser Lake. *HT 10 refers to non-backfilled pit habitat.

Habitat Type	Hectares	Habitat Units
1	0.00	0.00
2	2.57	0.72
3	15.24	6.03
4	0.00	0.00
5	1.49	0.87
6	1.83	1.39
7	0.00	0.00
8	0.71	0.37
9	7.60	5.14
10*	1.79	0.00
Total	31.23	14.53

Vault Lake Area Substrate Zones

Vault Lake Area Depth Zones



Legend

- Study Lakes (Post-Closure)
- Pit (Mine Plan, May 2015)**
 - Phaser (Backfilled)
 - BB Phaser (Not backfilled)
 - Vault (Not backfilled)
- Substrate Zone**
 - Fines
 - Mixed
 - Coarse
- Depth Zone**
 - < 2 m
 - 2-4 m
 - > 4 m

Substrate and Depth Zones Post-Closure



77 Wyndham Street South • Guelph ON N1E 5R3
T 519.822.1609 • F 519.822.5389 • www.dougan.ca

PROJECT: DA11-062-06

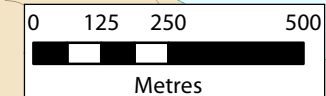
CLIENT: Agnico-Eagle Mines Ltd., Meadowbank Div.

	DATE: FEBRUARY 2016
	SCALE: 1:14,000
	DRAWN BY: LC
	CHECKED BY:

FIGURE: 4-1

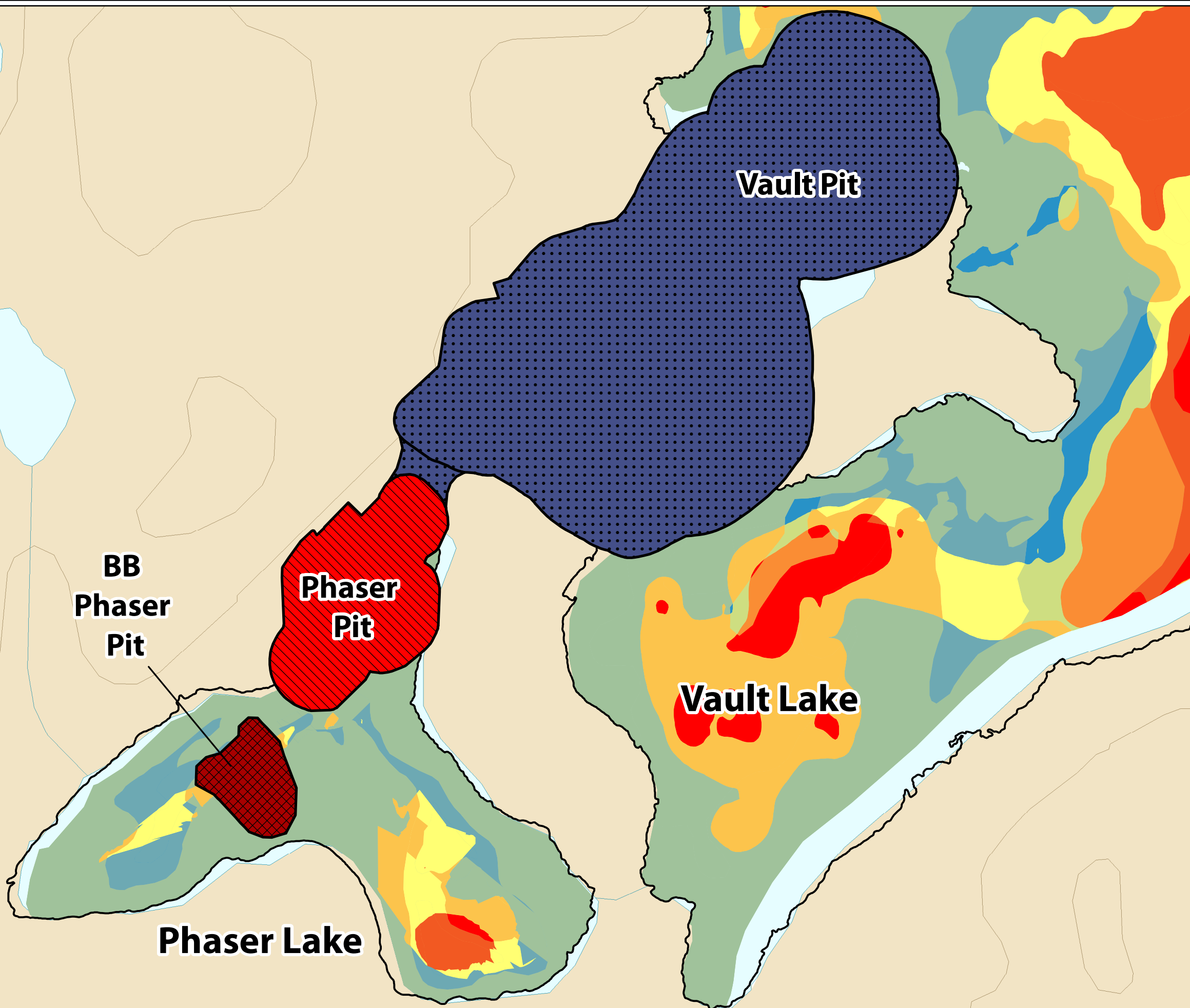
The information displayed on this map has been compiled from various sources. While every effort has been made to accurately depict the information, this map should not be relied on as being a precise indicator of locations, features, or roads, nor as a guide to navigation. MNR data provided by Queen's Printer of Ontario. Use of the data in any derivative product does not constitute an endorsement by the MNR or the Ontario Government of such products.

A



B

Vault Lake Area Habitat Types



Legend

- Study Lakes (Post-Closure)
- Pit (Mine Plan, May 2015)**
 - Phaser (Backfilled)
 - BB Phaser (Not backfilled)
 - Vault (Not backfilled)
- Habitat Type**
 - 1
 - 2
 - 3
 - 4
 - 5
 - 6
 - 7
 - 8
 - 9
 - 10

Habitat Type	Depth Zone	Substrate
1	<2 m	Fines
2	<2 m	Mixed
3	<2 m	Coarse
4	2-4 m	Fines
5	2-4 m	Mixed
6	2-4 m	Coarse
7	> 4 m	Fines
8	> 4 m	Mixed
9	> 4 m	Coarse
10	mixolimnion	Coarse

Habitat Types Post-Closure

77 Wyndham Street South • Guelph ON N1E 5R3
 T 519.822.1609 • F 519.822.5389 • www.dougan.ca

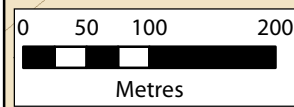
PROJECT: DA11-062-06

CLIENT: Agnico-Eagle Mines Ltd., Meadowbank Div.

<p>UTM Zone 14 NAD83</p>	DATE: FEBRUARY 2016
	SCALE: 1:6,000
	DRAWN BY: LC
	CHECKED BY:

FIGURE: 4-2

The information displayed on this map has been compiled from various sources. While every effort has been made to accurately depict the information, this map should not be relied on as being a precise indicator of locations, features, or roads, nor as a guide to navigation. MNR data provided by Queen's Printer of Ontario. Use of the data in any derivative product does not constitute an endorsement by the MNR or the Ontario Government of such products.



4.2 RESEARCH FUNDING

In 2013, Agnico Eagle re-assessed the No Net Loss Plan costs for the Meadowbank site (AEM, 2013). The originally estimated cost to construct access to Phaser Lake was \$462,611.12 using Reclaim V 6.0 software (AEM, 2013). Under the provisions of the DFO Fisheries Productivity Investment Policy: A proponent's guide to offsetting (DFO, 2013), as a complementary measure for offsetting the serious harm to fish associated with dewatering Phaser Lake, Agnico Eagle proposes to fund research projects using an estimated 10% (\$46,261) on top of the cost of constructing onsite Phaser Lake offsetting works. This may include both direct and in-kind contributions.

Agnico Eagle has been working with researchers at the University of Guelph and University of Alberta to understand aquatic and terrestrial foodwebs in the region, and have begun working with researchers at the University of Guelph on an eDNA study using Phaser Lake. Although the project is in early phases, all parties agree that this is considered a unique opportunity for such a study. Since Phaser Lake is a small, isolated lake with low biodiversity, that is has been dewatered following a fish-out program, biomass and/or species composition estimated through eDNA methods may be able to be compared to fishout data. This information will continue to contribute to an improved understanding of northern aquatic ecosystems and methods for habitat assessment. A summary of research questions, project activities to date and objectives is included as Appendix B. Communication of study results will be determined by the research team.

A second opportunity for research related to Phaser Lake is proposed in conjunction with Phaser Lake re-flooding. There are many questions related to fish habitat utilization that could begin to be answered during this project phase using fish tagging and telemetry. Agnico Eagle will work with academic researchers and contracted biologists to implement a study tracking fish habitat use in a lake basin which incorporates natural former lake bed, backfilled pit, as well as non-backfilled pit areas.

4.3 UNMAPPED AREA

As described in Section 3, resolution differences between substrate maps and base maps for Phaser Lake were found to produce an un-mapped zone of 2.23 ha around the northern perimeter of the lake, for which HUs could not be calculated for losses or gains.

The total area for which habitat units could not be calculated, associated with each mine feature within Phaser Lake (in-water area only) are shown in Table 4-5. Based on adjacent habitat types, all unmapped areas fall within the HT 3 zone (<2 m, coarse; see Figure 3-2). Since the Phaser Pit will be partially backfilled, and all substrate in impacted areas will remain coarse substrate post-construction, no significant changes in habitat quality in the unmapped areas is anticipated. In accordance with DFO's "Review of Habitat Evaluation Procedure (HEP) Input Parameters and Model Results for the Meadowbank Gold Mine Project" (Canadian Science Advisory Secretariat, Science Response 2016/038), the unmapped 2.23 ha of HT 3 was included in the total lost and gained habitat area in Phaser Lake.

Table 4-3. Total area of each mine feature in Phaser Lake and area for which habitat units could not be mapped.

Phaser Lake Feature	Total Area (ha)	Mapped Area (ha)	Unmapped Area (ha)
Phaser Pit	3.55	2.78	0.76
Phaser Pit Cap	0.66	0.66	0.00
Phaser Lake Basin	16.84	15.41	1.43
Roads	2.76	2.71	0.04
BB Phaser Pit	1.79	1.79	0
BB Phaser Pit Cap	1.69	1.69	0
Total	27.29	25.05	2.23

4.4 TIMELINE, DESIGN AND CONSTRUCTION OF THE OFFSETTING MEASURES

The duration of impacts to fish and fish habitat in Phaser Lake will extend from the initiation of dewatering and the fishout (July 2016), until re-flooding and fish introduction is complete. Phaser Lake is estimated to begin re-filling naturally from watershed run-off inflows during operations, and be completely re-flooded by natural inflows by 2027. No active pumping is planned at this time for Phaser Lake. Fish from Wally Lake will be transferred or the Vault Dike breached to allow fish access in consultation with DFO following re-flooding.

Phaser Pit and BB Phaser Pit operations will begin and will be completed in 2017; these operations will take place concurrent with Vault Operations, which are expected to finish in 2018.

Construction of in-basin mine-related features including roads, pit caps, and pit backfilling is based on mine construction requirements and, depending on the feature, will be re-contoured to promote mixed habitat types. While in-basin features will be constructed prior to re-flooding, offsets in fish habitat for onsite options will not be realized until fish are re-introduced and criteria for success are met through monitoring (see Section 4.5).

The proposed eDNA research project began in spring 2016, in anticipation of the limited opportunity for sampling prior to dewatering and the fish-out. Activities to date are detailed in Appendix B. The proposed study using fish tagging and telemetry to track habitat use is planned to occur when fish are re-introduced to Phaser Lake following re-flooding (date to be determined based on water quality monitoring).

4.5 MONITORING

Monitoring to confirm that offsetting measures have been properly implemented and are effectively counterbalancing the serious harm to fish occurring in Phaser Lake will be conducted as described in Agnico Eagle's Habitat Compensation Monitoring Plan. The duration and type of monitoring will allow for demonstration of full ecological functionality of the system (i.e. growth, reproduction and survival).

SECTION 5 • DISCUSSION AND CONCLUSIONS

5.1 SUMMARY

A total of 9.49 HUs are lost through the dewatering and mining of Phaser Pit and BB Phaser Pit in Phaser Lake. Implementation of the onsite offsetting measures proposed here results in a total gain of 14.53 HUs. These measures include re-flooding of the de-watered Phaser Lake, while creating access for Arctic char. In addition, Agnico Eagle is proposing to fund \$46,261 for research directly associated with Phaser Lake. Assuming this research funding is equivalent to a 10% increase in gained HUs, the total offsets for Phaser Lake are 15.98 HUs, or a 1.68:1 ratio of gains to losses.

5.2 ALLOTMENT OF GAINS

The DFO Fisheries Act Authorization NU-03-0191.4 was issued to AEM in 2013 for the dewatering of Vault Lake (losses of 27.73 HU), adjacent to Phaser Lake. At that time, Phaser Pit was planned not to be developed, so authorization was only sought for Vault Lake. However, NU-03-0191.4 included habitat gains associated with a variety of projects across the minesite, including a portion of the gains from re-flooding Vault Lake and Phaser Lake (total gains were 65.32 HU). I.e., in 2013, a Fisheries Act Authorization was not applied for or issued for the dewatering of Phaser Lake, but the offsets associated with re-flooding it were erroneously counted in the Vault Lake Fisheries Act Authorization. AEM communicated this to DFO at the time and will ensure the issue is equitably resolved.

REFERENCES

Cumberland, 2005. Meadowbank Gold Project – Water Quality Predictions. Cumberland Resources Ltd. October, 2005.

AEM, 2012. AEM Meadowbank Division – No Net Loss Plan. October 15, 2012.

AEM, 2013. Meadowbank Mine – Draft No Net Loss Plan Implementation Cost Estimate and Construction Schedule. Version 2. May 2013.

AEM, 2016. Meadowbank Gold Project – Habitat Compensation Monitoring Plan. AEM Meadowbank Division. Version 3. February, 2016.

Azimuth Consulting Group, 2005. Meadowbank Gold Project – Baseline Aquatic Ecosystem Report. Prepared for Cumberland Resources Ltd. October 2005.

DFO, 2013. Fisheries Productivity Investment Policy: A Proponent's Guide to Offsetting. Ecosystem Programs Policy, Fisheries and Oceans Canada. Ottawa, Ontario. November, 2013. ISBN: 978-1-100-22930-0

Golder Associates, 2004. Fish Habitat Compensation Plan for the Northwest Peninsula of the De Beers Snap Lake Diamond Project. Prepared for De Beers Canada Mining Inc. May 2004.

Golder Associates, 2007. Doris North Project No Net Loss Plan – Revision 6. Final Report. Prepared for Miramar Hope Ltd. December 2007.

Mainstream Aquatics Ltd. 2004. Fish Habitat No Net Loss Plan – Jericho Project. Prepared for Tahera Diamond Corporation. December 2004.

Appendix A –

HU Summary, Subtotals, and Totals by Species



TOTAL BY FEATURE - Summary

Phaser Lake				
Phaser Pit	Hectares		HU	
Habitat Type	Losses	Gains	Losses	Gains
1	0.23		0.05	0.00
2	1.23		0.32	0.00
3	1.80		0.64	0.00
4	0.19		0.06	0.00
5	0.09		0.04	0.00
6	0.01		0.01	0.00
7			0.00	0.00
8			0.00	0.00
9		3.54	0.00	2.40
10			0.00	0.00
Total	3.54	3.54	1.11	2.40

HU LOSSES - Species Totals

HU total per species x access weight						
Species >>	ARCH	LKTR	RNWH	BURB	SLSC	NNST
Access >>	0	1	1	1	1	1
1	0.00	0.00	0.01	0.00	0.00	0.03
2	0.00	0.05	0.06	0.06	0.05	0.09
3	0.00	0.13	0.09	0.11	0.22	0.07
4	0.00	0.01	0.02	0.01	0.01	0.01
5	0.00	0.01	0.01	0.01	0.01	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00
Total	0.00	0.21	0.19	0.20	0.29	0.21

HU GAINS - Species Totals

HU total per species x access weight						
Species >>	ARCH	LKTR	RNWH	BURB	SLSC	NNST
Access >>	1	1	1	1	1	1
1	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00
9	0.52	0.52	0.41	0.44	0.37	0.15
10	0.00	0.00	0.00	0.00	0.00	0.00
Total	0.52	0.52	0.41	0.44	0.37	0.15

Phaser Lake				
Phaser Pit Land-to-Lake	Hectares		HU	
Habitat Type	Losses	Gains	Losses	Gains
1			0.00	0.00
2			0.00	0.00
3			0.00	0.00
4			0.00	0.00
5			0.00	0.00
6			0.00	0.00
7			0.00	0.00
8			0.00	0.00
9		3.94	0.00	2.67
10			0.00	0.00
Total	0.00	3.94	0.00	2.67

HU total per species x access weight						
Species >>	ARCH	LKTR	RNWH	BURB	SLSC	NNST
Access >>	0	1	1	1	1	1
1	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00
Total	0.00	0.00	0.00	0.00	0.00	0.00

HU total per species x access weight						
Species >>	ARCH	LKTR	RNWH	BURB	SLSC	NNST
Access >>	1	1	1	1	1	1
1	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00
9	0.57	0.57	0.45	0.49	0.41	0.16
10	0.00	0.00	0.00	0.00	0.00	0.00
Total	0.57	0.57	0.45	0.49	0.41	0.16

Phaser Lake				
Phaser Pit Cap	Hectares		HU	
Habitat Type	Losses	Gains	Losses	Gains
1	0.02		0.00	0.00
2	0.26		0.07	0.00
3	0.36	0.64	0.13	0.25
4			0.00	0.00
5	0.03		0.01	0.00
6		0.03	0.00	0.02
7			0.00	0.00
8			0.00	0.00
9			0.00	0.00
10			0.00	0.00
Total	0.67	0.67	0.21	0.28

HU total per species x access weight						
Species >>	ARCH	LKTR	RNWH	BURB	SLSC	NNST
Access >>	0	1	1	1	1	1
1	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.01	0.01	0.01	0.01	0.02
3	0.00	0.03	0.02	0.02	0.05	0.02
4	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00
Total	0.00	0.04	0.04	0.04	0.06	0.04

HU total per species x access weight						
Species >>	ARCH	LKTR	RNWH	BURB	SLSC	NNST
Access >>	1	1	1	1	1	1
1	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00
3	0.03	0.05	0.03	0.04	0.08	0.03
4	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00
Total	0.03	0.05	0.04	0.04	0.08	0.03

TOTAL BY FEATURE - Summary				
Phaser Lake				
BB Phaser Pit	Hectares		HU	
Habitat Type	Losses	Gains	Losses	Gains
1	0.12		0.03	0.00
2	0.72		0.19	0.00
3	0.57		0.20	0.00
4			0.00	0.00
5	0.37		0.17	0.00
6	0.00		0.00	0.00
7			0.00	0.00
8			0.00	0.00
9			0.00	0.00
10		1.79	0.00	0.00
Total	1.79	1.79	0.59	0.00

HU LOSSES - Species Totals						
HU total per species x access weight						
Species >>	ARCH	LKTR	RNWH	BURB	SLSC	NNST
Access >>	0	1	1	1	1	1
1	0.00	0.00	0.01	0.00	0.00	0.02
2	0.00	0.03	0.04	0.04	0.03	0.05
3	0.00	0.04	0.03	0.04	0.07	0.02
4	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.04	0.04	0.05	0.03	0.02
6	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00
Total	0.00	0.12	0.11	0.12	0.13	0.11

HU GAINS - Species Totals						
HU total per species x access weight						
Species >>	ARCH	LKTR	RNWH	BURB	SLSC	NNST
Access >>	1	1	1	1	1	1
1	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00
Total	0.00	0.00	0.00	0.00	0.00	0.00

Phaser Lake				
BB Phaser Pit Cap	Hectares		HU	
Habitat Type	Losses	Gains	Losses	Gains
1	0.14		0.03	0.00
2	0.44		0.11	0.00
3	0.99	1.57	0.35	0.62
4	0.02		0.01	0.00
5	0.10		0.05	0.00
6	0.00	0.12	0.00	0.09
7			0.00	0.00
8			0.00	0.00
9			0.00	0.00
10			0.00	0.00
Total	1.69	1.69	0.55	0.72

HU total per species x access weight						
Species >>	ARCH	LKTR	RNWH	BURB	SLSC	NNST
Access >>	0	1	1	1	1	1
1	0.00	0.00	0.01	0.00	0.00	0.02
2	0.00	0.02	0.02	0.02	0.02	0.03
3	0.00	0.07	0.05	0.06	0.12	0.04
4	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.01	0.01	0.01	0.01	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00
Total	0.00	0.11	0.09	0.10	0.15	0.10

HU total per species x access weight						
Species >>	ARCH	LKTR	RNWH	BURB	SLSC	NNST
Access >>	1	1	1	1	1	1
1	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00
3	0.07	0.11	0.08	0.10	0.20	0.07
4	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	0.00
6	0.02	0.02	0.02	0.02	0.02	0.01
7	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00
Total	0.08	0.13	0.10	0.11	0.21	0.07

Phaser Lake				
Basin	Hectares		HU	
Habitat Type	Losses	Gains	Losses	Gains
1	0.29		0.06	0.00
2	2.29	2.57	0.60	0.72
3	11.08	11.08	3.92	4.38
4	0.51		0.15	0.00
5	0.98	1.49	0.46	0.87
6	0.87	0.87	0.53	0.66
7	0.52		0.16	0.00
8	0.20	0.71	0.08	0.37
9	0.11	0.11	0.06	0.08
10			0.00	0.00
Total	16.84	16.84	6.02	7.09

HU total per species x access weight						
Species >>	ARCH	LKTR	RNWH	BURB	SLSC	NNST
Access >>	0	1	1	1	1	1
1	0.00	0.01	0.01	0.01	0.00	0.04
2	0.00	0.10	0.12	0.12	0.10	0.17
3	0.00	0.81	0.58	0.69	1.38	0.46
4	0.00	0.04	0.04	0.03	0.02	0.03
5	0.00	0.11	0.11	0.12	0.07	0.04
6	0.00	0.14	0.13	0.11	0.12	0.04
7	0.00	0.04	0.05	0.03	0.02	0.02
8	0.00	0.02	0.02	0.02	0.01	0.01
9	0.00	0.02	0.01	0.01	0.01	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00
Total	0.00	1.27	1.07	1.14	1.74	0.80

HU total per species x access weight						
Species >>	ARCH	LKTR	RNWH	BURB	SLSC	NNST
Access >>	1	1	1	1	1	1
1	0.00	0.00	0.00	0.00	0.00	0.00
2	0.05	0.11	0.13	0.13	0.11	0.19
3	0.46	0.81	0.58	0.69	1.38	0.46
4	0.00	0.00	0.00	0.00	0.00	0.00
5	0.17	0.17	0.17	0.19	0.11	0.06
6	0.14	0.14	0.13	0.11	0.12	0.04
7	0.00	0.00	0.00	0.00	0.00	0.00
8	0.08	0.08	0.06	0.08	0.04	0.03
9	0.02	0.02	0.01	0.01	0.01	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00
Total	0.92	1.32	1.08	1.22	1.77	0.78

Phaser Lake				
Roads	Hectares		HU	
Habitat Type	Losses	Gains	Losses	Gains
1	0.05		0.01	0.00
2	0.50		0.13	0.00
3	1.40	1.96	0.50	0.77
4	0.31		0.09	0.00
5	0.16		0.08	0.00
6	0.32	0.80	0.20	0.61
7			0.00	0.00
8			0.00	0.00
9			0.00	0.00
10			0.00	0.00
Total	2.75	2.75	1.00	1.38

HU total per species x access weight						
Species >>	ARCH	LKTR	RNWH	BURB	SLSC	NNST
Access >>	0	1	1	1	1	1
1	0.00	0.00	0.00	0.00	0.00	0.01
2	0.00	0.02	0.03	0.03	0.02	0.04
3	0.00	0.10	0.07	0.09	0.17	0.06
4	0.00	0.02	0.03	0.02	0.01	0.02
5	0.00	0.02	0.02	0.02	0.01	0.01
6	0.00	0.05	0.05	0.04	0.04	0.01
7	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00
Total	0.00	0.22	0.19	0.19	0.27	0.14

HU total per species x access weight						
Species >>	ARCH	LKTR	RNWH	BURB	SLSC	NNST
Access >>	1	1	1	1	1	1
1	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00
3	0.08	0.14	0.10	0.12	0.24	0.08
4	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	0.00
6	0.12	0.12	0.12	0.10	0.11	0.03
7	0.00	0.00	0.00	0.00	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00
9	0.00	0.00	0.00	0.00	0.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00
Total	0.21	0.27	0.22	0.22	0.35	0.11

TOTAL BY FEATURE - Summary

Phaser Lake
BB Phaser Pit
Habitat Type Hectares Losses Gains HU Losses Gains
1 0.12 0.03 0.00
2 0.72 0.19 0.00
3 0.57 0.20 0.00
4 0.00 0.00 0.00
5 0.37 0.17 0.00
6 0.00 0.00 0.00
7 0.00 0.00 0.00
8 0.00 0.00 0.00
9 0.00 0.00 0.00
10 1.79 0.00 0.00
Total 1.79 1.79 0.59 0.00

Phaser Lake

BB Phaser Pit Cap
Habitat Type Hectares Losses Gains HU Losses Gains
1 0.14 0.03 0.00
2 0.44 0.11 0.00
3 0.99 1.57 0.35 0.62
4 0.02 0.01 0.00
5 0.10 0.05 0.00
6 0.00 0.12 0.00 0.09
7 0.00 0.00 0.00
8 0.00 0.00 0.00
9 0.00 0.00 0.00
10 0.00 0.00 0.00
Total 1.69 1.69 0.55 0.72

Phaser Lake

Basin
Habitat Type Hectares Losses Gains HU Losses Gains
1 0.29 0.06 0.00
2 2.29 2.57 0.60 0.72
3 11.08 11.08 3.92 4.38
4 0.51 0.15 0.00
5 0.98 1.49 0.46 0.87
6 0.87 0.87 0.53 0.66
7 0.52 0.16 0.00
8 0.20 0.71 0.08 0.37
9 0.11 0.11 0.06 0.08
10 0.00 0.00 0.00
Total 16.84 16.84 6.02 7.09

Phaser Lake

Roads
Habitat Type Hectares Losses Gains HU Losses Gains
1 0.05 0.01 0.00
2 0.50 0.13 0.00
3 1.40 1.96 0.50 0.77
4 0.31 0.09 0.00
5 0.16 0.08 0.00
6 0.32 0.80 0.20 0.61
7 0.00 0.00 0.00
8 0.00 0.00 0.00
9 0.00 0.00 0.00
10 0.00 0.00 0.00
Total 2.75 2.75 1.00 1.38

HU LOSSES -Species Sub-Totals

Habitat type area x HSI x species weight x life function weight
Species >> ARCH LKTR RNWH BURB SLSC NNST
Life Function >> SP NU FO OW SP NU FO OW SP NU FO OW SP NU FO OW SP NU FO OW SP NU FO OW
1 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.01 0.01 0.01 0.00
2 0.00 0.01 0.01 0.00 0.00 0.02 0.02 0.00 0.00 0.02 0.02 0.00 0.00 0.02 0.02 0.00 0.01 0.01 0.02 0.00 0.02 0.02 0.02 0.00
3 0.00 0.01 0.01 0.00 0.00 0.02 0.02 0.00 0.00 0.02 0.01 0.00 0.00 0.02 0.01 0.00 0.02 0.02 0.02 0.00 0.00 0.01 0.02 0.00
4 0.00
5 0.01 0.01 0.01 0.01 0.01 0.01 0.01 0.01 0.01 0.01 0.01 0.01 0.02 0.01 0.01 0.01 0.00 0.00 0.01 0.01 0.00 0.00 0.00 0.01
6 0.00
7 0.00
8 0.00
9 0.00
10 0.00
Total 0.01 0.03 0.03 0.01 0.01 0.05 0.05 0.01 0.01 0.05 0.04 0.01 0.02 0.06 0.04 0.01 0.04 0.04 0.05 0.01 0.02 0.03 0.05 0.01

Habitat type area x HSI x species weight x life function weight

Species >> ARCH LKTR RNWH BURB SLSC NNST
Life Function >> SP NU FO OW SP NU FO OW SP NU FO OW SP NU FO OW SP NU FO OW SP NU FO OW
1 0.00 0.00 0.00 0.00 0.00 0.00 0.01 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.01 0.01 0.01 0.00
2 0.00 0.00 0.00 0.00 0.00 0.01 0.01 0.00 0.00 0.01 0.01 0.00 0.00 0.01 0.01 0.00 0.00 0.00 0.01 0.00 0.01 0.01 0.01 0.00
3 0.00 0.02 0.02 0.00 0.00 0.04 0.03 0.00 0.00 0.03 0.02 0.00 0.00 0.04 0.02 0.00 0.04 0.04 0.04 0.00 0.00 0.01 0.03 0.00
4 0.00
5 0.00
6 0.00
7 0.00
8 0.00
9 0.00
10 0.00
Total 0.00 0.03 0.03 0.00 0.00 0.06 0.05 0.00 0.00 0.05 0.04 0.00 0.00 0.06 0.03 0.00 0.05 0.05 0.05 0.00 0.02 0.03 0.05 0.00

Habitat type area x HSI x species weight x life function weight

Species >> ARCH LKTR RNWH BURB SLSC NNST
Life Function >> SP NU FO OW SP NU FO OW SP NU FO OW SP NU FO OW SP NU FO OW SP NU FO OW
1 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.01 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.01 0.01 0.01 0.00
2 0.00 0.02 0.02 0.00 0.00 0.05 0.05 0.00 0.00 0.07 0.05 0.00 0.00 0.07 0.05 0.00 0.02 0.02 0.05 0.00 0.05 0.05 0.07 0.00
3 0.00 0.23 0.23 0.00 0.00 0.46 0.35 0.00 0.00 0.35 0.23 0.00 0.00 0.46 0.23 0.00 0.46 0.46 0.46 0.00 0.00 0.12 0.35 0.00
4 0.00 0.01 0.01 0.02 0.00 0.01 0.01 0.02 0.00 0.01 0.01 0.02 0.02 0.01 0.01 0.02 0.00 0.00 0.01 0.01 0.02 0.00 0.01 0.02
5 0.02 0.03 0.03 0.03 0.02 0.03 0.03 0.03 0.02 0.03 0.03 0.03 0.04 0.02 0.03 0.03 0.01 0.01 0.02 0.03 0.00 0.00 0.01 0.03
6 0.04 0.04 0.04 0.03 0.04 0.04 0.04 0.03 0.04 0.04 0.03 0.03 0.03 0.02 0.04 0.03 0.03 0.03 0.04 0.03 0.00 0.00 0.01 0.03
7 0.00 0.01 0.01 0.02 0.00 0.01 0.01 0.02 0.00 0.01 0.02 0.02 0.00 0.00 0.01 0.02 0.00 0.00 0.00 0.02 0.00 0.00 0.00 0.02
8 0.00 0.00 0.01 0.01 0.00 0.00 0.00 0.01 0.01 0.00 0.00 0.00 0.01 0.01 0.00 0.01 0.00 0.00 0.00 0.01 0.00 0.00 0.00 0.01
9 0.00
10 0.00
Total 0.07 0.35 0.36 0.11 0.07 0.60 0.50 0.11 0.06 0.50 0.39 0.11 0.08 0.58 0.37 0.11 0.53 0.53 0.58 0.11 0.06 0.17 0.46 0.11

Habitat type area x HSI x species weight x life function weight

Species >> ARCH LKTR RNWH BURB SLSC NNST
Life Function >> SP NU FO OW SP NU FO OW SP NU FO OW SP NU FO OW SP NU FO OW SP NU FO OW
1 0.00 0.00 0.00 0.00 0.00 0.00 0.01 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00 0.00
2 0.00 0.01 0.01 0.00 0.00 0.01 0.01 0.00 0.00 0.02 0.01 0.00 0.00 0.02 0.01 0.00 0.01 0.01 0.01 0.00 0.01 0.01 0.02 0.00
3 0.00 0.03 0.03 0.00 0.00 0.06 0.04 0.00 0.00 0.04 0.03 0.00 0.00 0.06 0.03 0.00 0.06 0.06 0.06 0.00 0.00 0.01 0.04 0.00
4 0.00 0.01 0.01 0.01 0.00 0.01 0.01 0.01 0.00 0.01 0.01 0.01 0.00 0.00 0.00 0.01 0.00 0.00 0.00 0.01 0.00 0.00 0.01 0.01
5 0.00 0.01 0.01 0.01 0.00 0.01 0.01 0.01 0.00 0.01 0.01 0.01 0.01 0.00 0.01 0.01 0.00 0.00 0.00 0.01 0.00 0.00 0.00 0.01
6 0.01 0.00 0.00 0.00 0.01
7 0.00
8 0.00
9 0.00
10 0.00
Total 0.02 0.06 0.06 0.02 0.02 0.09 0.08 0.02 0.02 0.08 0.07 0.02 0.02 0.09 0.06 0.02 0.08 0.08 0.09 0.02 0.01 0.03 0.07 0.02

Appendix B –

Phaser Lake eDNA Study Summary

Summary Report-eDNA analysis for Phaser Lake, Meadowbank**Date:** October 5, 2016

The objective of this study is to conduct a preliminary test of eDNA measurement methods in a small Arctic lake, and to explore potential relationships between eDNA and physical fish counts.

One set of water samples for preliminary eDNA analysis was collected by Agnico Eagle technicians on May 16, 2016, according to SOP-1 provided below. The preliminary water samples were received at the University of Guelph on May 19th. These samples were filtered (in aliquots of 0.5 L, 1 L, 2 L, and 4 L for both frozen and refrigerated samples) and DNA extracted using a Qiagen DNeasy Blood and Tissue Kit. The samples were stored at -80°C until the PCR was performed.

Results from the preliminary analysis indicated only a faint band for the 4 L refrigerated sample. As such, for the second set of water samples, 2 sites were sampled on August 17, 2016 (~12 L per site; see SOP-2 provided below) and the samples were refrigerated. The samples were received at the University of Guelph on August 19th and were filtered (using a smaller pore size of 0.5 µm) in 2 L aliquots the same day and stored at -20°C until extraction. The samples have been extracted and are now being stored at -80°C. Preliminary PCR will be performed shortly. The second PCR settings may also be modified to increase detection chances.

Primers were selected using the Primer Database in BOLD (http://www.boldsystems.org/index.php/Public_Primer_PrimerSearch). Primers used were VR1_t1 and VF2_t1, which amplify a 655 bp segment of the 5' region of the mitochondrial cytochrome *c* oxidase I (COI) gene (Ivanova et al. 2007; Ward et al. 2005). These primers were found to be suitable for all fish species (lake trout, round whitefish, Arctic char, burbot, ninespine stickleback, slimy sculpin) however no reference tissue for slimy sculpin was available for the first round of samples.

Provided there is successful detection of fish DNA in the second round of water samples, the products can be sequenced to determine what specific fish have been amplified. This would be carried out either at the in-house sequencing facility at the University of Guelph School of Environmental Sciences, or in conjunction with the Biodiversity Institute. At that point, the molecular tests could be compared to physical fish counts. However, it is noted that shedding/degradation rates will not be known.

References:

Ivanova, N. V., Zemlak, T. S., Hanner, R. H., & Hebert, P. D. N. (2007). Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes*, 7(4), 544–548. <http://doi.org/10.1111/j.1471-8286.2007.01748.x>

Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. N. (2005). DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 360(September), 1847–1857. <http://doi.org/10.1098/rstb.2005.1716>

Standard Operating Procedure for Preliminary Sampling (SOP-1)

Prepared by: Dylan Bowes
Reviewed by: Dr. Paul Sibley

Date: 2016-03-16
Date: 2016-03-19
Last Revised: 2015-05-16

eDNA collection protocol for Phaser Lake, Meadowbank

Sample Collection Protocol (Rees et al. 2014)

Sampling Summary

For the purposes of the preliminary analysis, we will sample water from mid-column, roughly 1 meter below the surface of the ice. Agnico will be provided with 1 cooler containing 4 L DNA-free sample bottles. A total volume of 20 L will be collected. Half of the samples will be frozen and half will be refrigerated to determine the impact of freezing on DNA detection capabilities.

Materials:

- 6 x labelled, 4 L autoclaved or DNA-free polypropylene screw-top bottles (supplied)
- 10% bleach solution
- Water pump and tubing
- Coolers and ice packs
- Field GPS
- Freezer (-20°C) and Refrigerator (4°C)
- Laboratory gloves (latex)

Sample Collection

1. To prevent contamination, all equipment used to sample and process the water should be sterilized using a 10-minute exposure to 10% bleach solution (Jerde et al. 2011). Sterilized gloves (sprayed with bleach) should be used during sampling collection and processing (Olson et al. 2012). In order to sterilize tubing, a sterilization circulation can be set-up using a bucket with bleach solution. Following sterilization and prior to taking samples, a large volume of water (i.e. roughly 10x volume of sterilization solution used) from the lake to be sampled should be pumped through the tubing to remove the bleach solution.
 2. At the site, record sampling location using GPS.
 3. Collect a total of 20 L of water from mid-column in DNA-free screw-top bottles by pumping water from approximately 1 meter below the surface of the lake using gloved hands (Olson et al. 2012).
 4. When filling the bottle, leave a small (4 cm) headspace to allow for expansion during freezing.
 5. During transport to field lab, keep the samples in the large coolers with ice packs to slow DNA degradation.
 6. Place half of the samples (3 x 4 L bottles) in a freezer and the remaining half of the samples in a refrigerator until shipping. Ship samples in coolers with ice packs to the University of Guelph for analysis.
-

References

- Jerde, C. L., Mahon, A. R., Chadderton, W. L., & Lodge, D. M. (2011). "Sight-unseen" detection of rare aquatic species using environmental DNA. *Conservation Letters*, 4(2), 150–157. <http://doi.org/10.1111/j.1755-263X.2010.00158.x>
- Olson, Z. H., Briggler, J. T., & Williams, R. N. (2012). An eDNA approach to detect eastern hellbenders (*Cryptobranchus a. alleganiensis*) using samples of water. *Wildlife Research*, 39(7), 629–636. <http://doi.org/10.1071/WR12114>
- Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R. M., & Gough, K. C. (2014). The detection of aquatic animal species using environmental DNA—a review of eDNA as a survey tool in ecology. *Journal of Applied Ecology*.
-

Standard Operating Procedure for eDNA Sampling (SOP-2)

Prepared by: Dylan Bowes
Reviewed by: Dr. Paul Sibley

Date: 2016-03-16
Date: 2016-03-19
Last Revised: 2016-07-30

eDNA collection protocol for Phaser Lake, Meadowbank

Sample Collection Protocol (Rees et al. 2014)

Sampling Summary

Based on the results of the preliminary analysis, large sample volumes are required and refrigerating samples will be preferred over freezing. We will sample water from mid-column, roughly 1 meter below the surface of the water. Agnico will be provided with 1 cooler containing 4 L DNA-free sample bottles. A total volume of 24 L will be collected.

Materials:

- 6 x labelled, 4 L autoclaved or DNA-free polypropylene screw-top bottles (supplied)
- 10% bleach solution
- Water pump and tubing
- Coolers and ice packs
- Field GPS
- Refrigerator (4°C)
- Laboratory gloves (latex)

Sample Collection

1. To prevent contamination, all equipment used to sample and process the water should be sterilized using a 10-minute exposure to 10% bleach solution (Jerde et al. 2011). Sterilized gloves (sprayed with bleach) should be used during sampling collection and processing (Olson et al. 2012). In order to sterilize tubing, a sterilization circulation can be set-up using a bucket with bleach solution. Following sterilization and prior to taking samples, a large volume of water (i.e. roughly 10x volume of sterilization solution used) from the lake to be sampled should be pumped through the tubing to remove the bleach solution.
 2. At the site, record sampling location using GPS.
 3. Collect a total of 24 L of water from mid-column in DNA-free screw-top bottles by pumping water from approximately 1 meter below the surface of the lake using gloved hands (Olson et al. 2012).
 4. During transport to field lab, keep the samples in the large coolers with ice packs to slow DNA degradation.
 5. Place samples in a refrigerator until shipping. Ship samples in coolers with ice packs to the University of Guelph for analysis.
-

References

- Jerde, C. L., Mahon, A. R., Chadderton, W. L., & Lodge, D. M. (2011). "Sight-unseen" detection of rare aquatic species using environmental DNA. *Conservation Letters*, 4(2), 150–157. <http://doi.org/10.1111/j.1755-263X.2010.00158.x>
- Olson, Z. H., Briggler, J. T., & Williams, R. N. (2012). An eDNA approach to detect eastern hellbenders (*Cryptobranchus a. alleganiensis*) using samples of water. *Wildlife Research*, 39(7), 629–636. <http://doi.org/10.1071/WR12114>
- Rees, H. C., Maddison, B. C., Middleditch, D. J., Patmore, J. R. M., & Gough, K. C. (2014). The detection of aquatic animal species using environmental DNA—a review of eDNA as a survey tool in ecology. *Journal of Applied Ecology*.
-