Report (Rev.0)

BIODIVERSITY IMPACT STUDIES - NORTHWESTERN ONTARIO REGION: BASELINE PROGRAM DESIGN

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PREPARED BY Zoetica Environmental Consulting Services

SUBMITTED TO Melissa Mayhew
Nuclear Waste Management Organization
22 St. Clair Avenue East
Sixth Floor, Toronto, ON
M4T 2S3, Canada

ZOETICA
ENVIRONMENTAL CONSULTING SERVICES

OFFICE 102-22351 St Anne Ave, Maple Ridge, BC, V2X 2E7
PHONE 604 467 1111
WEBSITE www.zoeticaenvironmental.com

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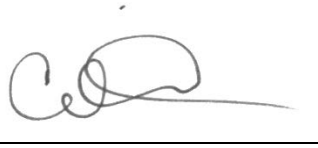
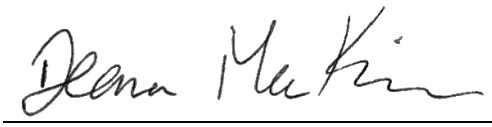
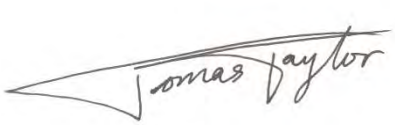
Prepared By:



Celia Chui
Wildlife Biologist (M.Sc., R.P.Bio., P.Biol.)



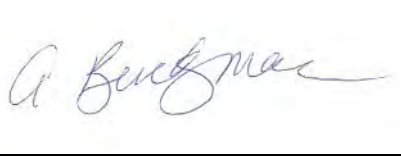
Deanna MacKinnon
GIS Specialist (B.Sc. Phys. Geog.)



Tomas Taylor
Wildlife Biologist (M.Sc.)

Jimmy Brett Allen
Senior Vegetation & Ecosystem Biologist
(R.P.Bio., R.P.P., M.C.I.P.)

Checked By:



Andrea Buckman
Senior Ecologist (Ph.D., R.P.Bio.)

Approved By:

Heather Bears
Principal, Senior Ecologist (M.Sc., Ph.D., R.P.Bio.)

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GLOSSARY AND ABBREVIATIONS

AOI	Area of Interest
APM	Adaptive Phased Management
ATV	All-Terrain Vehicle
BACI	Before-After-Control-Impact
BIS	Biodiversity Impact Studies
BPD Report	Biodiversity Impact Studies – Northwestern Ontario Region: Baseline Program Design Report
BPPA Report	Biodiversity Impact Studies – Northwestern Ontario Region: Best Practices and Preferred Approach Report
COSEWIC	Committee on the Status of Endangered Wildlife in Canada
COSSARO	Committee on the Status of Species at Risk in Ontario
CWD	Coarse Woody Debris
DGR	Deep Geological Repository
eDNA	Environmental DNA
ELC	Ecological Land Classification
EDMS	Electronic Document Management System
EMBP	Environmental Media Baseline Program
FRI / eFRI	Forest Resource Inventory / Enhanced Forest Resource Inventory
GIS	Geographic Information System
GPS	Global Positioning System
GRTS	Generalized Random Tessellation Stratified
IA	Impact Assessment
IK	Indigenous Knowledge
LIO	Land Information Ontario
LSA	Local Study Area
MAFA	Moose Aquatic Feeding Area
MNRF	Ontario Ministry of Natural Resources and Forestry
MTO	Ontario Ministry of Transportation
NHIC	Ontario Natural Heritage Information Centre
NWMO	Nuclear Waste Management Organization
PQP	Project Quality Plan
QA/QC	Quality Assurance/Quality Control
RIC / RISC	Resources Inventory Committee / Resources Information Standards Committee
RSA	Regional Study Area

SAR	Species at Risk
SARO	Species at Risk in Ontario
SOP	Standard Operating Procedure
SWH	Significant Wildlife Habitat (defined below)
TEM	Terrestrial Ecosystem Mapping
TISG	Tailored Impact Statement Guidelines for Designated Projects Subject to the *Impact Assessment Act and the Nuclear Safety and Control Act*
UTM	Universal Transverse Mercator
VC	Valued Component
WLON	Wabigoon Lake Ojibway Nation
A priori	"From what is before"; a theoretical deduction rather than empirical observation. With respect to the Biodiversity Impact Studies, *a priori* power analysis can be conducted prior to the study to estimate sufficient sample sizes to achieve adequate power.
Biodiversity value	All of the biotic environmental components that will be considered for study within the APM Project's Biodiversity Impact Studies. A subset of biodiversity values may ultimately be scoped into the Biodiversity Impact Assessment as Valued Components.
Herpetofauna	The reptiles and amphibians of a particular region, habitat, or geological period.
In media res	"In the middle of things." With respect to the Biodiversity Impact Studies, *in media res* analysis means testing statistical power or species detection saturation in real-time (e.g., at the end of each sampling day or each week of field work).
Lek	A communal area where animals congregate during the breeding season and males engage in competitive displays and courtship rituals to attract females. A variety of species use a lek mating system; grouse are amongst the most well-known examples.
Lentic	The term lentic refers to standing water habitats such as lakes and ponds, or swamps and marshes.
Lotic	The term lotic refers to running or moving water habitats such as rivers and streams.
Mast	Mast is the fruit of trees and shrubs, such as acorns and other nuts. Mast trees are important as food for certain wildlife, and Mast Producing Areas are considered Significant Wildlife Habitat in Ecoregion 5E and Ecodistrict 6E-14 in Ontario.
Midden (squirrel)	The preferred foraging location for squirrels, as indicated by a pile of leftover food such as cones. Middens tend to be centrally located in a squirrel's territory.
Mitigation hierarchy	A tool designed to help limit the negative impacts of development on biodiversity and ecosystem services. Involves a sequence of four key actions – avoid, minimize, restore, and offset - and provides a best practice approach to aid in the sustainable management of environmental resources by establishing a mechanism to balance conservation needs with development priorities.
Post hoc	"After the event." With respect to the Biodiversity Impact Studies, *post hoc* power analysis can be conducted after a study has been completed, and uses the obtained sample size and effect size to determine the power that was achieved.
Revell Batholith Area	Used to describe the general area surrounding the Revell Batholith Withdrawal Area within which the APM Project may be located. The Revell Batholith Area is near the

	Township of Ignace in northwestern Ontario. It is located in Treaty #3 in the traditional territory of Wabigoon Lake Ojibway Nation, among other Indigenous communities.
Rights-holders	First Nation and Métis communities who have asserted and or hold recognized treaty and/or Indigenous rights and whose Traditional Territories include the project location.
Seral stage	In forested landscapes, seral stage refers to the vegetation communities formed through succession (i.e., the process of forest aging) and adapted to the abiotic and biotic conditions of a site. Following a disturbance, succession typically progresses through establishment (early/young seral), thinning or stem exclusion (mid-seral), transition (mature seral), and a shifting mosaic (old seral).
Significant Wildlife Habitat	Defined in the Ontario Provincial Policy Statement, 2020 as: *Wildlife habitat* – areas where plants, animals and other organisms live, and find adequate amounts of food, water, shelter and space needed to sustain their populations. Specific wildlife habitats of concern may include areas where species concentrate at a vulnerable point in their annual life cycle; and areas which are important to migratory and non-migratory species. *Significant* – in regard to wildlife habitat, ecologically important in terms of features, functions, representation or amount, and contributing to the quality and diversity of an identifiable geographic area or natural heritage system.
The APM Project	The Deep Geological Repository and other required infrastructure for the safe, long-term management of Canada's used nuclear fuel.
Waterbody	A waterbody is an accumulation of water in an area and includes lakes, ponds, and wetlands that do not have a defined channel that flows through them.
Watercourse	A watercourse is a natural or artificial channel through which water flows and includes stream, rivers and wetlands that include a defined channel that flows through them.

1.0 INTRODUCTION AND STUDY DESIGN CONSIDERATIONS

In 2002, the Nuclear Waste Management Organization (NWMO) was established in accordance with the *Nuclear Fuel Waste Act* (S.C. 2002, c. 23). Under the *Nuclear Fuel Waste Act,* the NWMO is responsible for the safe long-term management of Canada's used nuclear fuel.

The NWMO recommended Adaptive Phased Management (APM) and this approach was accepted by the Canadian government in June 2007. APM is a combination of technical methods and a management system; the various components are summarized in Table 1-1 of the *Biodiversity Impact Studies – Northwestern Ontario Region: Best Practices and Preferred Approach* ('BPPA Report'; Zoetica Environmental Consulting Services 2020). The APM includes a site selection phase that aims to find a suitable location to host a Deep Geological Repository (DGR), which will store used nuclear waste deep below the earth's surface along with other required infrastructure ('the APM Project'). The APM Project will only proceed at a selected site with the involvement of the interested community, Indigenous (First Nation and Métis) communities in the area, and surrounding communities, working together to implement it. The NWMO has retained Zoetica Environmental Consulting Services ('Zoetica'™) to design and implement Biodiversity Impact Studies (BIS) for the Revell Batholith Area near the Township of Ignace ('Ignace'), northwestern Ontario, and Wabigoon Lake Ojibway Nation (WLON).

This report presents the Baseline Program Design for establishing the current biodiversity and ecosystem services that exist at one of the potential sites, which is associated with a geologic formation zone called the Revell Batholith, near the community of Ignace in northwestern Ontario. Within this Revell Batholith, the NWMO has identified an "Area of Interest" (AOI) within which the project could be located. In this report, the term 'Revell Batholith Area' will be used to describe the area within which baseline studies will be conducted.

The current *Biodiversity Impact Studies – Northwestern Ontario Region: Baseline Program Design* ('BPD Report') serves the primary purpose of presenting the methods, both desk- and field-based, to be used in undertaking baseline data collection and data management. Methods included in this report will follow the decisions and preferences outlined in the BPPA Report.

1.1 Baseline Program Objectives

The baseline data collection methods and analyses were designed such that they can achieve the following goals:

1. Establish important biodiversity and ecosystem services present prior to the development and operation of a potential APM Project, which will enable predictions of potential APM Project-specific and cumulative impacts as defined in the Tailored Impact Statement Guidelines (TISG) for the APM Project or the TISG Template[1];
2. Provide information on key biodiversity values and ecosystem services in the area to inform the APM Project design, and to ensure that the APM Project could be built in compliance with applicable regulations;
3. Integrate and trace community feedback into the baseline program;

[1] Since the APM Project-specific TISG will not be released until after the Project Description has been submitted, Zoetica will follow the general guidelines presented in the Template for Designated Projects Subject to the *Impact Assessment Act and the Nuclear Safety and Control Act* (IAAC 2019), hereafter referred to as the 'TISG Template'.

4. Inform project planning and facilitate the development of mitigation strategies to avoid and reduce negative impacts and identify opportunities for benefit to biodiversity values;
5. Establish baseline measures of biodiversity and ecosystem services against which select monitoring data, for effects that were evaluated with unacceptably low levels of certainty or for follow-up on select effects, can be compared to determine if the APM Project is having the type and degree of impacts predicted in the Impact Assessment (IA). Likewise, baseline data will enable the detection of unforeseen changes and adaptive management;
6. Inform environmental management performance monitoring and development of action levels; and,
7. Inform biodiversity conservation initiatives and regulatory and compliance monitoring programs.

1.2 Project Location and Context

Previous siting studies were conducted by the NWMO in collaboration with local communities and rights-holders[2]. These studies established an Area of Interest (AOI) for the APM Project, which is located approximately 20 km east of Wabigoon Lake Ojibway Nation (WLON), and 40 km west of Ignace, Ontario, within the northern portion of the Revell Batholith Temporary Withdrawal Area[3]. Through discussions with people in the area about a number of potentially geologically suitable repository areas identified based on results of desktop technical studies and field investigations (e.g., airborne geophysical surveys and geological mapping), the NWMO identified the AOI as the area within which they would focus ongoing investigations for APM Project feasibility.

The Revell Batholith Area is located in the Kenora District of Ontario, which is the most sparsely populated (0.2 people/km^2) and largest district in Ontario (over approximately 400,000 km^2 with a population of 65,533; Statistics Canada 2017). The region has a long history of mining and forestry. Tourism in the district is a more recent industry that continues to grow. There are no active or abandoned mines in the Revell Batholith Withdrawal Area at present (ENDM 2019). Forestry is still an active sector with logging activity occurring within the Revell Batholith Area. Popular activities for tourists are outdoor activities aimed at experiencing the natural beauty of the region, especially the abundant lakes. All of these activities could interact with the APM Project to create cumulative impacts, which will need to be considered in a cumulative effects assessment. For a more detailed review of past, current, and future activities, see Zoetica's BPPA Report, Section 1.5.

According to Ontario's Ecological Land Classification (ELC) System, the Revell Batholith Area and AOI developed for the APM Project are located within the Lake Wabigoon (4S) Ecoregion of the Ontario Shield Ecozone. The AOI, along with its context relative to various ecoregions, communities, roadways, and ecological features is presented in **Figure 1-1**.

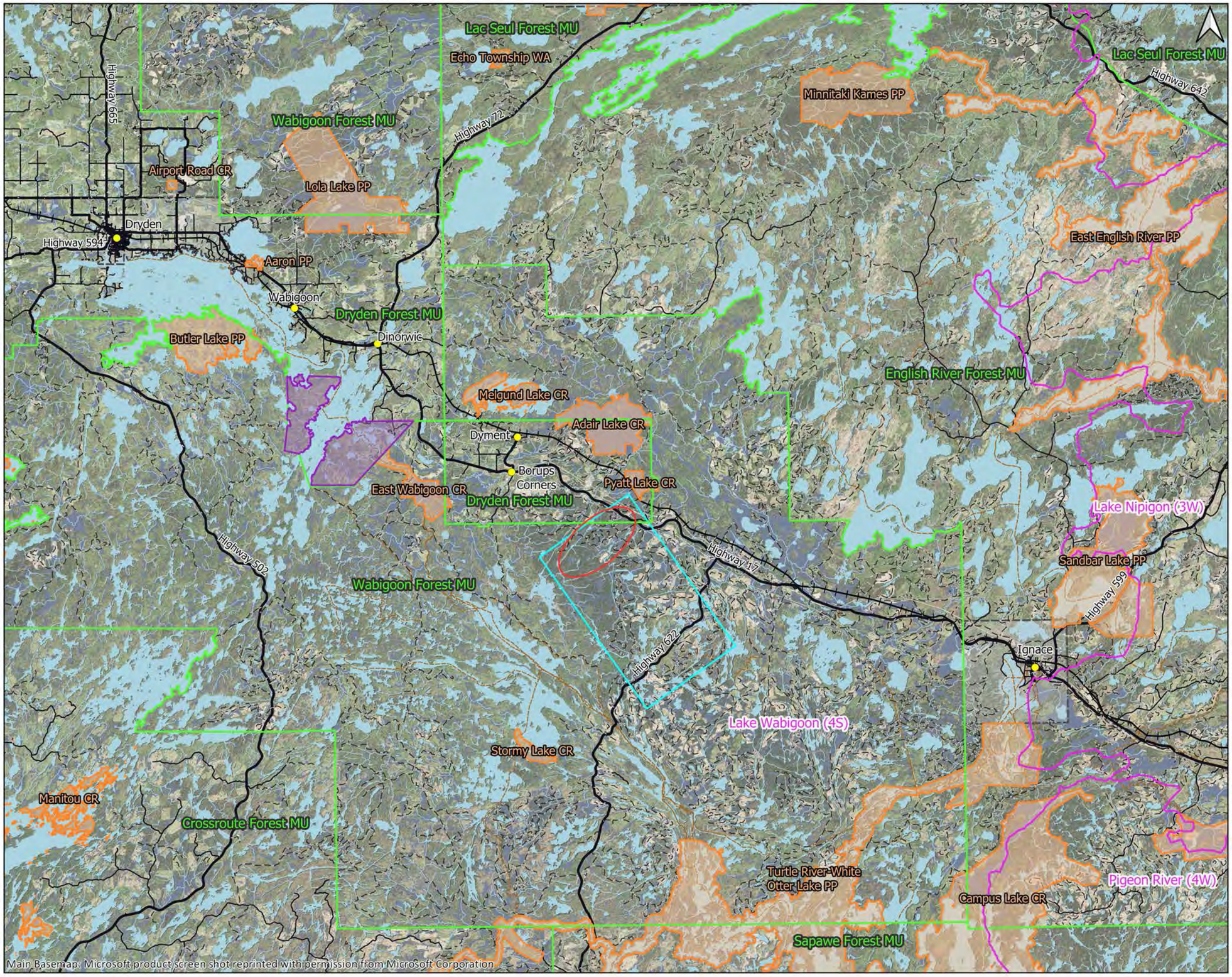
[2] As the NWMO has yet to identify a preferred site, it is acknowledged that the Impact Assessment process has not been initiated and the Duty to Consult has not been delegated. Throughout this document, the term stakeholder has been used to identify community members, agencies, private entities, etc., while rights-holder has been used to identify Indigenous peoples and communities (i.e., First Nations and Métis). These groups would be consulted in the event of an Impact Assessment.

[3] Several geographically large areas (areas temporarily withdrawn from mineral staking, i.e., temporary withdrawal areas) within the vicinity of the Township of Ignace were identified as potentially suitable for the long-term management of used nuclear fuel based on readily available geological information evaluated during Phase 1 desktop studies.

NWMO Biodiversity Impact Studies

Overview of Revell Batholith Area

Figure 1-1



- Area of Interest (AOI)
- Revell Batholith Withdrawal Area
- Provincial Park (PP), Conservation Reserve (CR), Wilderness Area (WA)
- Ecoregion
- Forest Management Unit (MU)
- Wetland
- Lake
- Watercourse
- Highway or Arterial Road
- Local Road
- Resource/Recreation Road
- Trail
- Railroad
- Community
- Municipal Boundary
- Wabigoon Lake No. 27

1:350,000

0 5 10 km

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Inset Basemap © OpenStreetMap contributors

Data received from:
Ontario Geohub - EcoRegion (MNRF), Forest Management Unit (MNRF), Indian Reserve (MNRF), OHN Waterbody (MNRF), OHN Watercourse (MNRF), ORWN Railway (MNRF), MNRF Road Segments (MNRF), Municipal Boundary - Lower and Single Tier (MMAH), Wetlands (MNRF)
NWMO - AOI, Revell Batholith Withdrawal Area, Trail Segments (MNRF)
IBAT - World Database on Protected Areas (UNEP-WCMC and IUCN)

Project CRS: NAD83 / UTM zone 15N	
August 14, 2020	Map ID: NWMO_BIS_010

No detailed project description for the APM Project is currently available. However, a conceptual, preliminary design of the APM Project was prepared by the NWMO and can be found in *Deep Geological Repository Conceptual Design Report Crystalline/Sedimentary Rock Environment* (Noronha 2016). A more detailed summary of the APM Project Description is available in Zoetica's BPPA Report, Section 1.6.

1.3 Overall Baseline Study Approach

The baseline studies have been designed with the whole impact study process in mind, from project planning through to IA and follow-up programs. Considering the whole process ensures that the baseline program will be properly contextualized and maximally informative. Prior to completing the current BPD Report, Zoetica produced a BPPA Report. The BPPA Report provided: initial scoping and rationale for potential biodiversity valued components (VCs), research and justification for best practices and preferred approaches for the BIS Program, and included a well-researched guidance document to select the most appropriate methods for conducting baseline data collection, the biodiversity IA, and the cumulative effects assessment. The BPPA Report is a living document that will be updated annually to reflect emerging technologies, continuing stakeholder and rights-holder feedback, and learning. As updates are made to the BPPA Report that are relevant to baseline methods, those updated details must also be reflected within the baseline program and BPD Report. A detailed description of the overarching process for the BIS Program is provided in the BPPA Report, Section 1.3.

The overall BIS Program that is endorsed includes a desk-based research exercise and iterative scoping, which will help to integrate new information and feedback annually as more is learned about the APM Project, community values and concerns, biodiversity values or ecosystem services that are likely or not likely to be impacted, and biodiversity values or ecosystem services requiring further study. Once baseline studies are sufficiently progressed, a VC (selection of biodiversity values likely to be included in the APM Project-specific TISG and carried through to an IA) and issues scoping exercise is undertaken to help focus studies to be undertaken for an IA. Next, impact predictions and decisions are made based on whether predicted changes are likely to fall within ranges that are acceptable to affected local communities, rights-holders, scientific experts, the regulator and other federal and provincial agencies, and other interested parties. The mitigation hierarchy[4] will be applied to effects that may fall outside of the acceptable range to reduce or limit changes such that they fall within an acceptable range, wherever possible. Residual effects (i.e., adverse impacts that remain after the application of the mitigation hierarchy) would be documented. Following the completion of the biodiversity impact assessment, follow-up monitoring programs would be proposed for selected valued components, focused on those predicted effects that are potentially significant and uncertain. The follow-up program will be designed to be suitable for detecting unanticipated impacts to enable adaptive management. While designing and implementing follow-up programs are beyond the scope of this work, the baseline data collection, analysis, interpretation, and reporting processes will be conducted with the goal of ensuring that those future programs will be well-informed, capable of monitoring the effectiveness of mitigation applied (following the mitigation hierarchy) as well as possible enhancements, and supported by a transparent impact assessment process.

[4] Mitigation Hierarchy is a set of guidelines that are nationally and internationally accepted as a best practice and provide a framework to follow a series of mitigation options in the order of avoidance, minimization, restoration, and offset to reduce development impacts and aim to achieve no net loss of biodiversity (BBOP 2012, IFC 2012, CSBI 2015).

As outlined in Section 4.2 of the BPPA Report, the biodiversity baseline program will follow a tiered approach. The baseline program will begin with the collection of broad-scale, foundational environmental data in Tier 1 (i.e., studies starting in Year 1), followed by more specific data collection in Tiers 2 and 3 (i.e., Year 2 onward). By using the results of Tier 1 studies to inform decision-making for later phases, this approach will enable the baseline program to move forward in a more efficient, focused, justified, transparent, and cost-effective manner. High quality data from the baseline study will allow for efficient issues scoping and VC selection for further assessment, which is the next step of the IA process.

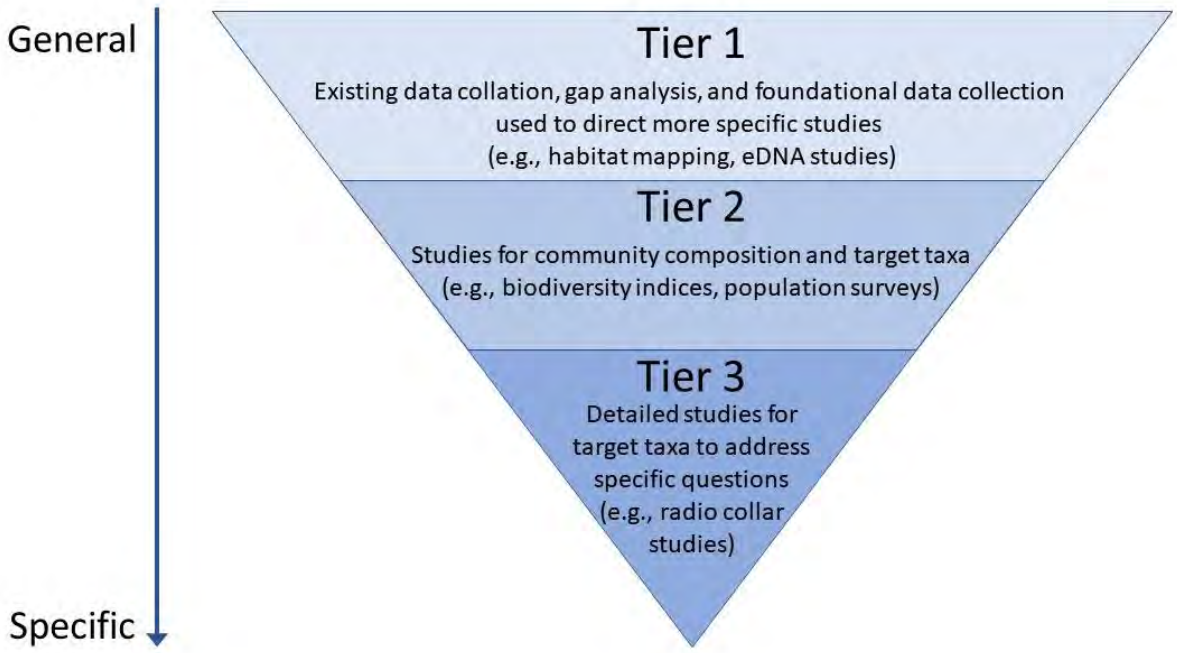


Figure 1-2. Graphical representation of the tiered approach for planning the BIS Baseline Program. Tier 1 studies involve more general information collection with increasing specificity and focus in Tiers 2 and 3. Tier 1 studies will focus on collecting foundational and broadly applicable data that will be used to support, justify, inform, and plan Tier 2 and 3 studies.

The overall process described herein is designed following a western science approach and interweaves Indigenous Knowledge (IK) and local input that has been made available. However, certain Indigenous communities, whose Traditional Territories fall within the study areas, may undertake their own studies that combine principles, values, and traditional approaches to arrive at their own conclusions about potential project impacts and mitigation options. At key points along this pathway, understanding and information from both processes will be interwoven to strengthen the APM Project, predictions, and mitigation, and to enhance positive outcomes.

The baseline program will make use of multiple sources of information, including available large datasets, desk-based research, IK consistent with the NWMO's IK policy, community input, previous work conducted for the APM Project, future field work proposed for other programs, and field work designed for the BIS. A key objective in the design of the baseline studies is to ensure that data on biodiversity values of importance to surrounding communities, stakeholders, and rights-holders are collected,

analyzed, and interpreted such that any potential effects of the APM Project on these biodiversity values can be effectively monitored over the long term, and will support an adaptive management program. The BIS Program will also be designed to collect evidence to support a 4-pillar sustainability-based IA framework (*Impact Assessment Act*, S.C. 2019, c. 28, s.1), which includes the following pillars: Environment, Social, Economic, and Health. The social pillar under the *IAA* framework also includes cultural considerations, which were discussed as a separate (fifth) pillar within the "Building a Common Ground" (2017) review of the Canadian Environmental Assessment Process, but is also integrated throughout the remaining four pillars where possible (*Impact Assessment Act,* S.C. 2019, c. 28, s.1). A key recommendation of the expert panel that reviewed the Canadian Environmental Assessment process was to interweave IK and community knowledge with the best evidence from science as a collaborative exercise between Indigenous communities, knowledge holders, and scientists (Gélinas *et al.* 2017). The BIS Program is designed such that it enables the interweaving of IK and community knowledge into the baseline studies, wherever possible, following the NWMO IK policy (NWMO 2016).

At this stage of the APM Project, the baseline program will focus on collecting data on biodiversity values that must be collected to ensure that the APM Project can be built without violating existing Acts or regulations and to show due diligence, and to examine ways in which the APM Project could be built to achieve "no net loss"[5] via application of the mitigation hierarchy to project planning.

The baseline program will also focus on biodiversity values likely to be required for study as valued components (VCs) by the APM Project-specific TISG, or based on community concerns not captured in the TISG, to support the biodiversity IA process. VCs are defined by the Impact Assessment Agency of Canada (IAAC) as "the environmental element of an ecosystem that is identified as having scientific, social, cultural, economic, historical, archaeological or aesthetic importance" (CEAA 2006). The conversion of a biodiversity value to a VC is particularly likely where the element represents a key value of interest to communities or to conservation (e.g., Species at Risk [SAR]), when it is present in the area, and where it can reasonably be expected to be impacted by the project, under certain circumstances. However, for the purposes of this BPD Report, elements to be studied are generally referred to as biodiversity values.

1.4 Inclusion of Stakeholder and Rights-holder Feedback

The development of the BIS is viewed as a collaborative process that involves engagement with, and input from affected local communities, rights-holders, scientific experts, the regulator and other federal and provincial agencies, and other interested parties. The development of the BIS will rely heavily on the incorporation of community concerns and knowledge. Indigenous Knowledge and local knowledge provide unique insight into the value of biodiversity and ecosystem functions for human well-being outside of knowledge that can be gained from a western science approach alone (Tengö *et al.* 2014). An understanding of the local environment provides clarity about locally cherished biodiversity values (e.g., fishing areas and favoured fish species, hunted and trapped species, valued plants and natural medicines, areas identified as sacred). Indigenous Knowledge and local knowledge can also identify holistic ecological interrelationships and observed changes in the local environment over a long period of collective memory.

[5] In the case of biodiversity values for the Biodiversity Impact studies, "no net loss" value would be defined as no acceleration in a stable or declining trajectory that is more pronounced than would be predicted to occur due to factors unrelated to the project. In the case that an area is improving over time from a downgraded condition, no net loss to biodiversity would also be defined as the avoidance of any interference with this improvement or recovery, and a net benefit in this case would be defined as an improving condition that is put on a more positive or timely trajectory by beneficial actions of the project proponent.

As IK has been transmitted through stories, ceremony, songs, cultural tradition, and art since time immemorial, this knowledge system is founded on a collective experiential basis that spans a much longer time frame than data collected through western science.

The development of the baseline studies and IA will involve continued and ongoing engagement and dialogue with interested and potentially affected communities to ensure that the program adequately reflects and incorporates feedback received. Inclusion of the results of engagement with communities into the APM Project from an early stage will strengthen the APM Project in the future, will ensure that studies required for the APM Project will be conducted in a culturally appropriate manner (e.g., inclusion of ceremony, where appropriate, or focusing on non-lethal sampling wherever possible), and will ensure that the NWMO remains accountable to the community. Ongoing dialogue will also help identify how community members participate in the baseline studies and the future IA. The results of the BIS Program will be discussed with stakeholders and rights-holders such that there is a better understanding of potential effects of the APM Project on biodiversity and ecosystem services as they relate to ecology and community values. The discussion of results with these groups will also provide for the ongoing improvement of knowledge about the environment associated with the APM Project.

The NWMO has been actively engaging with interested parties in the area since the Township of Ignace voluntarily joined the site selection process. Some of these engagement meetings were conducted to learn what types of questions and concerns stakeholders and rights-holders had regarding their environment, to gather information on the current stressors acting in their environment, and elements they felt were needed to develop a trustworthy and open environmental monitoring program (CanNorth 2020). Workshops to answer questions specifically related to the Environmental Media Baseline Program (EMBP) were initiated in 2018. A second round of workshops was conducted to present results from prior workshops and to present information on, and inform, the planning of the EMBP. Results from the first workshop were shared in a way that linked the community input to aspects of the draft program to maintain accountability of design decisions to the community. Workshop participants were asked if they could see their input in the program, and whether anything was missing. In some cases, where gaps were identified, the design was modified. Information gathered during these workshops that was relevant to the BIS Program was used to help inform the design of the biodiversity studies.

Zoetica reviewed all available community input collected to date from various past workshops held by the NWMO and stakeholders/rights-holders, and will continue to do so as engagement continues. In developing this Baseline Design Report, Zoetica reviewed information collected through previous workshops, including:

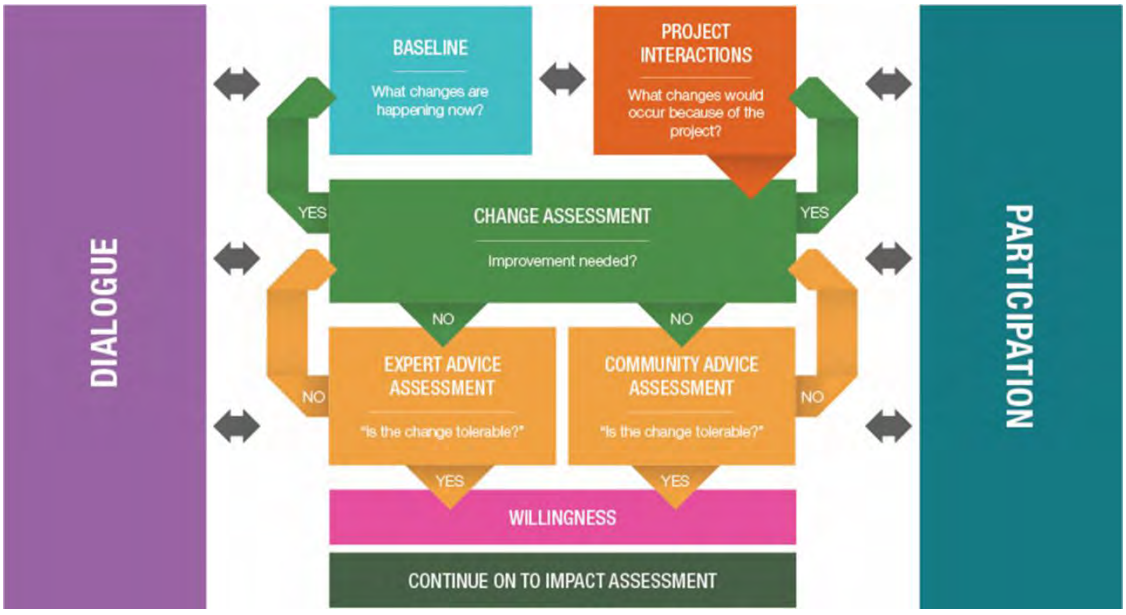
- The NWMO Environmental Media Baseline Program Design – Appendix B.1: Stakeholder and rights-holder input from Round 1 Workshops (CanNorth 2020)
- The NWMO Environmental Media Baseline Program Design – Appendix B.2: Stakeholder and rights-holder input from Round 2 Workshops (CanNorth 2020)
- The NWMO Adaptive Phased Management Project – Northwestern Ontario Region Environmental Media Baseline Program – Preliminary Sample Design Feasibility Assessment (Appendix F, CanNorth 2019a)
- Technical Memorandum (Final) – Conceptual Site Model for the Deep Geological Repository (January 24, 2020) (Appendix C, CanNorth 2020)

- Phase 1 Preliminary Community Well-Being Assessment – Township of Ignace, Ontario (SENES Consultants 2013)
- Phase 1 Preliminary Assessments, Summary Findings and Decisions: Creighton, Saskatchewan; Ear Falls, Ontario; English River First Nation, Saskatchewan; Hornepayne, Ontario; Ignace, Ontario; Pinehouse, Saskatchewan; Schreiber, Ontario; Wawa, Ontario (NWMO 2013)

Further, early engagement with stakeholders and rights-holders was conducted in April and May 2020 to solicit feedback on proposed baseline work for the BIS Program (summarized in Appendix B of the BPPA Report). Feedback received from these engagement sessions has been integrated into the studies and study design to be conducted in the Revell Batholith Area starting in Year 1. Not all stakeholder and rights-holder groups were available to participate during the April and May 2020 engagement events, due to issues related to the COVID-19 pandemic, and additional information will be sought from these groups via a meeting venue (online, telephone, or in person) and during a time period that is acceptable to them.

Interweaving previous and ongoing feedback received during stakeholder and rights-holder engagements into baseline programs is imperative to the success of satisfying requirements of communities, and for informing studies that include and assess community concerns. Feedback received from affected local communities, rights-holders, scientific experts, and other interested parties at previous engagement workshops, prior to the initiation of the BIS contract, identified several shared concerns. These concerns centred on the need for honesty and transparency of activities and studies conducted on behalf of the NWMO, facilitated by publicly accessible data, engagement with and involvement of local communities (especially with regard to engagement, training, and employment opportunities), the consideration of local input and knowledge in the design of the environmental baseline programs, potential impacts on various environmental and biodiversity values (including air, water, soil, fish, vegetation, and wildlife), the need for the involvement of experts, and the need to respect the land and Spirit (CanNorth 2020). **Figure 1-3** presents how the BIS fits into the various environmental components of the overall APM Project IA and how stakeholder and rights-holder engagement and participation will be sought during all phases and programs of the IA.

(a)



(b)

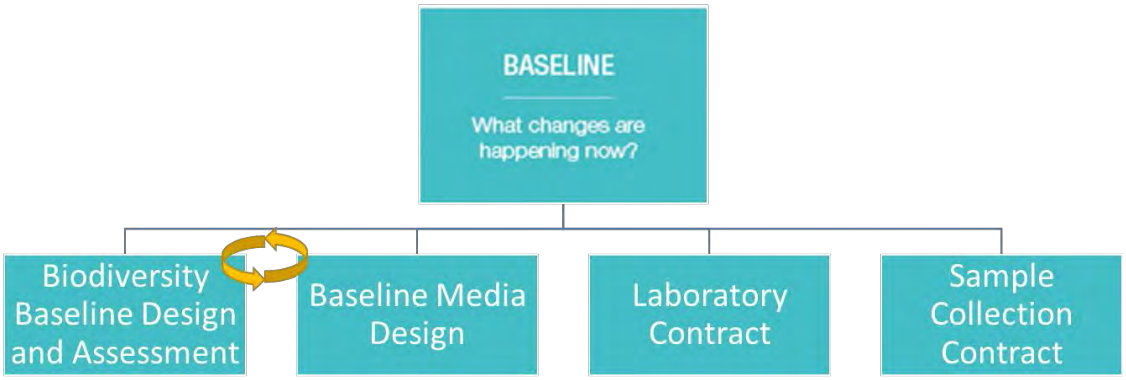


Figure 1-3. (a) Relationships between environmental components of the APM Project IA, along with continual and ongoing stakeholder and rights-holder engagement and participation. (b) Boxes indicate separate but related contracts for the work program. For example, Zoetica is responsible for the Biodiversity Baseline Design and Assessment (i.e., BIS), which focuses on the biotic environment (including ecosystem services), whereas CanNorth is responsible for the Baseline Media Design, which is mostly focused on the abiotic environment (e.g., water quality). However, the biotic and abiotic environments are inter-dependent and potential projects on one may affect the other. Therefore, close collaboration is needed to ensure the success of both programs and to enable a comprehensive IA.

Zoetica took stock of important values and observations of stakeholders and rights-holders that need to be considered as part of the BIS, and for making decisions in the BPPA Report. Zoetica understands that Indigenous communities in the Revell Batholith Area depend on the area for health, food security, and cultural/spiritual well-being. Zoetica also understands that communities and economies in the area rely heavily on the resource sector (e.g., forestry, mining), tourism (largely based on the outdoor opportunities in the region), and fish and guide-outfitting (key fished species: walleye, northern pike, and lake trout; key hunted wildlife: moose, black bear, white-tailed deer, and small game such as grouse and snowshoe hare). The key fished and hunted species will be considered as part of the biodiversity baseline studies as biodiversity values (see Zoetica's BPPA Report, Section 3.1). There is local recognition of the importance in maintaining a functional, balanced, and healthy ecosystem in the AOI and surrounding environment. Appropriate methods and study designs for capturing these values have been examined in Zoetica's BPPA Report.

As part of the BIS, important "ecosystem services" that benefit humans must also be considered, such that the NWMO can apply the mitigation hierarchy to project planning and implementation. Healthy ecosystem functions provide many, varied benefits ('services') to humans, such as tree and plant roots that filter and clean surface water runoff before it enters waterbodies and stabilize soils to reduce erosion risk; merchantable timber that can be used for building or economic gain; wetlands that store water and mitigate against extreme weather events like floods and droughts; large riparian trees that provide shade and maintain cooler water temperatures for fish; habitat that supports wildlife and fish that are favoured by hunters, trappers, and people who fish; plants for consumption and medicinal purposes; and outdoor opportunities that provide for mental, cultural, spiritual, recreational, and physical well-being (i.e., cultural services).

Ecosystem services are considered as part of the BIS because in an IA they are largely examined based on patterns found in relevant biodiversity values (e.g., studies of fish, vegetation, and wildlife, including

species of importance to communities), alongside a growing understanding of the human uses of the area (i.e., cultural services, to be studied as part of social, economic and/or health impact studies; and via discussions with Indigenous communities and local stakeholders). As ecosystem services are largely interdisciplinary, focusing the ecosystem services studies for the BIS will require thorough coordination and communication between potentially affected Indigenous communities and local stakeholders and other APM Project teams (e.g., health, social, economic, physical sciences). The ecosystem services that will ultimately be focused on will rely heavily on what Indigenous communities and local stakeholders perceive as important, and on those services that could be negatively impacted by the APM Project.

How community concerns of relevance to the biodiversity program noted thus far have been considered in the current BPD Report are outlined in **Table 1-1**. The methods proposed by Zoetica to study each of the listed values/concerns can be found in the sections noted in the table; however, the current BPD Report focuses on Tier 1 studies and detailed designs for Tier 2 and 3 studies will be developed at a later date to account for learning from Tier 1 studies. Additional information about community feedback is available in Section 1.4 and Appendix B of the BPPA Report.

Table 1-1. Community concerns and values reflected in the Biodiversity Impact Studies.

Value/Concern	**Description of how it is reflected/considered in the BIS**	**Baseline Study Section where Value will be Captured**
Important Country Foods Vegetation	Several vegetation species such as wild rice, various berry species, wild mushrooms (lobster, stump, and chanterelles), ginger, and other plants for tea were brought up during engagement meetings as culturally important and/or gathered by people for consumption and/or medicinal purposes. Starting in Year 1, Zoetica proposes to map vegetation in a biodiversity value-specific study area, including plant species of importance to community members. Additional information on vegetation of importance will be sought through workshops, and dialogue, and feedback will be sought prior to undertaking surveys.	Section 2.1 Section 3.0
Ecology of land and habitat for wildlife and fish	Ecology of the land was mentioned as a specific concern as well as habitat for biodiversity values. Starting in Year 1, Zoetica proposes to focus on mapping important habitat and vegetation and on identifying important wildlife habitat within biodiversity value-specific terrestrial and aquatic study areas. Further years of study will focus on more targeted studies of habitat specific to various biodiversity values.	Section 2.1 Section 2.2 Section 2.3 Section 2.5
Water supply and ecological features providing important services for life requirements of culturally important species, human use and consumption, and culturally harvested plants.	Ensuring the protection of water supply, and ecological features that provide habitat for various wildlife and fish as well as where culturally important plants are harvested were brought up in community feedback as important to consider during the design of baseline programs. In Tier 1 studies, Zoetica proposes studies for other biodiversity values that will feed into the ecosystem services information, along with desk-based studies. Hydrological studies will be conducted as part of the EMBP, and important water features and known/mapped aquifers will be identified within the biodiversity	Section 2.1 Section 2.3 Section 3.0

	baseline program. Future studies are proposed that focus on the assessment of ecosystem services using the TESSA toolkit. Michel Lake, which falls outside of the current $RSA_{AQU,}$ was identified as an important water source for the Township of Ignace. If human growth within Ignace as a result of the APM Project is predicted to impact the lake, it can be considered in future studies.	
Moose and deer	Monitoring of moose and deer populations and their overall health were noted as important study design considerations during community engagement meetings. In Tier 1 studies, Zoetica proposes to focus on field verification of potential Significant Wildlife Habitat (SWH) for both species, and seasonal habitat suitability modelling within a biodiversity value-specific study area for moose. Additional Tier 2 population-level studies may be designed based on results and needs identified from Tier 1.	Section 2.1 Section 2.2 Section 2.5 Section 3.0
Black bear, wolf, fox, and other carnivore species	Black bear has been noted as a hunted species by several community members. In addition, other carnivores such as wolves and foxes have been noted to increase and influence populations of other species that they take as prey. In Tier 1 studies, Zoetica proposes to determine the presence, distribution and abundance of suitable habitat and SWH for black bear through ecosystem mapping within biodiversity value-specific study areas. SWH for additional carnivores (i.e., den sites) will be noted if identified. Future years of study may focus on general community surveys for carnivores and on select, regionally important carnivore populations if warranted (e.g., if a SAR carnivore is detected during community surveys, and important/suitable habitat for that species is found within the AOI). Lynx was brought up in dialogue as an important local species; this species is best captured through studies of impacts of the project to snowshoe hare (see below), as lynx population sizes fluctuate very closely with the well-documented 8 to 11-year snowshoe hare cycle and are limited by the availability of this prey species.	Section 2.1 Section 2.2 Section 3.0
Snowshoe hare, red squirrel, and other small terrestrial mammals	Snowshoe hare and red squirrel were noted as country foods, and snowshoe hare act as the main and limiting prey species for lynx and are an important prey item for other carnivores. In Tier 1 studies, Zoetica proposes to focus on determining the presence, distribution, and abundance of suitable habitat for snowshoe hare and northern flying squirrel through ecosystem mapping within biodiversity value-specific study areas. Snowshoe hare are included due to their keystone role as a primary prey species for many carnivores in the boreal forest. Northern flying squirrel are included due to their role as keystone species that disseminate the spores of ectomycorrhizal fungi that associate with roots of pine trees and helps them obtain water and nutrients, and which are also important prey species for a variety of vertebrate predators. Future years may focus on population-level studies of local snowshoe hare populations and potentially other small terrestrial mammals and/or	Section 2.1 Section 2.2 Section 3.0

	key supporting habitat features (e.g., squirrel middens, mast trees) for those species of cultural and/or conservation importance.	
Beaver, muskrat, and other semi-aquatic mammals	Beaver dams have been brought up as a concern for the protection of local resources. Also, it was mentioned that at least one person in the community eats beaver and muskrat meat. In Tier 1 studies, Zoetica proposes to focus on determining 1) the presence of semi-aquatic mammal species through eDNA studies, and 2) the presence, distribution, and abundance of SWH for semi-aquatic mammals (i.e., den sites for mink, otter). Future years of study may focus on determining the numbers and locations of beavers in a biodiversity value-specific study area and their role in sustaining ecosystem services, and possibly additional semi-aquatic mammal species, where warranted.	Section 2.1 Section 2.2 Section 2.3 Section 2.5 Section 3.0
Ducks, geese, and upland game birds like grouse, raptors (e.g., owl)	During engagement, community members mentioned that several bird species were hunted. In Tier 1 studies, Zoetica proposes to focus on determining the presence, distribution, and abundance of birds, SWH features, and suitable habitat for eastern whip-poor-will (a SAR known to be present in the avian (bird) RSA; RSA_{AVI}) within a biodiversity value-specific study area. Future years of study may focus on ground-truthing potential SWH and conducting surveys for seasonal presence/not detected of Upland Breeding Birds, Upland Game Birds, Waterbirds, Shorebirds, and Raptors.	Section 2.1 Section 2.2 Section 3.0
Garter snakes, tree frogs, turtles, and other herpetofauna	During community workshops it was mentioned that garter snakes are abundant and should be considered for analysis (Appendix B.2, CanNorth 2020). In addition, studies for frogs and turtles were mentioned for consideration in the baseline program (Appendix B.1, CanNorth 2020). Tier 1 studies will focus on determining the presence, distribution, and abundance of SWH for herpetofauna in the biodiversity value-specific study area. Future studies may focus on ground-truthing potential SWH and conducting presence/not detected surveys for herpetofauna, with the potential for population surveys for species of importance that are detected and could be impacted by the APM Project.	Section 2.1 Section 2.2 Section 2.5 Section 3.0
Earthworms, bees, earwigs, wood ticks, spruce budworm, grasshoppers, and other terrestrial invertebrates	Concerns related to wood ticks, increases or decreases of several insects, and invasive species were brought up as concerns during community workshops (Appendix B.1 and B.2, CanNorth 2020). Further engagement conducted in May 2020 identified the main concern related to wood ticks as being a human health issue while acknowledging that there has been an increase in wood ticks with an increase in deer populations in the area. Thus, for the BIS, wood ticks will be considered as a current stressor on ungulate health, but focused studies on wood tick will not be conducted. In Tier 1, Zoetica proposes to conduct studies focusing on 1) determining the presence of SWH for monarch butterflies, and 2) recording incidental observations of target species during ecosystem mapping. Future studies may focus on 1) general community surveys and eDNA metabarcoding studies for terrestrial invertebrates, and 2) targeted surveys for at-risk or rare species if species or habitat are observed in Tier 1 studies.	Section 2.1 Section 2.2 Section 3.0

Walleye, lake trout, pike, sucker, perch, baitfish	Overfishing was raised as a concern during workshops conducted to gather community feedback (Appendix B.2, CanNorth 2020). Community input included good information on fish presence in lakes and identified fishing activity in the area surrounding Ignace (CanNorth 2020). Fish was identified as an important food source for local Indigenous communities (Appendix B.1 and B.2, CanNorth 2020). Additional engagement conducted in April and May 2020 identified several popular fishing areas where fishing could potentially increase in the future. Further, baitfish were identified as an important biodiversity value for recreational and commercial fishing, and participants mentioned that there may be bait blocks in the general area. Zoetica proposes that Tier 1 studies focus on determining the presence, distribution, and abundance of aquatic habitat types for fish in the biodiversity value-specific local study area (LSA) as well as the potential presence of various fish species (species composition) in various habitats within the LSA using eDNA analysis. Future studies may focus on determining fish community composition and demographics of fish populations and their habitat.	Section 2.3 Section 2.5 Section 3.0
Aquatic food web (e.g., benthic invertebrates and other primary and secondary producers)	Understanding the importance of food chains was brought up during workshops with community members (Appendix B.1, CanNorth 2020). In Tier 1 studies, Zoetica proposes a focus on determining the species composition (e.g., species presence) of aquatic primary and secondary producers in various waterbody types within the LSA_{AQU} through eDNA methodologies. Future studies may focus on additional community measures such as diversity indices which over time can, itself, be used as a proxy for determining project impacts on biodiversity), relative abundance of species, and measures of fish growth and health.	Section 2.5 Section 3.0

1.4.1 Future Planned Community Engagement for Biodiversity Impact Studies

Engagement with the communities surrounding the Revell Batholith Area has already begun (see Section 1.4 and **Table 1-1**, Section 1.5.1, and Section 1.5.2) and feedback has been interwoven into the early planning phase of the BIS. As noted, engagement will continue as an ongoing process throughout the BIS. Annual results of the biodiversity baseline reports and annual updates to the biodiversity IA will be presented to stakeholders and rights-holders in the area, the information from which may reveal concerns or opportunities for additional feedback from these groups. Such two-way information exchange will allow for the regular and structured refinement of the BPD Report. Feedback from communities will be sought during these refinement stages and will provide continued transparency of the BIS Program, and to maintain accountability of the program to local communities, rights-holders, scientific experts, the regulator and other federal and provincial agencies, and other interested parties. Ongoing engagement will ensure that community values and concerns, as they relate to biodiversity values, are accounted for in the development of the BIS Program.

1.4.2 Future Stakeholder/Rights-holder Involvement

Opportunities for community involvement in the collection of baseline data will be sought, identified, and interwoven, wherever possible, into the biodiversity baseline program. Any opportunities for stakeholder

and rights-holder involvement in baseline work will be documented and updated in the present living BPD Report. Community involvement may include, but is not limited to, the following:

- Designing and leading ceremony and Spiritual components of the BIS Program (focus on ceremony and Spirit);
- Providing cultural training or cultural protocols to field staff who will be involved in the baseline data collection;
- Providing guidance or local field knowledge including accessibility to sites, field logistics, and areas of importance;
- Working with field biologists to collect data in the field (where studies require trained biologists and consultants to lead the work);
- Pairing Indigenous youth and Elders with field biologists to encourage knowledge transfer and interweaving of traditional knowledge and western science; and,
- Being trained to collect baseline data independently of biologists, wherever possible (e.g., wildlife camera data collection).

1.5 Previous Biodiversity and Related Baseline Work for APM Project

The biodiversity baseline program planned by Zoetica seeks to build on environmental data available in the Revell Batholith Area, wherever possible. These datasets include past work done and information gathered for the APM Project by the NWMO with support from contractors who are experts in their field, Big Unobstructed Databases, and government sources. Previous work conducted on a more localized scale than available in Big Unobstructed Databases is outlined in Sections 1.5.1 to 1.5.5 below, the proposed EMBP studies (some of which will be conducted in collaboration with the BIS) are outlined in Section 1.5.6, and identification of gaps in data availability and data quality or extent for use in the BIS Program is presented in Section 1.5.7.

1.5.1 Phase 1: Desktop Studies and Engagement

The NWMO, supported by contractors who are experts in their field, worked with the community liaison committee and other community members to assemble information about the environmental, social, economic, and cultural conditions of the community, as well as objectives, issues and concerns. This information helped to provide a starting point for discussion with the community to explore the potential effects of the project on the community and the opportunities to achieve community objectives through the project. This work set the stage for subsequent deeper engagement and research on environment.

Phase 1 desktop studies and engagement were completed with the goal of gaining an understanding of the different potential APM Project areas, including the Revell Batholith Area, its surrounding environment, and the connections of people to the area. Assessments were conducted to determine whether environmental surroundings were sufficient for hosting a DGR and supporting infrastructure, and how to best cooperate with local communities throughout assessment and implementation of the entire APM Project and beyond.

Phase 1 desktop studies and engagement provided a foundation of information on land use, protected areas, heritage sites, topography, geological characteristics, sediment quality, terrain, groundwater quality, watershed boundaries, recreational and commercial fisheries, air quality, meteorology, and species of concern in the area, along with incidental reports on species present and pre-existing environmental conditions. During Phase 1 studies, scientific and technical studies investigated the

geological suitability of the area for hosting a DGR. Desk-based data were also compiled from existing data sources, including the Ontario Ministry of Natural Resources and Forestry (MNRF) and the Natural Heritage Information Centre (NHIC). Additional field data were gathered by Tulloch Engineering (Tulloch Engineering 2018a) on Ecological Land Classification (ELC), Significant Wildlife Habitat (SWH) features, stream reach classifications, and potential presence of SAR to produce environmental sensitivity maps.

1.5.2 Phase 2: Environmental Field Studies and Engagement

Phase 2 field studies and engagement further captured the suitability of potential repository areas for a DGR, and sought to reflect the input and interests of local stakeholders and rights-holders (Tulloch Engineering 2018b, 2018a, 2019a, 2019b). Field studies and further engagement activities were initiated in 2016 in potentially suitable sites, including the Revell Batholith Area. The environmental studies conducted within the Revell Batholith Area during this phase were focused on a limited spatial area that included three borehole sites and four potential access roads. Data were collected using general reconnaissance, trail cameras, song meters, night acoustic surveys, and select ground-truthing of terrestrial vegetation mapping data. Desk-based reviews (Tulloch Engineering 2018b) identified candidate SWH. Ground-based surveys rendered the identification of no SWH within a subset of these candidate SWH identifications within the 2018 Study Area (Tulloch Engineering 2018a). Non-significant moose aquatic feeding areas (MAFAs; Classes 1 and 2) were identified along an un-named tributary to Mennin Lake that parallels Trapline Road. Surface water, sediment, and soil were sampled as well. During these Phase 2 environmental field studies, potentially occurring SAR were determined (based on range maps and habitat conditions), and some presence/not detected studies for SAR were conducted within small areas (e.g., eastern whip-poor-will song recorded associated with boreholes). Phase 2 studies also allowed for the opportunistic identification of local flora, rare vegetation, amphibians, migratory birds, fish, and mammals, and the collection and analyses of environmental quality parameters. Engagement and discussion activities were carried out to better understand APM Project benefits, recognize areas where cooperation was possible and where concerns exist, and where mitigation may be required for potential negative effects of the APM Project.

Engagement sessions were conducted in 2018 and 2019 with stakeholder and rights-holder groups to solicit feedback regarding what is important to local communities and stakeholders in terms of the environment, what existing impacts are perceived, and what values are important for baseline monitoring. A summary of stakeholder and rights-holder concerns that were raised are presented in Appendix B.1 and B.2 of Canada North Environmental Services' ('CanNorth') EMBP Design Report (CanNorth 2020); those with particular relevance to the development of the BIS Program are summarized in Section 1.4 and **Table 1-1** of the BPPA Report.

1.5.3 Terrestrial Ecosystem Mapping

A preliminary natural features map was created by compiling external data sources during previous Phase 2 studies (Tulloch Engineering 2018b), which included ecosite classifications, rare vegetation communities, MAFAs, NHIC element occurrences, and sustainable forest licensee forestry data. Data were sourced from Land Information Ontario (LIO), Natural Resources Canada, Dryden District of the MNRF, NHIC, Ontario Forest Resource Inventory (FRI), and forestry information, and information was cross-referenced to create initial maps for ground-truthing. Select areas were then assessed in the field against the preliminary data gathered and identified knowledge gaps were investigated. The scope was further narrowed to three borehole sites and proposed access road routes where sensitivity mapping and environmental characterization were conducted (Tulloch Engineering 2019a). The scale of these data

compilations and mapping represents a small fraction of what is proposed for the scope of the BIS Program.

1.5.4 Aquatic Habitat Mapping

A desktop assessment was performed in 2016 in several study areas surrounding Ignace, including the Revell Batholith Area, to categorize streams according to the types of fish habitat they are likely to support (Tulloch Engineering 2018b, 2018a). Information for aquatic habitat mapping was acquired from LIO, ecosite information from the MNRF, and topographic information from GeoGratis. Streams were classified into eight categories including five wetland type "W" categories including: marsh (Wm), fen (Wf), bog (Wb), and swamp (Ws), as well as unclassified wetlands (Wu), where wetland type was unknown. Strahler stream order (Strahler 1957) was used for streams that did not pass through wetlands with headwater streams classified as Strahler orders 1 (H1) and 2 (H2). Higher order streams were classified as "O", or "U" if Strahler order was unknown. Waterbodies were not classified; however, where information was available, these waterbodies were denoted by thermal regime (cold, cool, and warm) from data acquired from the LIO database.

Field studies were conducted to ground-truth mapped aquatic habitat data (Tulloch Engineering 2018b). During field studies, biologists measured the following field habitat metrics: aquatic habitat type; water chemistry (temperature, dissolved oxygen, conductivity, pH); substrate (stream bottom) composition; stream channel morphology; habitat suitability for use by fish; vegetation types along the stream edge; presence of groundwater seeps; and confirmation of reach breaks identified during desktop mapping. Field observations were compared to desktop mapping of stream reach classifications and wetland evaluations to determine confidence in mapping. Results of field verification generally agreed with desktop mapping and the ecological importance of observed differences was determined to be minimal. The reclassifications were primarily due to the inability to differentiate between similar wetland types from aerial imagery, natural progression (changes) of wetland types over time, seasonal changes in water flow, and changes in habitat due to beaver activity. Some of these classified streams can be utilized in the BIS Program; however, stream classification for the proposed BIS will require classification over a much wider area.

1.5.5 Additional Wildlife and Fisheries Studies

Focused field studies for fish and wildlife were conducted in key areas within the AOI surrounding the borehole locations, the affiliated roads, and a buffer zone surrounding them (Tulloch Engineering 2018a, 2019b). Fish and wildlife data were collected through general reconnaissance, ELC studies, fisheries assessments, trail camera deployment, song meter and acoustic surveys, rare plant surveys, and recorded incidental observations. Field efforts focused on detecting SAR (species of conservation concern identified federally by COSEWIC and provincially by COSSARO), presence of migratory birds, and fish and fish habitat information. Surveys were conducted in several areas including Basket Lake, Indian Lake West, and Indian Lake East, as well as in some locations within the local and regional study areas identified for the BIS (see Section 1.8). Useful data on biodiversity values were identified through fish community surveys, MAFA assessments, habitat assessments for protected species, and incidental observations made during these studies.

1.5.6 Environmental Media Baseline Program Design

The APM Project EMBP has recently been designed by CanNorth to characterize the environmental media baseline conditions prior to commencement of the APM Project (CanNorth 2019a, 2019b, 2020). Environmental components that will be studied in the EMBP include:

- Tissue samples
- Hydrology
- Surface water parameters
- Air quality, noise, and light
- Shallow groundwater
- Soil quality

These environmental components (excluding tissue samples) collectively represent the abiotic environment on which living organisms depend. Any changes to the abiotic environment can have an impact on organisms that depend on environmental conditions to be maintained within a certain range of variation around their current state. Many potential impacts on biodiversity values selected for inclusion in the baseline study will occur through the alteration of the abiotic environment. For example, altering shallow groundwater levels could potentially reduce water levels within aquatic environments and alter vegetation, fish, and wildlife communities. While the abiotic environment has an impact on the biotic environment, the inverse is also true. For example, removal of vegetation can alter soil properties and increase the risk of slope failure on steep slopes, impacting habitat for other organisms and ecosystem services.

The inherent feedback between the biotic and abiotic environment will require collaboration between the BIS baseline program and the EMBP to ensure data are collected in an efficient manner that benefits both programs. Early communications (which have already begun through semi-monthly meetings) ensure all collected data can be efficiently utilized by both programs (e.g., benthic invertebrate and fish collection), as well as find potential efficiencies to reduce duplication of effort (e.g., for the proposed eDNA program).

1.5.7 Data Gaps

While previous field data and related programs provide a starting point for determining baseline study components that are likely to be important for the BIS Program, data collected to date have been collected with different scopes and objectives. Many past field studies were conducted within small, discrete spatial boundaries, such as within and directly surrounding borehole locations, rather than study areas of relevance for biodiversity studies. Larger scale studies are needed to first support community decision-making on willingness, and then an IA, if the community is willing to proceed to this stage. Therefore, data gaps exist that need to be filled via desk-based and field-based surveys within the greater area of relevance for the BIS Program (see Section 1.8). Big Unobstructed Databases, as well as government data sources, will continue to be explored by Zoetica to fill data gaps (see Table D-1 in Appendix D of the BPPA Report). However, it appears that many of the Big Unobstructed Databases contain data collected at spatial scales that are too coarse and/or using non-systematic methods, which limit their uses for filling gaps. Government data (see Table D-2 in Appendix D of the BPPA Report) include useful forest cover information from the MNRF that can be used as a starting point to begin planning surveys for the BIS Program; and data from the NHIC may include previous observations of SAR or species of importance to local stakeholders/rights-holders within or near proposed study areas, which can enable known species

in the area to be considered in baseline design planning. However, government data appear to be patchy in coverage and at a coarser spatial scale than needed for the BIS Program (see Section 2.1.2.3.1).

1.6 Biodiversity Values

Good practices for developing biodiversity studies include carefully defining the scope, i.e., the biodiversity values that will be included in the baseline study and potentially carried through the IA process as VCs. A well-thought-out baseline program will ensure due diligence can be demonstrated, will prevent wasting resources by collecting unnecessary information, will enable positive outcomes, and will reduce the risks to the APM Project cost, schedule, and/or performance. Ultimately, the BIS will need to meet the requirements to be laid out in the TISG for the APM Project, which will be written by the IAAC in discussion with the CNSC. Additional concerns of rights-holders and stakeholders may necessitate that supplemental studies be undertaken by the NWMO to facilitate public confidence in the project.

Scoping of biodiversity values to include in baseline studies is one of the earliest steps of the BIS, and is detailed in Zoetica's BPPA Report, Section 3.1, where the inclusion of various biodiversity components is rationalized. Biodiversity values selected through early scoping practices include those identified through community engagement processes, in consideration of regulatory requirements (e.g., listed species, protected areas, habitat features that must be avoided), and the inclusion of other potentially occurring species to i) allow for their potential use as indicator species, where needed, and ii) ensure that species that may become listed under the Ontario *Endangered Species Act*, or that may undergo a range shift into the APM Project area due to climate change in the reasonably foreseeable future, are accounted for. The biodiversity values recommended for inclusion in the BIS are presented within Zoetica's BPPA Report, and include:

- Vegetation
- Ungulates
- Carnivores
- Small Terrestrial Mammals
- Semi-Aquatic Mammals
- Bats
- Birds
- Herpetofauna
- Terrestrial Invertebrates
- Fish and Fish Habitat
- Primary and Secondary Aquatic Producers
- Ecosystem Services

1.7 Potential Project Interactions

A preliminary project component and biodiversity interaction matrix was included in Zoetica's BPPA Report, copied below as **Table 1-2**. This matrix was based on the proposed biodiversity values to include in the BIS and available information (with assumptions) about APM Project components, to date. Potential cumulative effects were also included in the BPPA Report, and details can be viewed in the BPPA Report, Sections 3.2.1.3 and 3.2.2.3.

Table 1-2. Preliminary APM Project component and biodiversity interaction matrix based on proposed biodiversity values to include in the APM Project BIS (to be refined during the VC scoping process) and available information (with assumptions) about APM Project components to date. A third dimension can be added to this scoping table that identifies indirect project impacts transmitted through other intermediary disciplines (e.g., physical sciences, traditional and local land use).

	Road Traffic	Linear Infrastructure	Vegetation Clearing	Construction Noise & Vibration	Communication Tower(s)	On-site Waste Management Facilities	Air Emissions /Dust	Water Quality	Water levels	Hunting/ Gathering/ Fishing Increase
Vegetation		x	x				x	x	x	x
Ungulates	x	x	x	x			x	x		x
Carnivores	x	x	x	x		x	x	x		x
Small Terrestrial Mammals	x	x	x	x		x	x	x		x
Semi-Aquatic Mammals	x	x (near water)	x	x			x	x	x	x
Bats	x	x	x	x	x		x	x		
Birds	x	x	x	x	x	x	x	x	x	
Herpetofauna	x	x	x	x		x	x	x	x	
Terrestrial Invertebrates	x	x	x				x	x	x	
Fish and Fish Habitat		x (water crossings)	x	x			x	x	x	x
Primary and Secondary Aquatic Producers		x (water crossings)					x	x	x	
Ecosystem Services*		x	x				x	x	x	x

* Cultural ecosystem services, such as those associated with spirit, enjoyment, and visual beauty of the landscape, are not addressed in the BIS but will be part of the APM Project's health and social impact assessment.

1.8 Study Areas

Study areas need to be established early in the development of baseline studies to first support community decision-making on willingness, then to support an IA (Gullison *et al.* 2015), if the community is willing to proceed to this stage. Identifying the spatial scale(s) for which to study biodiversity values is critical for ensuring that adequate information is collected to support the IA without the collection of erroneous information (Ross *et al.* 2006). Good practice suggests that study areas should encompass the extent of anticipated APM Project activities and impacts, and a consideration of the distribution of biodiversity values across the landscape (Gullison *et al.* 2015). Study areas should also be large enough to capture any potential cumulative effects that may occur in the region within the ranges of VCs present (IFC 2013). Zoetica began the process of defining a study area by mapping the AOI as defined by the NWMO, which represents the area within which the APM Project footprint area will be placed. The AOI was designed to include the area necessary to maintain design flexibility into and beyond the IA stage. Local and/or Regional Study Areas (LSA and RSA, respectively) for each biodiversity value were defined in Zoetica's BPPA Report, Section 5.2. Some of these maps are included again for the relevant baseline studies to be undertaken in the initial Tier 1 studies described in this BPD Report.

1.9 Statistical Considerations for Sampling Design

1.9.1 Site Selection

Sections 3.2 and 5.2 of the BPPA Report describe the potential project-related and cumulative effects that were considered when planning baseline field work. These effects, in turn, were considered when selecting survey sites and defining the spatial extent of survey effort focusing on each biodiversity value. Control sites in areas beyond the anticipated spatial extent of potential project-related effects will also be selected for each biodiversity value, as appropriate, to support a Before-After-Control-Impact (BACI) study design, as described in the BPPA Report, Section 3.2.3. The use of proper control sites will enable the disentanglement of environmental changes unrelated to the APM Project(e.g., natural sources of regional variation such as weather or climate change) from project-related impacts.

In developing baseline data collection maps for the BIS, considerations were made for locating data collection sites, such that there is proportional representation within and around areas that are currently impacted, or relatively pristine. It is important that background information gathered and data collection sites selected support the understanding of a dynamic system that has changed through the past and will continue to change into the future. The current baseline conditions represent the state of the current environment, which is the amalgamation of natural and anthropogenic changes, cycles, and processes that have occurred and changed from time immemorial until the present, and which will continue to change into the future. Therefore, it is important to collect data in both relatively disturbed (e.g., by roads, forestry roads, forest fires, forestry practices) and relatively pristine ecosystems (e.g., areas not in forestry rotation, areas not impacted by roads, relatively inaccessible areas) in proportion to their availability in each study area, which better allows for predictions about changes when those proportions begin to change, and to consider various scenarios. The consideration of currently impacted and non-impacted sites, and how both will be captured in the baseline study design, along with how the impacts of future projects will be considered, are discussed Section 7.0, Table 7-2 in the BPPA Report.

Biodiversity baseline field programs are optimized by determining survey locations in advance, based on a solid foundation of ecosystem maps. Zoetica does not recommend arriving on site and deciding study locations fully *in media res*, as biases will inevitably occur. Although more logistically difficult, care should

be taken not to bias the selection of study sites (e.g., based on access alone). Zoetica generally supports using a stratified random approach to allocate sampling effort (Gullison *et al.* 2015) using the results from ecosystem mapping done in Tier 1 data collection (described in Section 2.1). Stratified random selection of sampling locations and control sites, including replacement sites that can be visited should a random site not be accessible (e.g., black bear present), can be realized using a Generalized Random Tessellation Stratified (GRTS) (Stevens and Olsen 1999, 2004), which can be performed using the 'spsurvey' package in R (R Core Team 2016). The GRTS study design is further described in Zoetica's BPPA Report, Section 4.3.1.

Decisions about model selection and the use of data following data collection will likely need to be refined over time, in discussion with modeling statisticians (e.g., Dr. Carl Schwarz, Professor Emeritus of Actuarial Sciences, Simon Fraser University), and alongside a growing exploration of available data, and data that can be collected during baseline surveys (with considerations of sample sizes, data normality, equality of variance, and other factors that determine the types of statistics that are valid). However, using a proper GRTS study design provides promise that biodiversity data can be analyzed using Species Distribution Models (SDM); Stacked Species Distribution Models (SSDM), Joint Species Distribution Models (JSDM), and Ordination-based Models (ORD), which are all available to accomplish the task of linking species to environmental covariates, while open source climate data are available for interpolation to local site conditions, and which can be calibrated based on local climate variables. Data collected at the Atikokan meteorological station (associated with the Township of Ignace) between 1971-2000 are also summarized in Golder Associates (2013), which included an analysis of monthly daily averages for mean, minimum, maximum, extreme maximum and extreme minimum temperatures; monthly average rainfall or snowfall, including extreme daily rainfall and snowfall amounts; and average seasonal wind speeds and direction. Golder Associates recently completed a study for the NWMO to assess climate change impacts on the probable maximum precipitation (PMP) and intensity-duration-frequency (IDF) precipitation amounts for the Ignace study area (Schardong *et al.* 2020); this report summarizes the analyses of current and future (2050s and 2080s) climate on PMP and IDF estimates and provides a qualitative climate assessment beyond 2100.

1.9.2 Power Analyses

Data collected in Tier 1 should ideally be sufficiently powerful to demonstrate whether or not an effect has occurred, with a reasonable level of certainty. Statistically valid baseline data collection will be defined as achieving a theoretical/aspirational benchmark of a "good" statistical power of 0.8-0.9 (Jones *et al.* 2003), assuming that the goal is to detect a small to medium (d(0.2)-d(0.5)) effect size change due to the APM Project (Cohen 1990, Sawilowsky 2009) over various time frames of relevance (e.g., the program should be able to detect a large effect size (major impact) over a short time frame (e.g., 1-2 years) and more subtle and incremental, smaller impacts (medium to small effect sizes) over longer periods of time (e.g., 3-10 years)), and that baseline data will be used in BACI or gradient BACI designs (Smith 2002). In some cases, such as where species are in very low abundance, it may not be feasible to collect a sufficiently high sample size to achieve this goal; however, other considerations will be made to select study organisms for the purposes of statistical analyses to reduce the probability of this issue. If data cannot be generated to achieve this power within the project timeline, a more conservative threshold (e.g., higher alpha value) for detecting statistical change can be adopted, such that early adaptive management can be employed in response to a potential adverse effect.

Statistical methods for field survey design (*A Priori* Power Analyses), testing sampling effort (*In Media Res* Analyses), sampling intensity (*Post Hoc* Analyses), and statistical goals are reviewed in the BPPA Report, Section 4.3.2. The use of the general approaches outlined in the BPPA Report will ensure that the baseline program is neither under- nor over-sampled and proceeds in a scientifically valid and efficient way. More detailed decisions on statistical tests and models to use for baseline data analyses will be determined at a later date, as many tests require an initial examination of the structure of the data before optimal decisions can be made.

1.10 Data Management

1.10.1 Data Management and Transfer from Field Data Collection Contractor

The field data collection contractor will be expected to create their own Project Quality Plan (PQP) to meet the needs and standards of the NWMO. The field data collection contractor will also be expected to submit their data to the NWMO directly; data transmittal from the field data collection contractor to the NWMO will include their datasets, a transmittal letter, and a metadata file, examples of which will be provided by the NWMO through their instructional guidance form NWMO-INS-01390-0201. Currently, the NWMO uses a DAP data management program, and data will undergo a check by the NWMO on receipt. Data would then need to be requested by Zoetica (e.g., for use in analysis and reporting) from the NWMO, and the DAP-associated protocols for data transfer from the NWMO to Zoetica would be followed, including a transmittal letter, datasets, and metadata file to be included with the transfer in accordance to NWMO-INS-01390-0201.

The NWMO is in the process of procuring a more automated environmental data management system (EDMS). When that system has been procured and is in place, digitized data forms will be created by the NWMO based on templates of data collection forms supplied by Zoetica. Data that are filled out using digital forms will automatically be sent to the NWMO, and Zoetica will obtain automated emails noting when new, quality-checked data are available. Data will then be downloadable by Zoetica. Zoetica will not conduct an additional QA/QC check on data that have come from the field data collection contractor; it will be expected that data will have already undergone a rigorous QA/QC check and will be useable on delivery. However, if any obvious errors are noted by Zoetica, they will be reported to the NWMO for feedback through their compliance management protocols.

To adhere to the proposed baseline reporting schedule, the field data collection contractor will be required to submit their final field data to the NWMO, following the NWMO data transfer protocols, within a maximum of two weeks of completing field work, such that the NWMO can process the data and enter it into their own data management system, and send the data to Zoetica within 8 days of receiving it. Once data are received from the NWMO, Zoetica will follow NWMO data management protocols and verify that the data received match what is documented on the *Data Clearance Form*. Once the data are verified, a signed version of the *Data Clearance Form* is stored on ZoeticaNet in the data clearance form category as a permanent record and backup, which the NWMO can access as needed.

1.10.1 Data Management and Transfer from Zoetica

For data, reports, and other deliverables generated by Zoetica (e.g., maps, reports, analyzed data), data management will follow Zoetica's internal data management procedures, which are detailed in the Biodiversity Impact Studies PQP. Briefly, prior to supplying data to the NWMO, all files will be uploaded

to ZoeticaNet. New versions or iterations of documents are 'stacked' through ZoeticaNet as they are edited, such that each iterative change is preserved, in case a previous version needs to be restored or revisited. When files are uploaded to ZoeticaNet, the privacy for the file can be selected that will control who is able to see and download the file. Therefore, sensitive or confidential data can be stored on ZoeticaNet with restricted access such that we comply with the terms of data sharing agreements associated with various data sources. ZoeticaNet currently has a 100 GB capacity and can be expanded as needed; we also possess a dedicated GIS desktop computer and several multi-terabyte external hard drives for backup storage, which are stored securely at the Zoetica office. All deliverables to the NWMO (e.g., report, maps, shapefiles) are uploaded to ZoeticaNet as a permanent record and backup that the NWMO can access as needed. Data transferred from Zoetica to the NWMO will follow NWMO protocols. If the DAP system is still in place, the data transfer will follow NWMO-INS-01390-0201, including datasets, a transmittal letter, and metadata files. NWMO will provide the DAP Metadata Editor to Zoetica to create metadata for all data deliverables, including any data acquired directly by Zoetica from a third party or open data source. Once the EDMS system is procured and operational, data transfer from Zoetica to the NWMO will follow accompanying procedures to be developed and disseminated by the NWMO. The NWMO is currently exploring data management software for statistical code (e.g., GitHub), and Zoetica will submit their statistical code to the platform ultimately adopted and using associated data transfer protocols (to be developed).

All acquired data will be stored in two locations in its original form. First it is stored in the 'raw data' folder within the project-specific folder on the GIS desktop. It is then copied to the project-specific folder on the data storage designated hard drive. All external storage devices are stored in a locked area within the Zoetica office. If file size allows, a third copy can be saved to ZoeticaNet. In cases where file sizes are excessively large, such as what can be the case for orthoimagery and drone-generated visual files, additional external storage devices will be used to back up materials, and they will be stored in a locked area within the Zoetica office. Outputs from the data or edited versions are saved in separate folders to ensure original copies of all data are maintained. Any data that are considered sensitive (e.g., NHIC occurrence data) will be stored in separate 'sensitive raw data' folders so that data sensitivity is known when accessed. All products from sensitive data (e.g., shapefiles, Excel outputs, maps) that are uploaded to ZoeticaNet will have the necessary permissions in place so only authorized personnel can access them, and will have a clear label that it contains sensitive data and is not to be shared outside Zoetica, NWMO, or those with explicit permission to view.

All datasets that are acquired in anticipation of use in the BIS will have their source documented in the *Data Source Master List* which will be saved on the GIS desktop and a copy saved to ZoeticaNet. Data can be obtained from open sources online (e.g., Ontario GeoHub) or issued from an organization (e.g., MNRF). All data from open online sources will document the details of the download including the dataset name, source, data format, folder path name, date of download, most recent date the data were updated (if available), the source URL, and any applicable notes. All data issued from organizations will document the details of the dataset name, date the data were issued, most recent date the data were updated (if available), source organization, contact details for the organization personnel who issued the data (if applicable), folder path name, any use restrictions, data sensitivity or licensing information, and any applicable notes. Any data that are considered sensitive will have the line of information highlighted to ensure that sensitive data are obvious at a glance.

1.11 QA/QC

All baseline data collection will follow the appropriate Standard Operating Procedures (SOPs; see **Appendix A**) and QA/QC protocols provided, along with the internal PQP produced by the field data collection contractor to satisfy NWMO quality standard requirements. This section focuses on the general QA/QC procedures that will apply to the overall BIS baseline program. For Tier 1 studies, Zoetica anticipates that only eDNA studies will have method-specific as well as data-specific QA/QC protocols, which are described in Sections 2.5.3.4 and 2.5.3.6.

1.11.1 Quality Assurance (QA)

The QA procedures herein apply only to procedures undertaken by Zoetica prior to submission of data to the NWMO. Separate QA measures will be developed by the field data collection contractor as part of their PQP development prior to undertaking field work.

Quality assurance protocols are a set of activities for ensuring quality in processes; QA involves prevention of quality issues through planned and systematic activities, including documentation. For the BIS baseline studies, measures for QA include:

- Application of best practices, guidelines, and standards for studying biodiversity values, with consideration of community input, the regulatory environment, and data requirements for the APM Project IA.
- Baseline studies will be designed to collect sufficient sample sizes (e.g., including appropriate samples per unit area/habitat grouping, replicates, spatial and temporal coverage) to enable detection of potential future project effects.
- Production of Zoetica's draft BPPA Report and BPD Report, which provides documentation of research and steps taken to arrive at endorsed best practices and preferred approaches. The BPPA Report will be reviewed by the NWMO's APM Environmental Review Group (the ERG), which is comprised of IA and biodiversity experts and includes Indigenous representation.
- Additional community engagement (see Section 1.4.1) to ensure that local stakeholder and rights-holder input and concerns are integrated into the baseline study design.
- Development of baseline study design and future analyses and reporting will be completed by qualified, experienced, and knowledgeable staff, with senior review by registered professional biologists (R.P. Bio. designation in British Columbia).
- Expert subcontractors or specialized services (e.g., statisticians, air photo interpretation) may be retained, as needed and with the approval of the NWMO, to ensure that study design and future analyses are completed to a high standard of quality.
- Verification processes for data inputs and outputs, including checklists and documentation requirements.
- Recommendations for field data collection contractor qualifications and experience to assist with the NWMO's procurement process.
- Provision of sufficiently detailed SOPs, data forms, field maps, and other instruction (e.g., training by Zoetica staff) to the field data collection contractor and local field assistants prior to deployment.
- Requirements for redundancy in key field navigation gear, critical information (e.g., SOPs, data forms), and collected data (i.e., hard copies and electronic backups) to prevent loss.

- Increased training and oversight (e.g., quality checks by team leads) at the beginning of the field program to ensure that protocols are understood and being followed, and that modifications or corrective actions can be made early on, if needed, to ensure that the field program is completed to a high standard of quality.
- Regular reporting/communication schedule between the field data collection contractor, Zoetica, CanNorth, and the NWMO to track progress and ensure that any logistical problems be addressed in a timely manner.
- Regular data transfer schedule (rather than a "data dump" at the end of the field season) to enable periodic quality checks and as an extra backup, and identification of opportunities for continuous learning.
- Inclusion of contingency sampling/survey sites in survey plans, to ensure that if access is prohibitive (extremely difficult to impossible access) justified and ordered replacement sites are available.

The contracted services (e.g., field data collection contractor, laboratory services) will have or will develop their own project-specific QA/QC protocols (i.e., PQP) for equipment calibration, verification, and preventative maintenance; data entry and management, including record keeping; specific laboratory procedures and analyses (e.g., replication, controls, acceptance thresholds, storage conditions); good practices for cleaning and safety procedures; and other relevant tasks in their regular workflow; as well as the competency of staff completing the work. It is also expected that sample data being handled by contracted services will meet the data quality standards of the NWMO prior to being delivered to Zoetica for analysis.

1.11.2 Quality Control (QC)

The QC procedures herein apply only to procedures undertaken by Zoetica prior to submission of data to the NWMO. Separate QA measures will be developed by the field data collection contractor as part of their PQP development prior to undertaking field work. Quality control refers to a set of activities for identifying errors and ensuring quality in deliverables; QC involves activities or techniques used to achieve and maintain the product quality, process, and service. For the APM Project BIS baseline studies, measures for QC include:

- Review of data and verification documents produced by the field data collection contractor.
- Verification of data completeness prior to conducting statistical/data analyses or developing mapping products (as part of study design as well as for the baseline report). It is expected that verification of data quality will be part of the QC protocols of the field data collection contractor.
- Documentation of all data assumptions, limitations, and manipulations (i.e., metadata), as well as any recommendations to improve upon uncertainties.
- Verification and version tracking of software used in analyses (e.g., GIS tools, statistical programs) as described in the *Project Quality Plan* developed for the BIS.
- Internal peer review of baseline results and reporting (including mapped data) by qualified and experienced professionals to ensure that summarized information is accurate and that data interpretation is sound, scientifically valid, and defensible.
- Presentation of all raw data, calculations, and statistical steps as appendices in the baseline report to ensure transparency and allow for reproducibility of results.
- Inclusion of any statistical script used (e.g., R-script) in analyses within baseline report appendices.

- Document control and revision history tracking tables for reporting deliverables; project management through ZoeticaNet system.
- Regular communication within Zoetica and between Zoetica, subcontractors, field data collection contractor, the NWMO, and other technical programs, to discuss any quality issues identified and to propose solutions.
- Monthly summaries of work will continue to be provided to the NWMO for review throughout the baseline program, including discussions of any non-conformities affecting quality and corrective actions taken.
- Review of Year 1 of the BIS baseline program to assess the progress of filling data gaps and meeting data objectives, and to find opportunities for quality improvement and inform study design changes, if needed, for the next year.

2.0 TIER 1 STUDIES TO SUPPORT ALL BIODIVERSITY VALUES

This section presents the plans and details for each Tier 1 study planned for the BIS. Each study is designed to collect key information which will be required to plan or select Tier 2 studies. See Section 1.3 for details on the tiered approach. All Tier 1 studies will begin with desk-based work as part of pre-field work planning. The desk-based work will be completed by Zoetica. This desk-based work will include the collection, analysis, and mapping of available data. All field-based work will be completed by the field data collection contractor. This field-based work will include the collection of data according to the SOPs in **Appendix A**, and then digitizing and quality checking the data. After going through the NWMO's data management system (see Section 1.10.1), these data will then be provided to Zoetica for further analyses and interpretation, and ultimately incorporated into the biodiversity baseline reports and Impact Assessment (IA). **Table 2-1** provides a summary of the Tier 1 field programs, the approximate timeframe to complete them, and the skills that are required for the personnel that will conduct the data collection.

Figure 2-1 presents a conceptual project schedule for the Tier 1 and Tier 2 biodiversity baseline field programs. It is expected that with the exception of eDNA studies, which is proposed to include multi-seasonal sampling, the other Tier 1 studies can be completed within a single field campaign/season in Year 2. Due to the current COVID-19 delays, most of the Tier 1 field studies will need to be conducted at the same time as Tier 2 studies in Year 2. However, Zoetica will be undertaking desk-based work in the fall and winter of Year 1, which will help inform the Tier 2 field studies (e.g., survey locations). During the Year 2 field season, Zoetica will attempt to use the results of Tier 1 studies to make any necessary changes to Tier 2 studies; however, if real-time modifications are not possible, then Tier 1 results will be used to inform the next seasonal campaign for that study (as applicable).

Table 2-1. Outline of timeframes and required skills for the Tier 1 biodiversity field programs. Years herein pertain to a Jan 1 to Dec 31 calendar year.

Program	Timeframe	Frequency/Timing	Skills required for field-contractor personnel
Terrestrial Ecosystem Mapping	Year 2: July – August	• Generally conducted in July or August when vegetation is evident • Subsequent years of field work must be conducted during the same window • Year 1 studies will focus on collecting desk-based data within the aquatic LSA • Year 2 studies will focus on vegetation RSA	• Vegetation specialists capable of identifying majority of vegetation species in the region and classifying ecosites • Soils specialists capable of describing soil profiles and classifying ecosites • Field assistants with knowledge of the region and its flora and fauna
Habitat Suitability Modelling & SWH Identification	Year 2: July – August	• Similar rationale as TEM; to be conducted at the same plots • But consider optimal timing for specific biodiversity values; e.g., MAFAs should be assessed from mid-June to end of July	• Wildlife biologists capable of assessing wildlife habitat suitability in the region • Familiarity with assessing Significant Wildlife Habitat
Aquatic Habitat Mapping	Year 2: May – September	• During summer low flow periods (May to September) when visibility and access are best, and vegetation is evident • Year 2 studies will focus on the LSA, and may focus on additional comparable control sites in the aquatic RSA; spring visits can also detect non-permanent water bodies	• Fisheries biologist capable of assessing fish habitat and fish habitat suitability in Ontario • Familiarity with wetland classification (e.g., bog, fen, marsh, swamp) in accordance with definitions outlined in the *Ontario Wetland Evaluation System – Northern Manual* (MNRF 2014a) • Knowledgeable in aquatic vegetation species identification
Drone Pilot Program	Year 2: May – September	• Seasonal timing will depend on objectives • Currently proposed to be done concurrently with aquatic habitat mapping and TEM in Year 2 • Program may expand to multiple years	• Skilled drone pilots with previous experience working and flying in remote locations • Pilots must carry a valid Transport Canada issued drone pilot certificate • Drones must be marked and registered and must fly following the Canadian Aviation Regulations
Environmental DNA Studies	Year 1-Year 3: Seasonal	• Seasonally (fall, spring, summer) in Years 1-2 at minimum, coordinated with surface water component of the EMBP • Reduced program for fall in Year 1; more intensive efforts for spring and summer sampling in Year 2	• Experienced field biologists with knowledge of local wildlife and fish biology and ecology • Familiarity with wetland classification (Ontario Wetland Evaluation System) and stream reach identification • An understanding of best practices in eDNA sample collection, e.g., requirements for strict decontamination protocols and detailed record keeping

	• Winter sampling to occur in Year 3 only if other seasonal results indicate presence of species of interest in AOI • May be refined in subsequent years of baseline data collection (study area and/or frequency)	• Required qualifications and experience should be coordinated with the needs of the surface water component of the EMBP; eDNA sample collection includes water quality measurements

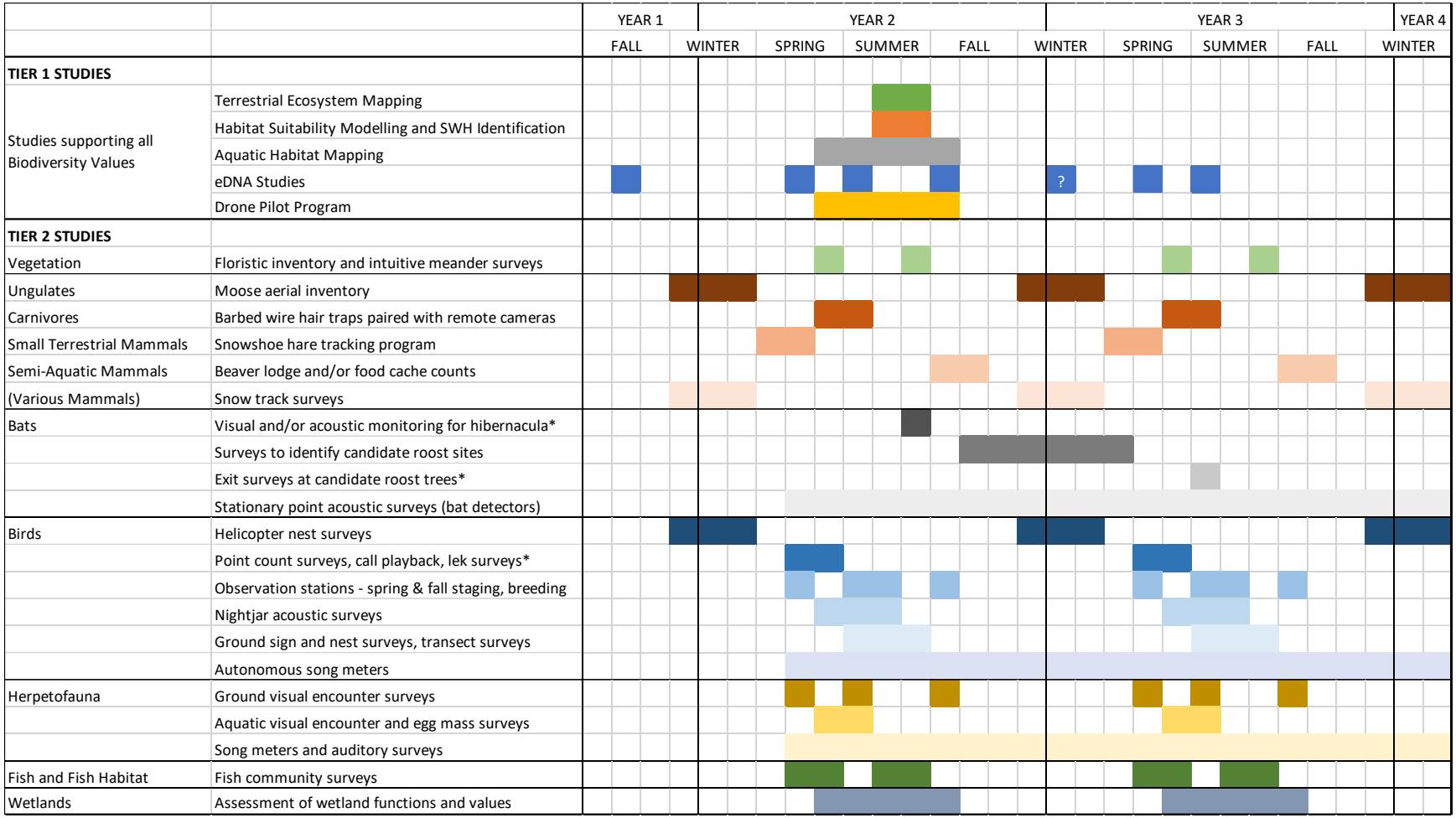


Figure 2-1. Tier 1 and 2 biodiversity values baseline studies proposed for the APM Project Biodiversity Impact Studies. An asterisk (*) indicates that surveys are only needed if important features, sign or animals are found. The question mark for winter eDNA studies in Year 3 is to show that sampling in winter will only occur if previous seasonal results indicate that species of interest are present in the AOI and would be expected to overwinter within certain habitat types in the AOI. Note: Ecosystem services studies will rely on learning from other studies and engagement to be undertaken in Years 1 to 3.

2.1 Terrestrial Ecosystem Mapping

2.1.1 Overview

To understand and characterize the environment surrounding the APM Project, Terrestrial Ecosystem Mapping (TEM) will detail the various ecosystems present in the Revell Batholith Area. An ecosystem is a community of plants, animals, and other organisms and their interactions with their abiotic (physical) environment. Ecological classifications will be defined by topography, landscape gradients and patterns, terrain, vegetation, and soils.

2.1.1.1 Data Objectives

Completing TEM for the Revell Batholith Area will provide the required detail about the various types of ecosystems present. It will enable the identification of associations between the presence and abundance of terrestrial species detected in baseline studies with habitat features. Further, TEM will provide suitable habitat groupings for planning stratified random baseline surveys for biodiversity values, such as the design to be used for eDNA collection (see Section 2.5). Since ecosystems are complex communities of organisms interacting together, stratifying the landscape into ecosystem types will allow for an understanding of the co-varying organism clusters that are present across ecosystems.

2.1.1.2 End Use

The results of TEM will be utilized as input into various biodiversity programs for the BIS. TEM data will form a key base layer for habitat suitability modelling (see Section 2.2). TEM habitat groupings will be used to plan randomized, stratified study locations for use in collecting data via a GRTS design for Tier 2 studies of various biodiversity values. Finally, TEM is used to describe and assess the habitat that is available within the LSA and the greater RSA[6] (see **Figure 2-2**), and to determine the area (ha) of various habitats that will be lost or indirectly impacted due to the APM Project. TEM from the larger regional area will also assist in determining the local rarity of lost or impacted habitat.

2.1.1.1 Best Practice Guidance

The BPPA Report details the rationale for the decision of the selected best practices. The best practices that will be used while planning and conducting the TEM will depend on which stage of the TEM study is being undertaken. The various best practices that will be used within the TEM studies are detailed in **Table 2-2**.

Table 2-2. Summary of best practices and when they will be applied during Terrestrial Ecosystem Mapping.

Best Practice	**When Best Practice is Used**
Ontario's Ecosite Guidelines: Ecosites of Ontario (ELC Working Group 2009) Boreal Ecosite Factsheets (OMNR 2014) Field Guide to the Substrates of Ontario (OMNR 2015)	Conducting air photo interpretation to classify a polygon into ecosites; when at the site to confirm or reclassify a site; when completing the Site and Soil Description Form
Standard for Terrestrial Ecosystem Mapping (RIC 1998)	Determining survey effort; general project planning
Field Manual for Describing Terrestrial Ecosystems 2nd Edition (BC MFR and BC MOE 2010)	Determining field methods; developing field forms and protocols

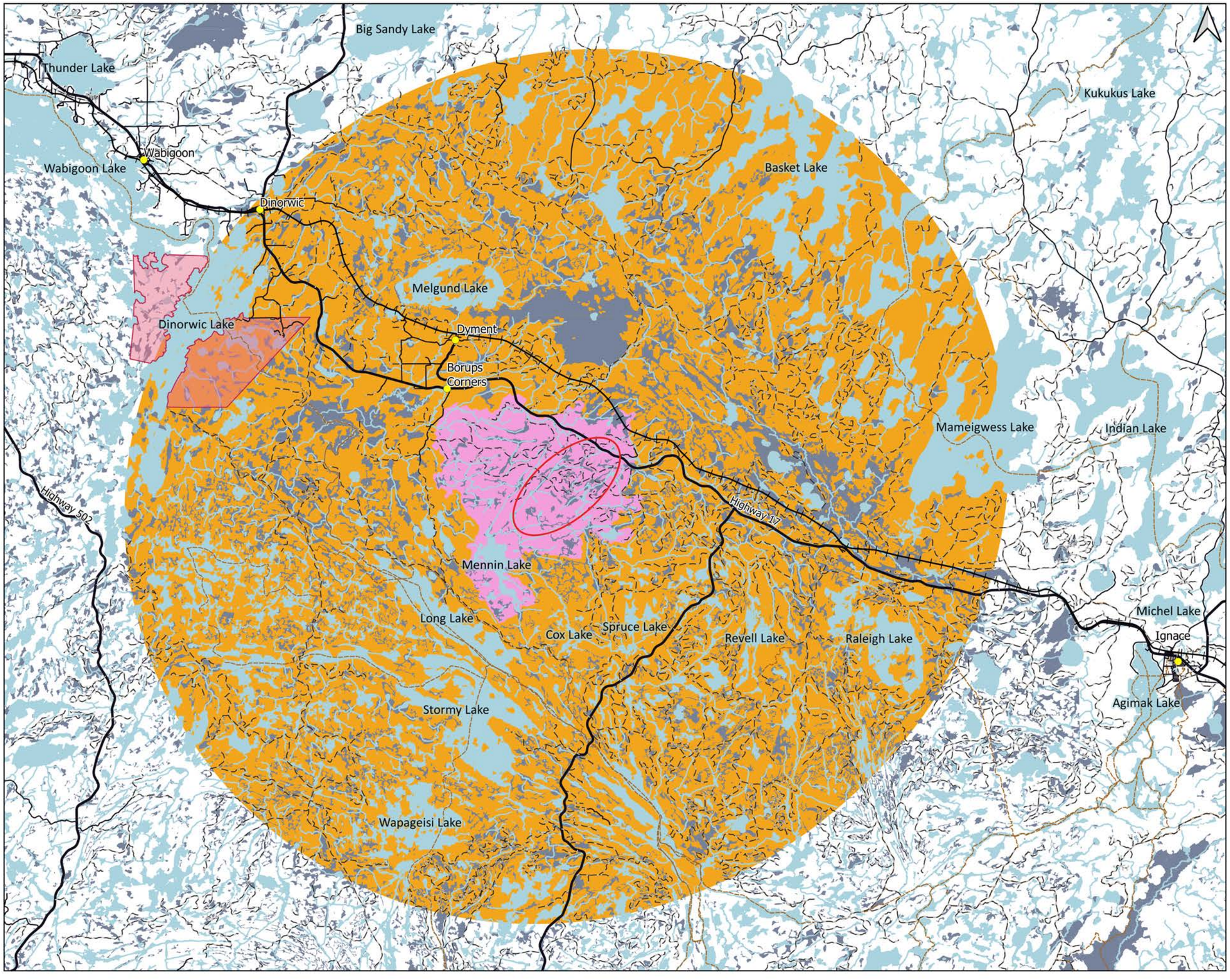
[6] We note that the RSA will not be mapped in Year 1; mapping will first be focused on the AOI and LSA and then progress outward for mapping habitat for species with representative habitat within the RSA.

2.1.2 Desk-based Program

Before any TEM field work can begin, a collation of desk-based data will be used to create a preliminary map of terrestrial ecosystems. The desk-based maps produced will act as a first draft, which will be validated and refined through field assessments (see Section 2.1.3). Validation will determine how accurate the desk-based map is, and refinement will increase the accuracy of the map by filling data gaps and fixing errors.

2.1.2.1 Study Area

As the TEM data will inform the habitat suitability of other biodiversity values (e.g., moose) and will be used to stratify survey locations for various Tier 1 and 2 field studies, the study area for TEM must be large enough to provide regional context to all other biodiversity value study areas. The TEM study areas will ultimately cover the largest area that will be used to study vegetation in Tier 2 (see Section 3.0), and TEM data will provide an initial characterization of the vegetation of the Revell Batholith Area. The TEM study areas will include a local study area, which will cover the area required to plan other studies (e.g., eDNA and aquatic habitat mapping), defined as the aquatic LSA (LSA_{AQU}), and a regional study area for vegetation defined as RSA_{VEG} (see **Figure 2-2**; see Section 3.2.3 in the BPPA Report for the general considerations and information for constructing biodiversity value-specific study areas). The RSA_{VEG} is thus a summation of the areas of all other terrestrial RSAs for the biodiversity values included in the BIS, ensuring that accurate vegetation data are available for planning studies for and assessing impacts to biodiversity values that are dependent on the habitats that vegetation provides. Mapping efforts will first focus on the LSA_{AQU} in Year 1 and will be expanded outward into the RSA_{VEG} in future years. As the Revell Batholith Area represents one of two remaining sites that are being considered for the APM Project, this expansion approach provides partial assurance that the decision to map a very large area, which is a time consuming and costly endeavour, will occur in step with decisions to narrow down the selection of a final site.



NWMO Biodiversity Impact Studies

Terrestrial Ecosystem Mapping Study Areas (LSA_{AQU} and RSA_{VEG})

Figure 2-2

- Regional Study Area (RSA_{VEG})
- Local Study Area (LSA_{AQU})
- Area of Interest (AOI)
- Wetland
- Lake
- Watercourse
- Highway or Arterial Road
- Local Road
- Resource/Recreation Road
- Trail
- Railroad
- Community
- Wabigoon Lake No. 27

1:250,000

0 5 10 km

ZOETICA

Inset Basemap © OpenStreetMap contributors

Data received from:
Ontario Geohub - Indian Reserve (MNRF), OHN Waterbody (MNRF), OHN Watercourse (MNRF), ORWN Railway (MNRF), MNRF Road Segments (MNRF), Wetlands (MNRF)
NWMO - AOI, Trail Segments (MNRF)

Project CRS: NAD83 / UTM zone 15N

August 14, 2020 | Map ID: NWMO_BIS_019d

2.1.2.2 Data Sets

The preliminary data source for ecosystem information is the Enhanced Forest Resource Inventory (eFRI) developed by the MNRF. The eFRI dataset divides the landscape into polygons and classifies them according to the ELC system. The ELC system classifies Ontario into ecosystem types at various levels of detail and scale. The hierarchy of the ELC system is shown in **Figure 2-3**; the polygons in the eFRI dataset have been classified at the ecosite level. This dataset will be used to provide an understanding of ecosystems expected in the area and for preliminary planning.

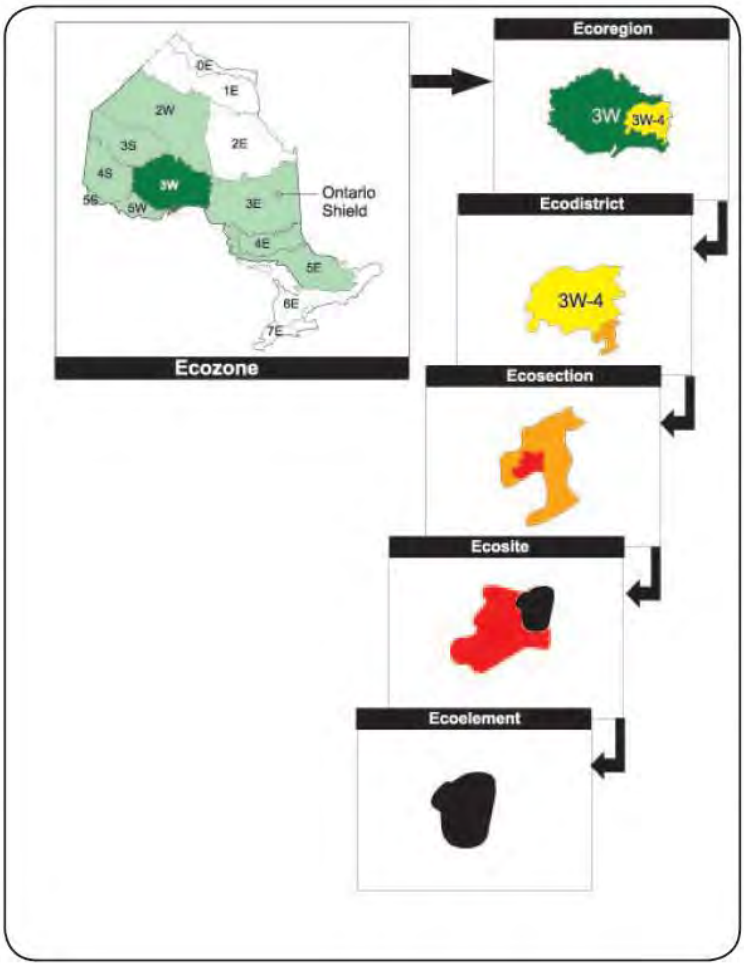


Figure 2-3. Levels in the Ontario Ecological Classification hierarchy. Reproduced from *The Ecosystems of Ontario, Part 1: Ecozones and Ecoregions* (Crins *et al.* 2009).

Through the desk-based work described in Section 2.1.2.3.1, a more refined version of the eFRI dataset has been developed. This work used aerial imagery previously collected by the NWMO to create a more detailed and updated ecosite dataset (which includes wetlands). Other data will be used with the refined ecosite data to provide more ecosystem information. This will include watercourse and waterbody data from the Ontario Hydro Network (available from Ontario GeoHub); observations and occurrence data (available from NHIC) will be used to identify potential habitat use and locations of rare plants; and all data collected from previous NWMO field programs for the variety of information they have already collected, which include some already ground-truthed ecosites.

2.1.2.3 Methods

2.1.2.3.1 Desk-based Ecosite Refinement

After collation of the datasets in Section 2.1.2.2, the next task involves developing a more detailed and accurate ecosite dataset, which will better suit the needs of the BIS. The new ecosite dataset has been created by retyping the area using more recent imagery collected by the NWMO in 2017. The ecosite classification was refined with a smaller minimum polygon size. The original minimum polygon size used by the MNRF to classify the eFRI dataset was 8 ha. For the BIS, a smaller minimum polygon size will be required, as some important vegetation communities smaller than 8 ha may have been missed and would be underrepresented. According to RIC (1998), polygons within the LSA_{AQU} should be refined to a land area of a minimum size of 0.5 ha at a 1:10,000 scale. Within the RSA_{VEG}, polygons should be refined to a minimum size of 2.0 ha at a 1:20,000 scale. This desk-based task was completed by a Sumac Geomatics, a sub-contractor to Zoetica, as they are skilled air-photo interpreters who have extensive experience delineating ecosite polygons in the Revell Batholith Area. To date, Sumac Geomatics has completed ecosite refinement within the LSA_{AQU}, and will gradually extend the analysis to the RSA_{VEG}. The desk-based exercise will result in a more accurate ecosite dataset, which will be referred to as the 'refined ecosite data' in the following sections. The refined ecosite data will be used by the field data collection contractor when ground-truthing.

2.1.2.3.2 Pre-Field Work Planning

Using the refined ecosite data to create field sampling plans will be completed by Zoetica, as sample locations and effort information will be required to plan the field-based activities presented in Section 2.1.3. Zoetica will perform GRTS on the refined ecosite data to stratify and randomize the locations of the plots, as well as the survey type for each plot (i.e., full, ground, or visual plots). The ratios for the proportion of each survey type recommended by RIC (1998) are presented in **Table 2-3**. The results of the GRTS survey design will be provided to the field data collection contractor.

During the first field season, the focus will be on verifying the refined ecosite data within the LSA_{AQU}. As the LSA_{AQU} delineates the area expected to be potentially affected by the farthest-reaching project effects (i.e., dust, noise, and water withdrawal), verification of the LSA_{AQU} should be done at survey level 2 at a ratio of 3:17:80 (**Table 2-3**) for full/ground/visual plots, respectively (RIC 1998). Details on the differences between full, ground, and visual plots can be found in Section 2.1.3.4. Zoetica will determine all survey sites for the LSA_{AQU} before the baseline field program begins; however, sites will not be visited in Year 1 due to the available survey window and delays that have occurred due to the COVID-19 pandemic (delays were announced following the near completion of the present report). The NWMO hopes to begin environmental field work for the APM Project in the fall of Year 1, which is too late to accurately assess vegetation, as many of the plants will have undergone senescence. Therefore, the first field season that consists of ground-truthing the mapped ecosite data would occur in the summer of Year 2, and sites not verified during the first field season will be verified in subsequent field seasons. Once a project footprint has been developed and finalized, all polygons within the project footprint that were not surveyed will be selected for surveying to ensure all habitat expected to be lost is properly classified.

In future field seasons, and if the Revell siting location is selected for continued investigation for the APM Project, the RSA_{VEG} will be surveyed at a survey level 4 at a ratio of 5:20:75 (**Table 2-3**) for full/ground/visual plots, respectively (RIC 1998). The selection of study sites in the RSA_{VEG} will be completed once the first field season is complete, as the information collected in the field will be used to improve the desk-based ecosite mapping in the RSA_{VEG}.

Table 2-3. Survey intensity levels for Terrestrial Ecosystem Mapping. From RIC (1998). Survey intensity level 2 will be used in the LSA_{AQU}, and survey intensity level 4 will be used in the RSA_{VEG}.

Survey Intensity Level	Percentage of Polygon Inspections	Ratio of Full Plots: Ground Insp.: Visual Checks	Suggested Scales (K=1,000)	Range of Study Area (ha)
1	76 – 100%	2 : 15 : 83	1:5 K to 1:10 K	20 – 500
2	51 – 75%	3 : 17 : 80	1:10 K to 1:20 K	100 – 10,000
3	26 – 50%	5 : 20 : 75	1:10 K to 1:20 K	5,000 – 50,000
4	15 – 25%	5 : 20 : 75	1:20 K to 1:50 K	10,000 – 500,000
5	5 – 14%	5 : 20 : 75	1:20 K to 1:50 K	10,000 – 1,000,000
R*	0 – 4%	0 : 25 : 75	1:20 K to 1:50 K	50,000 – 1,000,000+

The refined ecosite data will be screened against various criteria to flag potentially important ecosite polygons that will require verification; these polygons will then be specifically chosen while planning the field work detailed in Section 2.1.3. The types of potentially important ecosites will include candidate or potential: rare and exemplary plant communities; ecosites which are likely to contain rare plants, culturally significant plants, and weeds, introduced and invasive plants; and ecosites where candidate SWH is more likely to be present. By ensuring field work targets potentially important ecosites, the required data to confirm their importance will be collected and more targeted Tier 2 and 3 studies can be planned to provide further detail for important ecosites.

2.1.3 Field Program

2.1.3.1 Survey Timing

The survey window to conduct TEM field work is dependent on the timing to identify the most species of vegetation. TEM field work is generally conducted in July or August when vegetation is the most visible; however, considerations of local seasonal conditions need to be made when determining the survey window (RIC 1998). The field data collection contractor will determine the ideal survey windows; this may include input from a local botanist, Indigenous guide, or through IK. All subsequent years of field work must be conducted during the same timing window to ensure seasonal consistency.

Field studies can be performed in moderate weather conditions that pose no safety concerns; however, rainy conditions could make walking between sites more difficult and reduce survey efficiency. If a helicopter is used for pick-up and drop-off, or ferrying between sites, then weather conditions must be suitable for safe flight and landing. Some helicopter landing sites may only be suitable for helicopter landing under dry conditions, which could result in the inaccessibility of some survey sites.

2.1.3.2 Survey Crew

Field survey crews for TEM will be paired with survey crews for habitat suitability modelling (Section 2.2). Ideally, each survey team will consist of one experienced ecosystem biologist (terrestrial vegetation specialist), one experienced soil specialist, one wildlife biologist with experience in habitat suitability modelling, and one local field assistant with general knowledge of wildlife (if community interest and availability allow). The required experience, roles and responsibilities of the vegetation specialist, soil

specialist and local field assistant are detailed below, and the roles and responsibilities of the wildlife biologist can be found in Section 2.2.3.2.

The local field assistant will aid in the navigation of the crew between sites, complete the Site Description Form upon arrival, and document the study location with photographs. The local field assistant will provide their local knowledge and expertise to the other field staff as needed. The local field assistant can also assist other field staff in setting up or executing their survey if required (e.g., digging soil pit, marking off vegetation plot, recording data).

The vegetation specialist should have extensive field experience in the region and will be expected to be able to identify most vegetation species that could be encountered during the survey. Any plants that could not be identified during the survey can be identified with a field guide or photographed, described, and sampled for later identification; however, this should be minimized for survey efficiency. Knowledge of rare plants will not be required; however, it is considered an asset for incidental observations and increasing survey efficiency.

The vegetation specialist will be required, with assistance from the local field assistant, to mark the plot boundary or plot centre (depending on plot type detailed in Section 2.1.3.4). They will then complete the Vegetation Form, Tree Attributes for Wildlife Form, and Coarse Woody Debris (CWD) Form and also ensure the Site Description Form is completed. Zoetica will develop a list of plant species of interest with input from stakeholders, rights-holders, and local botanists. Based on their experience in the area, the field data collection contractor's vegetation specialists may also provide recommendations of species that should be included on the list of species of interest. The list of important vegetation will include potentially present but rare plants, culturally significant plants, and any weeds, introduced, or invasive plants that have potential to occur in the region; this list will be provided to the field data collection contractor. It is expected that the field data collection contractor will know how to identify these species before beginning the survey and incidental observations of the species will be recorded.

The soil specialist should have extensive field experience describing the soils in the region. The soil specialist will be expected to have the experience and skills necessary to classify soils according to the *Field Guide to the Substrates of Ontario* (OMNR 2015)*;* however, experience using this guide would be considered an asset and is not mandatory.

The soil specialist will be required, with assistance from the local field assistant, to dig a soil pit or mark out their visual inspection plot (depending on plot type detailed in Section 2.1.3.4). They will then complete the Site and Soil Description Form.

Once the field forms are completed, but before leaving the site, the vegetation and soil field staff will need to discuss what ecosite classification they will assign to the plot.

While the field protocol necessitates walking between sites and documenting observations, the use of a helicopter can increase field efficiency by dropping the field crew off at a starting location and picking them up from their ending location. A helicopter will likely be required to gain access to some areas with limited ground access. This benefit can be cost-effective if the TEM occurs concurrently with other studies that will require the helicopter to access difficult areas. Coordination with other concurrent field studies, those conducted as part of the BIS and other disciplines, will be required to share the use of the helicopter to limit waiting time (e.g., staggering start and end time). The helicopter pilot will require experience and expertise in landing in similar difficult terrain.

2.1.3.3 Equipment and Materials

Gear checklists are provided in the SOPs in **Appendix A** to help the field data collection contractor ensure that they are field-ready. Standard data collection gear will include hard copies of field forms, SOPs, list of survey location coordinates and field maps printed on waterproof paper, pencils, waterproof notebook, clipboard, binoculars, and camera. Navigational gear will include a GPS unit, tablet or smartphone with georeferenced digital field map of survey locations and routes between survey locations, and a compass. In case the tablet or smartphone stops working in the field, the list of survey location coordinates can be used along with the hard copy field map and GPS to find the survey locations and continue the survey. The field data collection contractor will ultimately be responsible for preparing their own health, safety, and environment plan for acceptance by the NWMO.

Each field specialist will require gear to complete the tasks related to their roles in the surveys. For the soil description, the required gear will include a shovel, measuring tape, trowel, gold tees, pH testing kit with 10% HCl, pruners, water bottle (for texturing), Munsell colour charts, and hand lens. For the vegetation description, the required gear will include plant identification guides, hand lens, measuring tape, diameter tape, Releskop or prism set, increment borer, straws, tape, flagging tape, flagging stakes, and critical distance tables. All field specialists should have their own camera, sample bags and a marker to mark the bags.

As discussed, helicopter services are likely needed to enable cost-effective and efficient coverage of the study area for habitat suitability modelling studies. Ideally, the aircraft will be able to accommodate the TEM and habitat suitability modelling survey crews; however, staggering crew pick-up and drop-off may be needed and will be determined when additional details about logistics are available. Alternatively, if helicopter services are not used – for example, if the NWMO determines that ground travel to the pre-determined sampling locations is feasible – then other modes of transportation will be required from home base to the study sites. In this case, a truck and/or ATVs will likely be required.

2.1.3.4 Field Protocol

Survey plots visited on the ground will be assessed using full, ground or visual methods at a ratio of 3:17:80, respectively (see Section 2.1.2.3.2). Which method to employ at each survey location will be pre-determined by Zoetica during the pre-field work planning. All field methods are detailed in the SOP in **Appendix A** and are based on the methods in *Field Manual for Describing Terrestrial Ecosystems 2nd Edition* (BC MFR and BC MOE 2010).

For vegetation surveys, a full plot involves recording all observed vegetation within a 400 m^2 plot; completing two transects within the plot to record coarse woody debris; and, recording all trees within the plot and noting attributes for wildlife. When completing a ground plot vegetation survey, only the dominant and indicator plant species are recorded. For a visual plot, no vegetation information is recorded, and only the ecosite code is confirmed.

For soil descriptions, site topography details are recorded along with a general soil profile from a soil pit. When completing a ground plot survey, the site topography details are all recorded, but the details for the soil profile are reduced. For a visual plot, no site topography or soil information is recorded, and only the ecosite code is confirmed.

For the field protocol for the wildlife habitat descriptions, see Section 2.2.3.4.

2.1.3.5 Data Collection and Recording

The data forms to be used in the field will be adapted from those in the *Field Manual for Describing Terrestrial Ecosystems 2nd Edition* (BC MFR and BC MOE 2010). There are 6 total Ecosystem Field Forms that the field data collection contractor will need to complete; however, not all forms are completed for every survey. All Ecosystem Field Forms need to be completed by the field staff member assigned to each form (see Section 2.1.3.2 for crew member responsibilities). The forms that are completed for each survey type (full, ground, or visual) are summarized in **Table 2-4** and detailed in the SOP found in **Appendix A**.

When completing a full plot, all forms are thoroughly filled out to provide maximum detail. During a ground plot, not all forms are completed, and the level of detail required is reduced for the forms that need to be completed. When completing a visual plot, no site, soil, or vegetation descriptions are required to be recorded; only the basic site information is required to confirm the ecosite code and detail the rationale for the change from the pre-determined ecosite. The Wildlife Habitat Assessment Form is completed for all survey types.

Table 2-4. Summary of Ecosystem Field Forms and the type of survey for which they are completed.

Form Name	Survey Type		
	Full	**Ground**	**Visual**
Site and Soil Description Form	X	X*	X*
Vegetation Survey Form	X	X	
Tree Attribute for Wildlife Survey Form	X		
Coarse Woody Debris	X		
Wildlife Habitat Assessment Form	X	X	X

* When completing the Site Description Form, only the required spaces are completed

2.2 Habitat Suitability Modelling and Identification of Significant Wildlife Habitat

2.2.1 Overview

Habitat suitability maps can be created from ecosystem maps (see Section 2.1), and can be used as a habitat surrogate approach for protecting other biodiversity values that may not be directly assessed (e.g., due to species rarity and seasonal mobility). Habitat suitability maps can also be used for determining the quality of habitats that provide the requirements needed by various species at different times of the year and life history requisites, and for measuring the APM Project impacts on these habitats. The latter consideration is valuable for calculating/predicting direct and indirect habitat loss and to evaluate options for achieving "no net loss" of biodiversity (Gullison *et al.* 2015).

2.2.1.1 Data Objectives

The objectives of the habitat suitability modelling are to 1) assign wildlife habitat suitability ratings to various ecosite classifications based on their relative importance to wildlife populations, and 2) assist in the selection of survey locations for more detailed Tier 2 biodiversity value community studies. Results of the habitat suitability modelling conducted at select polygons in the refined ecosite dataset can be applied to areas where ground-truthing has not been conducted.

2.2.1.2 End Use

Mapping of available low- to high-quality habitat, based on currently understood species-habitat associations for various life requisites and signs of use by various biodiversity values, can be used to rank

habitats spatially, over large areas. Mapped areas may include extrapolations of species-habitat relationships to areas where ground-truthing or surveys were not directly conducted. Habitat suitability maps can help evaluate the availability, spatial arrangement, uniqueness (compared to a regional context), and quality of habitat that could be directly or indirectly affected by a project. Habitat suitability maps can be fed into project design decisions (e.g., siting of infrastructure), and can help identify key locations for more detailed and targeted biodiversity surveys to be conducted as part of Tier 2 activities. Ultimately, habitat suitability ratings and mapping products can assist in the prediction of habitat loss and alteration, and can help to direct appropriate mitigation strategies early in the design of the APM Project.

2.2.1.3 Best Practice Guidance

In Ontario, there are no provincial standard protocols or guidelines for biodiversity habitat suitability modelling aside from tools developed for forest management. The general approach to habitat suitability modelling in Ontario involves comparing species habitat descriptions to eFRI data, ELC data (e.g., ecosites), other remotely sensed land cover data (e.g., Provincial Land Cover 2000, derived from Landsat-7), and/or field-based assessments. However, wildlife habitat assessments using remotely sensed data alone may be insufficient, as important habitat characteristics such as subcanopy and understory features are not visible to photo interpreters (Boan *et al.* 2013).

To develop more accurate and precise habitat suitability maps, field-based surveys are required to verify potentially suitable areas identified through desktop studies. Identification of SWH in Ontario also uses this approach; Appendix G in the SWH Technical Guide (OMNR 2000) presents habitat matrices for all native vertebrate (amphibians, reptiles, birds, mammals) and vascular plant species in Ontario. The habitat matrices include habitat and general habitat features associated with the probability of use by each species and how the species may use them. SWH Criteria Schedules are available in several ecoregions in the province but not within Ecoregion 4S where the AOI and surrounding study areas are located.

While the guidelines for identifying and evaluating SWH in Ontario will be useful for the APM Project, protocols for more detailed habitat suitability models are lacking. We recommend following the *British Columbia Wildlife Habitat Rating Standards* (RISC 1999), which detail the requirements for wildlife habitat assessment data collection as well as the development of wildlife habitat suitability maps. These Resources Information Standards Committee (RISC) standards define the habitat rating criteria for different map scales, along with the standardized level of detail needed while describing life requisites and seasonal habitat use to develop species-habitat criteria and preliminary habitat ratings. The refined ecosite dataset, developed through the desk-based ecosite refinement outlined in Section 2.1.2.3.1, must then be ground-truthed through field surveys, typically in coordination with other discipline teams that are assessing other terrestrial ecosystem components (e.g., vegetation, soils). A detailed, standardized form is used for recording data during wildlife habitat assessments (BC MFR and BC MOE 2010). Finally, the RISC standards provide guidelines for developing a final ratings table incorporating the additional field-based data on species-habitat relationships, which can then be used to develop habitat suitability models and maps for the BIS Program.

2.2.2 Desk-based Program

The desk-based program will be conducted by Zoetica scientists and will support the field components to be conducted by the field data collection contractor.

2.2.2.1 Study Area

Study areas for habitat suitability modelling will differ among biodiversity values selected. For field data collection, study areas for habitat suitability modelling will be the same as those described in Section 2.1.2.1, as the wildlife crew will be paired with the field crew conducting ground-truthing for verification of TEM. These areas were defined by using the largest areas for any of the biodiversity values (see rationale in Section 2.1.2.1).

For the creation of seasonal biodiversity value-specific habitat suitability maps for use in the development of future baseline studies (e.g., Tier 2 studies), as well as for use in impact studies, study areas will be clipped to those proposed for relevant biodiversity values selected for Tier 2 studies.

2.2.2.2 Data Sets

The datasets used for the selection of sampling areas to conduct habitat suitability modelling are the same as those derived for the TEM field program and are outlined in Section 2.1.2.2. Zoetica will create habitat suitability maps for calculating habitat measurements for baseline and impact studies purposes after updating the initial ecosystem mapping with results obtained during the vegetation surveys conducted as part of the TEM field program by the field data collection contractor (Section 2.1.3).

2.2.2.3 Methods

2.2.2.3.1 Rating Criteria

A habitat rating is a value assigned to a habitat based on its potential to support a particular species for a season and life requisite (e.g., breeding, shelter) compared to the best habitat in the province that is used for that species for the same season and life requisite. In Ontario, there are no provincial benchmarks for seasonal habitats for which all other habitats for that species are rated against. However, in Ontario, the identification of SWH has been conducted for many species, and habitat matrices for native amphibians, reptiles, birds, mammals, and vascular plants have been developed. The habitat descriptions of SWH include general habitat features that are associated with each species, such as wetlands, open water, riparian areas, grasslands, parklands/suburban areas, thickets, and second growth, old growth and mature stands, forest edges, downed woody debris, seeps, and cliffs, talus slopes, and ravines. While habitat features associated with SWH are described for some species, the level of detail may not be sufficient to relate habitat ratings to specific life histories for each species (i.e., SWH is generally more seasonal and area specific); further, information in the habitat descriptions may not be at a fine enough scale to rate habitat within each polygon in the refined ecosite dataset (SWH is not rated for quality but either identified as not present, candidate SWH based on habitat description, or confirmed SWH typically based on species presence and abundance). While candidate (potential) SWH will be documented when identified during field activities, the establishment of benchmarks against which other seasonal/life requisite habitats will be rated will require development through additional literature reviews of local and regional habitat use and seasonal abundance and density data for the biodiversity values.

Habitat suitability ratings are ultimately designed to reflect the value of a habitat (i.e., its relative potential use by animals of a particular species for certain life history activities) rather than actual numbers of animals using the habitat; hence habitat suitability maps tend to be a more conservative and point out all potentially available habitat for each biodiversity value, regardless of whether it is currently occupied by that species (e.g., if the landscape is far below the carrying capacity for a species). In addition, Indigenous knowledge holders and local experts that are well-versed in species habitat requirements in northwestern Ontario will have a good idea of “ideal” habitat conditions for each wildlife value. For habitat suitability

modelling for the biodiversity impact studies, Zoetica proposes to use the *Significant Wildlife Habitat Technical Guide* and the SWH Criteria Schedules available for the nearest ecoregion to the APM Project (i.e., 3W) as a starting point to identify "ideal" habitat features for certain seasons and life history requisites; however, additional literature research and input from Indigenous knowledge holders, and local and regional experts will be required to establish "ideal" habitat for all species, seasons, and life histories of interest for mapping. This process will ensure that the habitat within the study area of relevance to the species or group being considered is rated consistently with habitat models currently in use within the province, but that the additional information builds out habitat maps to provide more detail than the SWH Criteria Schedules.

For the rating of habitat for most species, Zoetica proposes to use the 4-class rating scheme that rates habitat suitability as high (H), moderate (M), low (L), and nil (N) for defined seasons and habitat uses, wherever reasonable to do so (Table 2-5). The 4-class rating system is suggested for species for which there is an intermediate knowledge level of habitat preferences. In the case where more detailed knowledge of seasonal habitat preference is available for the species (e.g., black bear and ungulates), a 6-class system will be attempted (RISC 1999). However, if not enough is known about seasonal habitat preferences of a wildlife species, the 4-class system will be used. A four class system results in fewer inconsistencies among field crew when ranking habitat and avoids conveying false precision where that precision is difficult to define (e.g., differentiating between "High" and "Moderately High" rated habitat). In some cases, research and input may reveal the need to use a 4-class system, where knowledge about species habitat preferences is more limited.

Table 2-5. Habitat suitability rating schemes for three levels of knowledge about a species' use of habitat. From RISC (1999)

% of Regional Best*	Substantial Knowledge of Habitat Use (6-class)		Intermediate Knowledge of Habitat Use (4-class)		Limited Knowledge of Habitat Use (2-class)	
	Rating	**Code**	**Rating**	**Code**	**Rating**	**Code**
100 – 76%	High	1	High	H	Habitat Useable	U
75 – 51%	Moderately High	2	Moderate	M		
50 – 26%	Moderate	3				
25 – 6%	Low	4	Low	L		
5 – 1%	Very Low	5			Likely No Value	X
0%	Nil	6	Nil	N		

* "Regional Best" is the regional benchmark habitat for species against which all other habitats for that species are rated. For the biodiversity impact studies, Regional Best will be based on the Ontario SWH Criteria Schedule for Ecoregion 3W (OMNRF 2017) for particular seasons/life history requisites for some species where data are available, or will be developed from research and literature for other seasons/life requisites where data are not available.

The use of habitat by a species is closely related to the season or time of year, and specific activity or life requisite (e.g., breeding) that they will be using the habitat for. Thus, habitat ratings for species are typically conducted for a specified season and life requisite. Seasons may be defined at several different levels of detail (**Table 2-6**): 1-season (all combined), 2-season (Winter and Growing), 4-season (Winter, Spring, Summer, and Fall), and 6-season (Early Winter, Late Winter, Early Spring, Late Spring, Summer, and Fall). Decisions on the level of detail are specific to the species studied. Within any type of rating class, not all seasons in a year require being rated.

Table 2-6. Four levels for describing seasons of habitat use. From RISC (1999). Often, specific dates will be used to define each of the seasons listed in column 3, based on species-specific life history patterns and when unique seasonal habitat use patterns are observed.

Level	Code	Description*	Application
1-season	A	All seasons	• When habitat use between seasons cannot be differentiated (small map scales and/or species with low mobility)
2-season	W	Winter	• When seasonal habitat use can only be roughly differentiated • Small map scales
	G	Growing (spring, summer, fall)	
4-season	W	Winter	• When four distinct seasons of habitat use can be differentiated (medium to large map scales) • Species for which there is an intermediate or substantial knowledge level • When species occur in the province only part of the year (migratory species – only 3 of the 4 seasons rated)
	P	Spring	
	S	Summer	
	F	Fall	
6-season	WE	Early Winter	• When distinguishing detailed seasons for black bear and ungulates (for most of these species, only 4 or 5 of the 6 seasons will be rated; e.g., food habitat for bears may be rated for Early Spring, Late Spring, Summer, and Fall)
	WL	Late Winter	
	PE	Early Spring	
	PL	Late Spring	
	S	Summer	
	F	Fall	

* Winter, as an entire season, can be used for either a 2-season or a 4-season rating but is differentiated into early and late seasons using a 6-season rating scheme. Similarly, Spring is differentiated into early- and late- seasons using the 6-season rating scheme.

Life requisites are special habitat requirements that are needed to sustain and perpetuate the species and may include food/cover and specific life requisites (e.g., reproduction, migration, hibernation) for a given species. Similar to seasons, life requisites may be defined at several different levels of detail (**Table 2-7**): Habitat can be rated generally as "Living" for food/cover life requisites (food, security, thermal, and security/thermal), or for specific life requisites (courtship, hibernating, migrating, reproducing, and staging).

Table 2-7. Life requisites used in suitability ratings: definitions and codes. From RISC (1999).

Life Requisite	Code	Definition
Food/Cover		
Food	FD	• Habitat used for consuming food items, including searching for and consuming food simultaneously (such as done by grazers, browsers, flying insectivores, and ducks)
Security	SH	• Habitat used for protection or hiding from predators
Security/Thermal	ST	• Habitat used for security and/or thermal values (this category used when differentiation between thermal and security values is difficult or impossible
Thermal	TH	• Habitat used for protection from heat, cold, precipitation, or wind

Specific		
Courtship/Mating	CO	• Habitat used for courting, pair-bonding or mating (when separate from reproducing habitat)
Hibernating	HI	• Habitat used for hibernating
Living	LI	• Habitat used for general living activities and includes other life requisites such as FD, ST, CO, HI, MS, RB, RE, or SG
Migrating (seasonally)	MS	• Habitat used for regular, annual travel (e.g., habitat used by deer for spring and fall migrations)
Reproducing (birthing)	RB	• Habitat used specifically for giving birth to live young (mammals); may or may not include courtship/mating, depending on the animal species
Reproducing (eggs)	RE	• Habitat used for building a nest, laying eggs, incubation, hatching, and feeding non-mobile young (amphibians, birds, and reptiles); may or may not include courtship/mating, depending on the animal species
Staging	SG	• Habitat used for staging during spring and fall migrations

Habitat suitability ratings based on the combination of seasons and life requisites, and the level to which they are applied depend on: 1) the particular requirements of each species, 2) scale of the map, and 3) objectives of the project. The minimum required life requisite is "Living" except for birds that breed in the area when "Reproducing" must also be rated. For most species, the minimum requirement for seasonal ratings is two seasons. Often, the seasons selected for rankings are those that may limit the population size via seasonal reductions in the carrying capacity of the landscape. **Table 2-8** provides an example of the minimum requirements for habitat suitability modelling for selected species as described in the RISC standards (RISC 1999).

Table 2-8. Minimum requirements for rating habitat use (life requisites and seasons) and the rating scheme to use for some commonly rated vertebrate species at two map scales. From RISC (1999).

Map Scale:	1:50,000		1:20,000	
	Rating Scheme	Habitat Use	Rating Scheme	Habitat Use
Mammals				
Ungulates	6-class	Living-Winter Living-Growing	6-class	Living-Winter Living-Growing
Bears	6-class	Living-Growing	6-class	Living-Growing
Mustelids: Marten, Fisher, Wolverine, Badger	4-class	Living-Winter Living-Growing	4-class	Living-Winter Living-Growing
Birds				
Residents	4-class	Living-All	4-class	Living-All
Breeding Visitants	4-class	Living-Growing	4-class	Living-Growing Reproducing
Amphibians and Reptiles				
Pond-dwelling amphibians	4-class	Living-All	4-class	Living-All
All other amphibians; Reptiles: Turtles & Lizards	2-class	Living-All	4-class	Living-All
Reptiles: Snakes	2-class	Living-All	4-class	Living-Growing Hibernating

2.2.2.3.2 Procedures for Developing Wildlife Habitat Ratings

In this section, the process for applying the habitat criteria to polygons in the refined ecosite dataset is described through the development of species accounts and ratings tables as part of the work required to support the baseline reporting. This information will be developed by Zoetica in Year 1 as part of the desk-based work for the Tier 1 baseline program and will be provided to the field data collection contractor before field verification.

2.2.2.3.2.1 Level of Survey Intensity

Survey intensity and sampling are typically used to conceptualize the inventory scale. The *Standard for Terrestrial Ecosystem Mapping in British Columbia* (RIC 1998) provides guidance on the survey intensity and field plot inspection density for various map scales. For the baseline studies, the survey intensity will be tied to the field survey intensity for TEM outlined in Section 2.1.2.3.2, as the field crews will be composed of surveyors for ground-truthing the refined ecosite dataset and wildlife personnel for the identification and rating of wildlife habitat. Field verification will begin during the first field season within the LSA_{AQU} and may be expanded in future years within the RSA_{VEG} (see Section 2.1.2.3 for rationale). The LSA_{AQU} delineates the area expected to be potentially affected by the farthest-reaching project effects (i.e., dust, noise, water withdrawal). Based on the size of the study areas for the baseline program, and guidelines presented in RISC (1999), field verification within the LSA_{AQU} (5,030 ha) will be conducted at a survey level of 2 and a ratio of 3:17:80 (see **Table 2-3**) for full/ground/visual plots, respectively (RIC 1998). Details on the differences between full, ground, and visual plots can be found in Section 2.1.3.4. Zoetica will perform GRTS to stratify and randomize the type of plots to be conducted at each location, to meet the required ratio; this information will be provided to the field data collection contractor. All survey sites for the LSA_{AQU} will be determined at this stage; however, not all sites might be visited in the first field season due to limitations of the number of sites able to be visited each day and the length of the survey window. Any survey sites not verified during the first field season will be verified in subsequent field seasons. Once a project footprint has been developed and finalized, any refined ecosite dataset polygons within the project footprint not surveyed, will then be selected for surveying (level 1 intensity – 100% of plots) to ensure all habitat that will be lost will be properly classified by habitat value for the selected biodiversity values.

In future field seasons, the RSA_{VEG} (269,937 ha) will be surveyed at a survey level of 4 and a ratio of 5:20:75 (**Table 2-3**) for full/ground/visual plots, respectively (RIC 1998). The selection of study sites will be completed once the first field season is complete as the information collected in the field will be used to improve the ecosite mapping in the RSA.

2.2.2.3.2.2 Wildlife Species Selection

Careful consideration was given to the selection of wildlife species (biodiversity values) for habitat suitability modelling. Species are normally chosen to represent those that have economic or social value or that are at-risk. In general, ungulates and bears should always be included on the list of species as they are wide-ranging, require large tracts of seasonally different habitats, and represent species of economic, social, and ecological importance. Not all species are well suited for habitat suitability inventory. For example, habitat suitability modelling may not be the best inventory methodology for species where habitat requirements and distributions are not well understood. In addition, some species may have habitat needs that do not correlate well with polygons in the refined ecosite dataset (e.g., peregrine falcon breeding sites). Predatory species (e.g., lynx, wolf) may also be difficult to map when primary prey (e.g.,

snowshoe hare, moose) are not considered. Prey species are typically more abundant and provide more detectable signs.

General considerations in the selection of species for habitat inventory outlined in RISC (1999) include:

- Ungulates and bears;
- Species that use a variety of habitats over their annual range;
- Species where there is a good understanding of the relationship between life requisites and habitat attributes;
- Species whose habitat requirements correlate well with the polygons in the refined ecosite dataset;
- Species where there is a reasonable likelihood of observing the animals or detecting evidence of use in the field;
- Prey species – when predator species is considered of importance for the project;
- A list of no more than approximately 6 to 10 species (as the time to rate more within the field will be too time-consuming and hold up the TEM team);
- Species for which there is very little known about their distribution and habitat requirements should be excluded; and,
- Wide-ranging, opportunistic carnivores that do not relate well to ecosystem map attributes should be excluded.

For the baseline studies, a selection of species was considered for Tier 1 habitat suitability assessment that covers a variety of habitat types. Many of the species selected cover the habitat attributes required for other species not included in the list, including potential species at risk. The species proposed for habitat suitability modelling according to RISC (1999) standards are presented in **Table 2-9**.

Table 2-9. Proposed terrestrial wildlife species for habitat suitability modelling following RISC (1999) standards.

Proxy/Surrogate Species for Suitability Maps	**Habitat Considerations Relevant to Other Species (protection of high-quality proxy species habitat leads to protection of the following)**	**Surrogate Species Beneficiaries**
Moose	• Wetlands (MAFAs) • Matrix of different forest ages • Riparian shrub habitat • Early seral stage habitat (forage) • Late seral stage coniferous forest habitat with a high canopy cover (for snow interception in late winter) • Mineral licks (SWH) • Seeps and springs (SWH) • Cervid movement corridors (SWH) – riparian areas, woodlots, areas of physical geography (ravines or ridges)	<u>Early successional stage forest (moose forage habitat)</u> White-tailed deer Black bear A wide variety of upland breeding birds reliant on early successional forest, including: Red-eyed vireo Alder flycatcher Common yellowthroat Winter wren Ruffed grouse Spruce grouse <u>Mature coniferous forest (late winter moose habitat)</u> American marten Fisher

		Northern flying squirrel A variety of late seral stage bird species, including: Winter wren Blackburnian warbler Brown creeper Spruce grouse Pileated woodpecker <u>Wetlands (and riparian areas)</u> Beaver Muskrat Waterfowl A variety of amphibians and reptiles with aquatic life history phases (e.g., frogs, salamanders, turtles) <u>Mineral licks</u> White-tailed deer Porcupine <u>Seeps and springs</u> Spotted salamander Blue-spotted salamander Ruffed grouse Spruce grouse Black bear <u>Cervid movement corridors</u> White-tailed deer Smaller mammal species
Black bear	• Early green-up locations • Area high in berry productivity (more open areas, edges) • Large tracts of forest interspersed with early successional vegetation • Forest with dense understorey and variety of tree and shrub species (nut-bearing and fruiting) • Secure den sites for winter hibernation and rearing of young (e.g., hollow trees); mammal denning sites are SWH	<u>Denning carnivores</u> Gray wolf Red fox Coyote Canada lynx Bobcat
Snowshoe hare	• Early successional forest with dense understorey, interspersed with old forest with many gaps • Dense conifer stands in winter (thermal cover and protection from predators) • More open habitats with abundance herbaceous cover in summer (forage)	<u>Primary predators of snowshoe hare</u> Canada lynx American marten

Northern flying squirrel	• Mature and old stands of conifer or mixedwood forest with high structural diversity, abundant snags, and tall trees • Mature coniferous trees, live and dead cavity trees for maternal dens and shelter • Shrub layer that provides shelter for ground foraging	<u>Late successional stage forest</u> American marten Fisher Winter wren Blackburnian warbler Brown creeper Spruce grouse Pileated woodpecker
Eastern whip-poor-will (Threatened)	• Dry, open, deciduous woodlands of small to medium trees • Oak or beech with lots of clearings and shaded leaf-litter • Wooded edges, forest clearings with little herbaceous growth • >100 ha forests; may require 500-1,000 ha to maintain population • Shrubland or successional fields that are not being actively farmed (SWH)	<u>Shrub/early successional bird breeding habitat</u> Yellow-bellied cuckoo Northern hawk owl Philadelphia vireo House wren Northern mockingbird Brown thrasher Clay-coloured sparrow Black-billed cuckoo Tennessee warbler Palm warbler Connecticut warbler Wilson's warbler Lincoln's sparrow Ruffed grouse Eastern kingbird American woodcock

For other species of interest that are suboptimal for detailed habitat suitability modelling (the general considerations described above), SWH identification will be used (OMNR 2000, OMNRF 2017). Any SWH that has already been identified or is encountered in the field will be documented and mapped. Examples of SAR of relevance to the Revell Batholith Area that have SWH defined in OMNRF (2017) are presented in **Table 2-10**, along with a list of other species that would benefit from the protection of the habitat. Note that all potential SWH (not just those listed below) identified through desk-based or field-based studies will be documented and mapped as part of Tier 1 and/or Tier 2 studies for various biodiversity values.

Table 2-10. Proposed species at risk for mapping of Significant Wildlife Habitat and other species that will benefit from the protection of that habitat (beneficiary species).

Example Species of Interest	Habitat Considerations that are also Significant Wildlife Habitat	Other Beneficiary Species
Little brown myotis Northern long-eared myotis **(Endangered)**	• Suitable caves and mine shafts that are used as hibernacula (SWH) • Mature deciduous or mixed forest stands with >10/ha large-diameter wildlife trees (maternity colony; SWH)	Eastern small-footed myotis **(Endangered)** Big brown bat Silver-haired bat
Snapping turtle **(Special Concern)**	• Suitable sand gravel beaches adjacent to undisturbed shallow weedy areas of marshes, lakes, and rivers; located in an open and sunny area, e.g., south-facing slopes that are used by turtles for nesting areas (SWH)	Western painted turtle

	• Permanent waterbodies, large wetlands, and bogs and fens with water deep enough not to freeze and/or have soft mud substrates for burrowing (turtle wintering area; SWH)	
Short-eared owl (**Special Concern)**	• Grasslands, open areas or meadows that are grassy or bushy • Also nests in bogs, fens, and marshes • Large grasslands, fields, meadows that are not being actively farmed and serves as open country bird breeding habitat used for ground-nesting (SWH)	Bobolink **(Threatened)** Eastern bluebird Vesper sparrow LeConte's sparrow Savannah sparrow Horned lark Northern harrier American kestrel
Yellow rail **(Special Concern)**	• Large, freshwater or brackish grass and sedge marshes with dense vegetation • Wetlands with shallow water and emergent aquatic vegetation used readily by marsh birds for breeding habitat (SWH)	Black tern **(Special Concern)** American bittern Sora Red-necked grebe Northern shoveler Ring-necked duck American coot Pied-billed grebe Marsh wren Sedge wren Common loon Sandhill crane Solitary sandpiper Trumpeter Swan Green-winged teal Spotted sandpiper
Bank swallow **(Threatened)**	• Sand, clay or gravel riverbanks or steep riverbank cliffs • Lakeshore bluffs of easily crumbled sand or gravel • Gravel pits, road-cuts, grasslands, or cultivated fields that are close to the water • Areas with exposed soil banks, undisturbed or naturally eroding that is not a licensed/permitted aggregate area (colonially-nesting bird breeding habitat – bank and cliff; SWH)	Cliff swallow Northern rough-winged swallow

Additional species of interest (e.g., SAR, species of importance to stakeholders/rights-holders) are more suited for direct study than employing a habitat suitability modelling approach. For example, eDNA sampling in multiple seasons may help to identify important amphibian breeding habitats (considered SWH) and aquatic overwintering habitats (see **Table 2-15** in Section 2.5.3.1), as well as habitats for cryptic waterbirds and other difficult-to-detect species. For amphibian species that inhabit both woodland and wetland habitats at different times of the year, eDNA metabarcoding results indicating these species' presence could trigger further studies to identify potential amphibian movement corridors (also considered SWH).

The reader is reminded that the current BPD Report focuses on the first tier of field studies, and that results of Tier 1 along with ongoing engagement will inform future tiers, which will result in the release of

additional baseline design report SOPs, and updates to the current, living document. Detailed baseline designs proposed for Tier 2 and 3 studies will likely include aerial surveys to locate raptor stick nests, point counts and/or automated song meters to identify important shorebird and waterfowl migratory stopover areas. Habitat suitability modelling for additional species, including SAR, may also be conducted in future years of study if detection of required habitat and/or species through field observation and eDNA studies indicates the potential use of the applicable study area and the project could result in potential effects. Changes in understanding of how far-reaching potential project effects could extend may also trigger the mapping for additional species.

Future studies will be identified and refined based on the consideration of information collected during Tier 1 results, and through ongoing engagement needed to address concerns of local communities, rights-holders, scientific experts, the regulator and other federal and provincial agencies, and other interested parties.

2.2.2.3.2.3 Preliminary Species-Habitat Criteria and Maps

Before field sampling occurs, preliminary species-habitat criteria will be developed and mapped by Zoetica. Two main components of the preliminary species-habitat criteria for wildlife habitat suitability modelling include:

1. **The Species Account**: Provides general background on the biology of selected species. Habitat requirements for each life requisite and associated season are identified.
2. **The Habitat Rating**: Relates the habitat requirements described for each species in the species account to relevant ecosystem attributes. All assumptions used for assigning ratings are described and a preliminary ratings table will be developed by Zoetica. The preliminary habitat ratings present the hypothesis of the relationship between species and habitat attributes in the project area. Field verification is conducted alongside TEM ground-truthing by the field data collection contractor.

As part of the baseline program, Zoetica will develop desk-based preliminary habitat criteria for each of the selected species. The preliminary habitat criteria are refined at several stages during the baseline program. During Tier 1 studies, where sufficient wildlife sign is observed, refinement of the ratings criteria may be possible. Suitability ratings would then be updated for each unique polygon in the refined ecosite dataset (Section 2.1.2.3.1). Criteria and maps may also be refined as more data are gathered on species occurrence and relative abundance during Tier 2 studies.

<u>Collation of Existing Data</u>

The development of preliminary habitat criteria and maps include the collation of existing data and includes the following data sources:

- **Existing Species Models**: While no known habitat suitability maps exist for the relevant biodiversity value-specific local and regional study areas, a criteria list for SWH has been developed for Ecoregion 3W (for particular species, some of which are at risk), which outlines what constitutes an SWH. These SWH criteria will be applied to find and investigate candidate

SWH within the various study areas around the APM Project.[7] Criteria developed for species-specific SWH will be assessed for applicability of use in the development of preliminary species-habitat models.

- **Ecosystem Mapping**: The RISC standard for wildlife habitat ratings (RISC 1999) requires broad-scale ecosystem mapping (1:250,000). The eFRI dataset, which contains polygons classified into ecosites, could be used since it is available for the entire RSA_{VEG} and exceeds the scale requirement detailed in the RIC standard. However, a refined ecosite dataset is being produced through the desk-based work outlined in Section 2.1.2.3.1; this refined dataset will have a smaller minimum polygon size than the eFRI dataset and will greatly exceed the requirement in RISC standard for wildlife habitat ratings (RISC 1999). Using the refined ecosite dataset will allow for more accurate habitat assessments due to more homogeneous polygons and ecosite information that is based on much more recent imagery than the eFRI dataset. The refined ecosite dataset will be created in stages beginning with the LSA_{AQU} and moving out into the RSA_{VEG} gradually; the coarser eFRI dataset will be used where the desk-based refinement has not yet been completed. The refined ecosite dataset will be ground-truthed along with completing wildlife habitat suitability ratings. Preliminary habitat suitability maps may be created from the refined ecosite dataset if sufficient time allows before ground-truthing.
- **Provincial Benchmarks**: No known Ontario provincial benchmarks exist for species; however, a criteria list for SWH has been developed for Ecoregion 3W (for particular species, some of which are at risk), which can be applied to the habitat within the various study areas around the APM Project[7] to help determine certain key habitats for select species.
- **Literature Review**: A literature review using references supplied by species experts, governmental reports, online searches including the Web of Science is critical in the development of species-habitat criteria, and assists in updating the most recent relevant information on local species and habitat requirements.
- **Interweaving Indigenous Knowledge**: Indigenous Knowledge provided by Indigenous communities, Elders, and knowledge holders can provide valuable information for building local species-habitat criteria and maps.
- **Interviewing Species Experts**: Knowledge provided by species and habitat experts (western science) can provide valuable information for building local species-habitat criteria and maps.

Development of Species Accounts

A species account is a description of the life history, biology, and habitat requirements of a given species, and for habitat suitability modelling, it refers to the life requisites and habitat requirements relevant to the project area. Information obtained during the review of the literature and from interweaving IK and information from species experts can provide the basis of information used to develop the species account. The focus of the species account should be on what habitat requirements are relevant to the boreal forest conditions similar to those found in Northwestern Ontario and include as much detail as possible when relating life requisites to habitat requirements and ecosystem attributes. The information included in **Table 2-11** will be included in a species account, where available (adapted from RISC 1999):

[7] The APM Project is located within Ecoregion 4S; however, a SWH Criteria Schedule has not yet been developed by the MNRF for this ecoregion. Given the proximity of Ignace to Ecoregion 3W, the MNRF has confirmed that it is likely more appropriate to adopt the draft 3W schedule.

Table 2-11. Information to be included in a species account. Adapted from RISC (1999).

Information Type	Description of Details
Name	• Include the scientific and common name of the species, as well as the standard species codes (Cannings and Harcombe 1990).
Status	• As listed by the Ontario *Endangered Species Act* and federal *Species at Risk Act*, as well as any COSEWIC designations.
Distribution	• Include provincial range and detail whether the species, as it occurs in the project area, is at the periphery or at the centre of its range.
Study Area (e.g., AOI, LSA, RSA) & Map Scale	• Identify the ecoregions, ecodistricts, and ecosites for the study area. • Identify the map scale for which the species-habitat criteria are being developed.
Ecology & Habitat Requirements	• Include a brief description of the species' life history and ecology as it relates to the use of habitats in the project area. • Describe the general seasonal habitat requirements of the species.
Habitat Use (Life Requisites and Seasons)	• Identify the season level (e.g., 1-, 2-, or 4-season) and the combination of life requisites and seasons that will be rated for the project. • Note: food and cover life requisites are generally required throughout the year and the season must always be identified explicitly in the ratings table. Most other life requisites (e.g., reproduction, hibernation, migration) are confined to specific times of the year and are specific to each species – the season for these specific life requisites must be identified in the Species Account. • Identify months of habitat use for each life requisite and the season for which it is rated: o For resident species, all months of the year may be accounted for and rated. o For migratory species, only months for which it occurs in the province are rated. o For those migratory species in which some individuals or populations regularly overwinter, all months of the year should be accounted for and rated.
Habitat Use & Ecosystem Attributes	• For each life requisite-season combination to be rated, describe the specific ecological attributes (e.g., ecosite classification, important plant species, canopy closure, age structure, slope, aspect, terrain characteristics) which provide the required life requisites. • Ensure these attributes are identified in the ecosystem database to be created and maintained by Zoetica.

Development of Preliminary Habitat Ratings

After developing species accounts to describe the species' life history and habitat requirements, the next step is to relate how that information will be used to develop the preliminary habitat suitability ratings. The guidelines for rating the species' habitat are outlined in **Table 2-12**.

Table 2-12. Criteria and description of information used to develop preliminary habitat suitability ratings. Adapted from RISC (1999).

Criteria	Description
Rating Scheme	• Three habitat suitability rating schemes are outlined in RISC (1999): o 6-class rating scheme – for detailed knowledge of species' habitat use. Note: Zoetica endorses the use of 4-class rating scheme where it is too difficult to differentiate between the rating classes as it is less prone to inconsistencies in rating across survey crew and plots and is suitable for this project o 4-class rating scheme – for intermediate knowledge of species' habitat use o 2-class rating scheme – for limited knowledge of species' habitat use • Note: The level of detail sets the parameters for developing both the preliminary and final ratings tables.
Provincial Benchmark	• Identifies the highest suitability habitat for the species in the region, against which all other habitats for that species are rated. • No formal provincial benchmarks exist for habitat suitability modelling in Ontario; however, species-specific SWH will be used as a starting point for ideal habitat features supporting species in certain seasons and for certain life history requisites, but this information will need to be supplemented with habitat evidence gathered from literature and local and regional studies and knowledge to develop relationships between species life histories and important habitat requirements to identify benchmarks for rating all habitat against.
Ratings Assumptions	• Provide a descriptive account of the ratings assumptions, including: o The effects of ecosystem attributes (e.g., slope, aspect, structural stage) on the ratings; o The highest potential rating for each habitat attribute and/or ecosite classification expected in the project area; and, o The minimum and maximum ranges of expected ratings for each habitat attribute and/or ecosite classification. • Assumptions should always accompany the preliminary ratings table and should reference the information source wherever possible.
Preliminary Ratings Table	• Provide suitability ratings (by season and life requisite) for all structural stages, for features such as: o Landscape position (slope, aspect, crest, special habitats) o Moisture regimes (floodplain, dry average, moist, deep/shallow soil, rock outcrop) o ecosite classification o Special features (e.g., lakes, cliffs, wetlands) o Structural stages may be grouped (e.g., shrub/herb, young forest, mature/old forest)

Ratings Adjustments Considerations	• Create a list of habitat attributes that may not be included in the ratings table but may be important for the species. o Size and proximity effects, or attributes that may be used to adjust ratings table to produce a more accurate habitat map. For example, if literature demonstrates that a species avoids habitat within 1 km of roads, habitat ratings for that species can be downrated when a habitat unit is within 1 km of a road. o Listing expected adjustments at this stage provide further direction for field sampling and verification.

2.2.2.3.2.4 Final Species-Habitat Maps

Draft/preliminary species habitat maps will be developed after creating a rating scale and relating it to ecosites. Final species-habitat maps will be developed following field verification (Year 2). The species-habitat maps may be revised again after conducting Tier 2 studies, which will capture more detailed information on species presence and composition within the relevant species study areas. The final species-habitat maps will differ from the preliminary species-habitat criteria and maps in that they include the final ratings tables as developed from the additional data collected during field sampling and the field-verified refined ecosite data. The final species-habitat maps will be accompanied by:

1. The revised species account;
2. Revised ratings assumptions; and,
3. The final ratings table developed from:
    - Project species list
    - Standard ratings schemes required for each species
    - Standard life requisites and seasons required for each species
    - Preliminary ratings tables that have been revised from field sampling in future years
    - Field data as summarized in baseline reporting
    - Ecosite modifications and subdivisions based on field-based TEM results.

The final species-habitat ratings maps are typically assigned a reliability qualifier that reflects the level of information available for a species' life requisite, as well as the understanding of the species-habitat relationships, and applicability to ecosystem mapping. The qualifier indicates the accuracy and reliability of the species-habitat ratings and resultant maps. General criteria for assigning a reliability qualifier to species-habitat ratings and habitat maps include (adapted from RISC 1999):

- Low Reliability: Available information used in the habitat ratings is based on studies in other provinces or countries with some or little local information on species-habitat relationships. No verification has been done.
- Moderate Reliability: Available information used in the habitat ratings is based mainly on studies, reports and expertise on the species-habitat relationships gained within Ontario. Some information from ecosystems in the study area, but mostly extrapolated from similar ecosystems. No verification or limited verification has been done.
- High Reliability: Available species-habitat relationship information used in the habitat ratings is based mainly on detailed studies, reports and expertise gained within Ontario and pertaining directly to the ecosystems in the study area. Ratings have been verified. Verification includes

testing the ratings against actual data (e.g., nest records, element occurrence records) or by ground-truthing and sampling.

2.2.3 Field Program

The field program will be undertaken by the field data collection contractor, using rating criteria and desk-based habitat suitability criteria developed by Zoetica and outlined in Section 2.2.2.

2.2.3.1 Survey Timing

The timing of field surveys will be coordinated with the timing of TEM field efforts (Section 2.1.3.1) in July or August when vegetation is the most visible and easily identified. A wildlife biologist will be part of the field team and responsible for conducting the wildlife habitat suitability assessments alongside the vegetation specialists who will be ground-truthing the mapped TEM. During the Year 2 field season, the focus will be on verifying habitat suitability within the LSA_{AQU} (see Section 2.1.2.1 for the rationale for using the LSA_{AQU}). Field-based identification of potential SWH will focus on breeding habitats, permanent features (e.g., mineral licks), MAFAs, and other SWH that are identified through vegetation communities and characteristics. However, some types of SWH may only be identifiable in non-summer seasons (e.g., stopover and staging areas, wintering areas and hibernacula); the potential presence of these SWH can first be investigated through discussions with local stakeholders, rights-holders, and other study teams (e.g., geoscience) on the ground in other seasons. Tier 2 studies can then be designed to help identify and confirm SWH.

In future seasons, the RSA_{VEG} (see Section 2.1.2.1 for the rationale for using the RSA_{VEG}) may be surveyed if warranted. The selection of survey locations will be undertaken once the first field season is complete as the information collected in the field will be used to improve the ecosite mapping in the RSA_{VEG} (see Section 2.1.2.3.2).

2.2.3.2 Survey Crew

The field survey contractor for habitat suitability modelling will be paired with survey crews for TEM (Section 2.1.3). Ideally, each combined survey team will consist of one experienced ecosystem biologist (terrestrial vegetation specialist), one experienced soil specialist, one wildlife biologist with experience in habitat suitability modelling, IK holder with expertise pertinent to habitat suitability and use, and one local field assistant with general knowledge of wildlife and habitat use(if community interest and availability allow). The roles and responsibilities of the vegetation and soil crew and local field assistants are detailed in Section 2.1.3.2.

For habitat suitability modelling, the wildlife biologist will be responsible for the field identification of potential wildlife habitat, and its suitability for seasonal habitat use by identified biodiversity values, including the value of the plot-type for each species on a seasonal basis. The wildlife biologist will also be responsible for recording habitat data on all relevant forms (see SOPs and forms in **Appendix A**). Minimum qualifications for wildlife personnel on a wildlife habitat mapping project include an understanding and experience in wildlife habitat analysis with a preference of knowledge of *BC Wildlife Habitat Rating Standards* (RISC 1999) and knowledge of the habitat needs of wildlife in northwestern Ontario, how those needs relate to ecosite classifications and how ecosite classifications change over time, and an understanding of how to identify and evaluate SWH (OMNR 2000). Additional crew members are responsible for notifying the wildlife biologist in the case where wildlife sign is observed. Knowledgeable field assistants may also assist in applying local knowledge of wildlife seasonal habitat use, where applicable.

Field work will likely require contracted helicopter services. The pilot is not expected to be directly involved in habitat suitability modelling studies but will be relied on to find appropriate landing spots for the survey team. The helicopter may also be required to move field crews from location to location multiple times per day.

2.2.3.3 Equipment and Materials

Standard field gear and data collection materials (e.g., binoculars, camera, notebook, pencil, clipboard) should be carried to record incidental observations of wildlife and wildlife sign (e.g., tracks, scratch marks, bedding sites, signs of feeding, kill sites, animal trails, nests, dens, burrows, and scat), information about site access, or other pertinent notes.

Helicopter services are likely needed to enable cost-effective and efficient coverage of the study area for habitat suitability modelling studies. Ideally, the aircraft will be able to accommodate the TEM and habitat suitability modelling survey crews; however, staggering of crew pick-up and drop-off may be needed and will be determined when additional details about logistics are available. Alternatively, if helicopter services are not used – for example, if it is determined by the NWMO that ground travel to the pre-determined sampling locations is feasible – then other modes of transportation will be required from home base to the study sites. In this case, a truck and/or ATVs will likely be required.

Field work for habitat suitability modelling studies will require general navigational equipment to travel from the helicopter landing spot or road-based access point to the pre-determined sampling site, including GPS unit (also used to record geographic coordinates of important features, and plot locations), compass, and tablet or smartphone with georeferenced digital maps as well as hard copy field maps as a backup. The field data collection contractor will be required to develop a comprehensive health, safety and environmental management plan that is accepted by the NWMO before the initiation of any field activities.

2.2.3.4 Field Protocol

The habitat suitability field protocol will be conducted in coordination with the TEM field methods (Section 2.1.3). Detailed SOPs are provided in **Appendix A**. The species accounts and preliminary ratings tables will be provided by Zoetica before field activities and should be used as references in the field. As data are collected, the preliminary ratings tables and ratings assumptions should be updated and revised if required. Additional sampling may be required to ensure important habitat for selected species is represented.

The field activities for the assessment of habitat suitability are conducted for both full plots and ground plots as outlined in Section 2.1.3.4. Habitat suitability assessments are completed separately for each species based on the habitat requirements for each species for various seasons and life requisites that have been pre-determined by Zoetica as important for rating.

2.2.3.5 Data Collection and Recording

A summary of field methods is outlined in Section 2.1.3.4 and detailed SOPs are provided in **Appendix A** and include field forms and instructions for filling in forms.

<u>Completing the Ecosystem Field Forms</u>

The Wildlife Habitat Assessment Form is part of a suite of Ecosystem Field Forms used to collect ecological mapping data. It is not a stand-alone form but must be used in conjunction with the Site and Soils

Description Form and Vegetation Forms for full plots. The Wildlife Habitat Assessment Form is designed to collect information at a detailed level to develop final habitat suitability ratings for each species and requisite/season.

For each species, habitat is assessed in two ways:

1) the plot type; and,
2) the plot-in-context.

Plot type refers to the combination of site, soil, and vegetation characteristics that describe the plot. Two sampling plots may have the same plot type if they share these characteristics. The plot type assessment is tied to the ecosystem field forms that have the ecosite classifications on which the final ratings table is based. Thus, food/cover ratings developed for the plot type guide developing the final ratings for the project area.

However, in the context of the surrounding habitats and features, plots may have different values to a wildlife species. The plot-in-context assessment provides a record of the habitat features occurring in the project area that have a synergistic effect on the wildlife values and helps in the formulation of rules about the spatial arrangement required for habitat use.

For full plots, all information on the Wildlife Habitat Assessment form must be completed with the exception that wildlife tree and coarse woody debris sections must be completed only for species that require these attributes. For ground inspection plots, the plot-in-context assessment is not required.

Completing the Significant Wildlife Habitat Survey Form

The SWH Survey Form and associated instructions are based on the *Significant Wildlife Habitat Criteria Schedules for Ecoregion 3W* (OMNRF 2017). As described in the Criteria Schedule, different types of SWH are more likely to be present within certain ecosites. Ecosite information can, therefore, help to refine the types of SWH that should be assessed in more detail, while other types of SWH can quickly be screened out. However, since SWH may not exclusively occur in the listed ecosites, the habitat criteria associated with each type of SWH should be reviewed at the survey plot to confirm the likelihood of candidate SWH being present.

As discussed in Section 2.2.3.1, some SWH constitute non-summer seasonal habitats. In these cases, the surveyor should indicate “Unknown” in the Habitat Criteria field of the SWH Survey Form, and provide rationale in the adjacent space on the form explaining that the survey timing was suboptimal for SWH identification. However, any wildlife or wildlife sign observed, especially for species of interest (e.g., SAR, species of importance to local stakeholders/rights-holders) should also be recorded. These plots can then be revisited during the appropriate season (e.g., as a Tier 2 study) to confirm the presence of SWH and/or species of interest.

2.3 Aquatic Habitat Mapping

2.3.1 Overview

Fish and fish habitat are valued by recreational and commercial land users, Indigenous communities, and the general public for social, recreational, commercial, domestic, and spiritual reasons. A popular activity in the region is fishing, highlighting the importance of fish and fish habitat, and the important link between these biodiversity values, ecosystem services, and socio-economic impact considerations. Fish habitat is

protected by the *Fisheries Act* (R.S.C. 1985, c. F-14; updated in 2019) and includes any watercourse, waterbody, or wetland that provides functions for life history stages of fish. Fish species that are listed under the federal *Species at Risk Act* (S.C. 2002, c. 29) or the Ontario *Endangered Species Act* (S.O. 2007, c. 6) may be afforded even greater protections for habitat.

To understand and characterize the aquatic environment surrounding the AOI, aquatic habitat mapping will detail the various ecosystems present in the Revell Batholith Area. Results of these studies will help to support the requirements of the *Impact Assessment Act* and provide the basis for designing additional biodiversity value-specific studies to understand biodiversity in the AOI, and "*to assess the potential adverse and positive environmental, health, social and economic effects and impacts arising from the designated project activities"* as per the TISG Template (IAAC 2019).

2.3.1.1 Data Objectives

The primary data objectives of the aquatic habitat mapping for the BIS are to:

1) Characterize the presence and distribution of fish habitat within the aquatic Local Study Area (LSA_{AQU}) and select control sites within the aquatic Regional Study Area (RSA_{AQU}) under baseline conditions (see Section 2.3.2.1 for study area delineations);
2) Detect any important fish areas including spawning, rearing or potential overwintering habitat in the LSA_{AQU};
3) Assess the potential for SAR habitat within the LSA_{AQU};
4) Evaluate the distribution of habitats within the LSA_{AQU} and select control sites within the RSA_{AQU} to identify suitable sampling sites for fish community characterization studies (Tier 2); and,
5) Provide the required baseline data for the development of a potential monitoring program(s) to address the environmental, regulatory, and stakeholder/rights-holder concerns relevant to the APM Project.

Aquatic habitat mapping will cover the LSA_{AQU} to collect information on the watercourses that may be directly or indirectly (i.e., downstream) influenced by the APM Project and will include the identification of different types of aquatic habitat (e.g., wetland, pond, lake, headwater tributary, stream, river). Additional aquatic habitat mapping may be conducted in select areas within the RSA_{AQU} to establish biologically-relevant control sites. Aquatic habitat mapping forms the foundation on which to build intensive inventories where identification of fish-bearing streams, fish species distribution and physical habitat data are required. Aquatic habitat mapping also provides the ability to identify stream reaches where site-specific detailed inventories are required to determine the presence of fish, and species present.

2.3.1.2 End Use

Aquatic habitat mapping will assist in the selection of suitable locations for future fish and detailed fish habitat baseline (Tier 2) studies. Aquatic habitat mapping can also be used to identify the location of critical and sensitive aquatic and riparian habitats. The products derived from the desk-based and field aquatic habitat mapping (RIC 2001a) will yield watershed-based mapping (1:20,000) showing known fish species presence and predicted distribution, lake characteristics (e.g., surface area, depth), stream reach boundaries and characteristics (e.g., stream width, fish habitat gradient), channel classification, and location and characteristics of obstructions. Aquatic habitat mapping, as outlined in RIC (2001a), can be used to help determine habitat suitability for assessing the potential of watercourses/waterbodies to support fish populations as well as identifying the location of critical and sensitive aquatic and riparian

habitats. Aquatic habitat mapping will be used to help derive the initial selection of best management practices for further studies and to focus decisions regarding additional information requirements (e.g., locations of fish community composition studies). In addition to fish habitat, aquatic habitat mapping will identify stream reaches where suitable riffle habitat exists for future benthic invertebrate collections conducted as part of the EMBP (CanNorth 2020).

2.3.1.3 Best Practice Guidance

The *Reconnaissance (1:20 000) Fish and Fish Habitat Inventory: Standards and Procedures* (RIC 2001a) will be used for desk-based watershed mapping and field verification methods; however, modifications will be made by using Ontario-specific mapping layers (at the 1:20,000 scale) and data requirements for forms outlined in the Ontario Ministry of Transportation's (MTO) *Environmental Guide for Fish and Fish Habitat* (MTO 2009). The RIC standards are designed to assess desk-based aquatic habitat mapping at a more detailed scale (1:20,000) relative to methods described by MTO (1:50,000) and are generally more comprehensive as they are directed at full inventory methods and not focused on highway and transportation projects as described in MTO (2009).

The mapping portion of the *Reconnaissance Fish and Fish Habitat Inventory* (RIC 2001a) is intended to provide information on potential fish distributions and stream reach and related biophysical data for interpretation of habitat sensitivity and suitability for fish production. The approach includes a desk-based mapping application, similar to that conducted during preliminary environmental studies for the APM Project (see Section 1.5.4), as well as field survey to supplement mapping by verifying connectivity between waterbodies and identifying potential ephemeral streams and waterbodies, pooled areas, and to collect more detailed habitat characteristics at a selection of reaches.

2.3.2 Desk-based Program

The *Reconnaissance Fish and Fish Habitat Inventory* (RIC 2001a) includes a pre-field preparation and planning component whereby information from maps and air photos is collected to build an initial aquatic habitat map classifying watercourses and waterbodies, including stream reaches, lakes, ponds, and wetlands. This section outlines the methods and approaches that Zoetica will follow for the preparation of interim maps for planning and field survey purposes. The desk-based program will be conducted by Zoetica before the implementation of the field survey conducted by the field data collection contractor in spring of Year 2.

2.3.2.1 Study Area

Aquatic habitat mapping for the collection of fish habitat information will be conducted at two scales: within the local study area, defined as the LSA_{AQU}, and within select areas of the regional study area, defined as the RSA_{AQU} (**Figure 2-4**) to capture appropriate control sites. The area for the collection of fish habitat information is typically much larger than the immediate project footprint area and considers aquatic connectivity, water flow direction, and linkages that extend well beyond the boundary of the project footprint. In determining the appropriate study areas, guidance outlined in the MTO's *Environmental Guide for Fish and Fish Habitat* (MTO 2009) was followed and includes the following considerations:

- The study area must encompass all waterbodies that potentially support fish and fish habitat that could potentially be affected by the APM Project.
- Aquatic ecosystems are continuous and inter-connected systems and fish and habitat variables (e.g., sediment, bedload, nutrients, food, cover) move throughout and along the fluvial

continuum. Therefore, the study area should be large enough so that the background information encompasses inter-connected waterbodies and adjacent portions or reaches of waterbodies.
- Knowledge about fish or habitat conditions in adjacent areas can be used to interpret or identify potential for specific fisheries or habitat conditions in the immediate project area.
- Impacts in the immediate project area can be transferred to adjacent areas, potentially affecting fish and habitat in those areas.
- Flexibility for the APM Project is maintained, particularly in planning and siting surface infrastructure, road alignments (and other linear corridors), and associated waterbody crossings.
- Information from the broader area provides context to assess relative representation and the importance of a particular type of habitat.

The LSA_{AQU} includes the watercourses and waterbodies with the potential of being impacted by the APM Project. The LSA_{AQU} is defined by the waterbodies contained within a 1 km buffer around the AOI plus Mennin Lake and the Mennin River drainage, as it is assumed that Mennin Lake could be used as a source for water withdrawal as well as potential discharge (CanNorth 2020). The waterbodies contained within the AOI for the APM Project generally flow to the southwest and contribute flow to Mennin Lake (CanNorth 2019a). The northwest area of the AOI crosses the drainage basin boundary between the Revell River and the Mennin River basins, both of which eventually flow into Wabigoon Lake via the Wabigoon River. The Revell and Mennin Rivers provide the largest flows in the area and are likely to be used as sources for water withdrawal and assimilating effluent discharges for the APM Project (CanNorth 2019a), and effects to water chemistry or hydrology within the AOI could potentially influence downstream areas. Thus, the LSA_{AQU} also includes the watershed drainage for the Revell River north of the AOI to the confluence with the Wabigoon River and includes the Wabigoon River to its confluence with the Mennin River. Areas within these drainages that are upstream of the potential for effects from the APM Project were eliminated from the boundary of the LSA_{AQU}.

The RSA_{AQU} covers a much larger area, which includes a larger boundary surrounding the LSA_{AQU}, outside of the direct influence of the APM Project but within the Wabigoon watershed (**Figure 2-4**). The RSA_{AQU} is designed to be large enough to provide a regional context of habitat in the Revell Batholith Area, provide an area within which suitable control sites can be selected, and to account for the potential need for the consideration of the movements of wide-ranging species, such as lake sturgeon (if found in the area using eDNA. Lake sturgeon is a SAR that can move up to 400 km in Ontario; Rusak and Mosindy 1997, Golder Associates Ltd. 2011). It is possible, though unlikely, that Lake Sturgeon could interact with the LSA_{AQU} at certain times of the year or during periods of its life cycle (e.g., spawning), if present in the larger Wabigoon watershed. The RSA_{AQU} also accounts for the area needed to accommodate the study of cumulative effects on fish and fish habitat.

NWMO Biodiversity Impact Studies

Aquatic Habitat Mapping and eDNA Study Areas (LSA_{AQU} and RSA_{AQU})

Figure 2-4

- Regional Study Area (RSA_{AQU})
- Local Study Area (LSA_{AQU})
- Area of Interest (AOI)
- Wetland
- Lake
- Watercourse
- Quaternary Watershed
- Tertiary Watershed
- Highway or Arterial Road
- Local Road
- Resource/Recreation Road
- Trail
- Railroad
- Community
- Wabigoon Lake No. 27

1:375,000

0 5 10 km

ZOETICA

Inset Basemap © OpenStreetMap contributors

Data received from:
Ontario Geohub - Indian Reserve (MNRF), OHN Waterbody (MNRF), OHN Watercourse (MNRF), ORWN Railway (MNRF), MNRF Road Segments (MNRF), Watershed Tertiary (MNRF), Watershed Quaternary (MNRF), Wetlands (MNRF)
NWMO - AOI, Trail Segments (MNRF)

Project CRS: NAD83 / UTM zone 15N

August 14, 2020 | Map ID: NWMO_BIS_014b

The RSA_{AQU} is defined by the southern portion of the Wabigoon tertiary watershed boundary clipped to the northwest side of Wabigoon Lake. As human population size increases in the area due to the APM Project and other potential projects, fishing pressure in lakes in the surrounding area may be impacted. Within the RSA_{AQU}, four lakes (Wabigoon, Dinorwic, Melgund, and Raleigh) have been identified as popular recreation areas that may experience fishing pressure with an increased human population. Additional lakes outside of the RSA_{AQU} (e.g., Basket, Kukukus, Mameigwess, Indian, Barrel, Paguchi, and Cecil) have been identified as popular areas for fishing and may also be important to consider if human population growth resulting from the APM Project is expected to lead to more fishing and use in those areas. Other lakes identified as important for additional ecosystem services (e.g., as a water source) have been included for potential study. The RSA_{AQU} includes the watershed boundaries or the sub-basins of the tributaries to and including Revell Lake and its outflow, as these may provide suitable control sites for Mennin Lake and its drainage (CanNorth 2020). The size of the RSA_{AQU} may be refined as more is learned about the potential for downstream effects due to the APM Project.

2.3.2.2 Data Sets

The first steps described in the *Reconnaissance Fish and Fish Habitat Inventory* (RIC 2001a) are the desk-based project planning phases, which will be conducted by Zoetica. **Figure 2-5** demonstrates the steps in the data review and desk-based project baseline program, adapted from RIC (2001a).

The first step in the development of an aquatic habitat mapping inventory is the review of all existing fish and fish habitat information. The most relevant information on fish and fish habitat in Ontario is data collected by the MNRF. Additional sources of information will be reviewed based on, but not limited to, references outlined in the *Environmental Guide for Fish and Fish Habitat, Section 3: Background Data Collection* (MTO 2009).

Base Map (1:20,000 Stream Network): The 1:20,000 base map and drainage that will be used for the project needs to be identified. Zoetica proposes that the Ontario Hydro Network (1:20,000) watercourse mapping (from Ontario GeoHub) be used.

Watercourse/Waterbody Referencing: All streams on the 1:20,000 base map need to be identified using a unique watercourse/waterbody identifier, which is used on all data forms (see Aquatic Habitat Mapping SOP in **Appendix A**). The following watercourse/waterbody information will be used to reference all waterbodies in the aquatic study areas (adapted from RIC 2001a):

- *Watercourse/Waterbody Identifier*: This will include an alpha-numeric string of characters that uniquely identifies a watercourse/waterbody within a watershed.
- *Gazette Name*: Official name of the waterbody as listed in the Gazetteer of Canada for Ontario. Use 'unnamed' if the waterbody is not gazetted.
- *Alias*: Locally used name for the waterbody and can be obtained from MNRF archives or local sources.
- *Geo-referencing*: The Universal Transverse Mercator (UTM) coordinate is used to identify the location of a lake, pond, stream, or wetland, and/or survey site. UTMs are recorded as three sets of numbers: zone – easting – northing, separated by periods, and can be obtained from the 1:20,000 map or Global Positioning System (GPS).
    - For stream reaches, use UTM of upstream reach break.
    - For survey sites in stream reaches, record UTM coordinates of the downstream end of the site.

  - For waterbodies (lakes, ponds, wetlands), the UTM normally refers to the location of the inlet stream on the waterbody. If more than one inlet exists, the main inlet is used, and in cases where no inlet exists, the UTM of the geographic centre of the waterbody is used.
  - For survey sites in waterbodies, UTM coordinates are recorded from the approximate centre of the site.
  - For features with a linear extent, UTM is for the downstream end.
- *Numeric Identifiers*: Each mapped feature e.g., reach breaks, survey sites) must be location referenced. This will be done by recording the UTM of the feature, or by assigning a unique numeric identifier (NID) to the feature. This can be recorded as a waypoint number using a GPS unit.
  - Record the NID for a mapped feature on the interim map.
  - All data forms (e.g., reach tables, survey forms; see Aquatic Habitat Mapping SOP in **Appendix A**) include the NID field.

Desk-based aquatic habitat mapping will be conducted using available aerial imagery and the desk-based refined ecosite dataset (see Section 2.1.2.3.1), as the classification data for wetlands based on the ELC system (ELC Working Group 2009) will be one of the datasets used to delineate reaches.

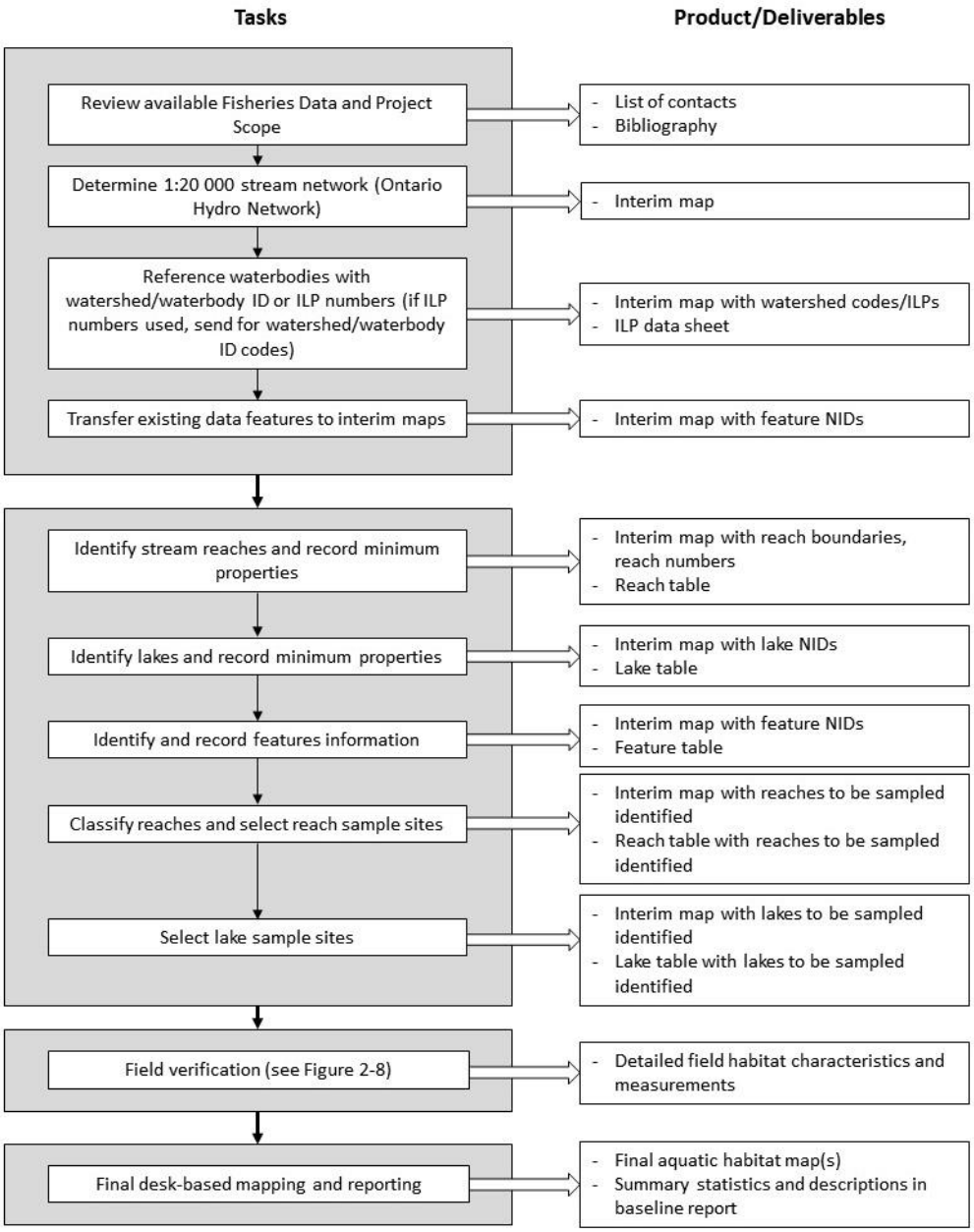


Figure 2-5. Flowchart of the tasks and products in the desk-based aquatic habitat mapping phase of the APM Project. Adapted from RIC (2001a).

2.3.2.3 Methods

All relevant features and information collated during the desk-based review phase conducted by Zoetica include:

- Previous fish sampling and distribution information including sampling sites, known upstream and downstream distribution limits;
- Waterfalls that may act as obstructions to fish movement;

- Chutes or cascades that may potentially act as obstructions;
- Culverts and other stream crossings that potentially alienate fish habitats;
- Major beaver dams;
- Logjams and sediment wedges;
- Landslides or major erosional events that affect the channel;
- Evidence of subsurface flow;
- Enhancement activities; and,
- Other information that may affect the survey objectives and plan.

After the data review phase, interim maps will be prepared by Zoetica following standards outlined in the *Standards for Fish and Fish Habitat Maps* (RIC 2001b) with the adaptations described in this protocol. These interim maps are to be used throughout the field survey by the field data collection contractor and will include:

- Watercourse/waterbody codes for all waterbodies with information (streams, lakes, and connected wetlands); and,
- Features from data review, referenced with NID.

2.3.2.3.1 Classification and Survey Design

All waterbodies and watercourses in the LSA_{AQU} will be classified and mapped using maps and air photo analysis. The steps in classification and survey design are presented in **Figure 2-5**. Additional waterbodies and watercourses in the RSA_{AQU} may also be classified, for areas that will be selected as control sites. It is not necessary to survey all waterbodies and watercourses in the LSA_{AQU}, as the classification of water features in the LSA_{AQU} allows for the extrapolation of information collected in the field to reaches that were not surveyed. However, due to greater probability of project effects acting on aquatic habitats nearer to the potential project, a higher proportion of aquatic habitat will be surveyed within the AOI, with a lower proportion further away.

The *Reconnaissance Fish and Fish Habitat Inventory* (RIC 2001a) provides a framework to classify stream reaches, lakes, and wetlands, to allow for the implementation of a design to survey within these various waterbody types based on a stratified approach. Reach channel types and habitat characteristics within each class are assumed to be consistent. Field survey locations within these reaches and habitat types will be selected after desk-based waterbody classification (Year 1) and before field data collection beginning in spring of Year 2 using a statistical randomized design (e.g., GRTS; see Section 1.9.1). Additional sites will be added to help characterize potential fish species presence and distribution limits in reaches containing barriers (e.g., above and below falls) and areas where potential impacts require being assessed at specific locations (e.g., near proposed project infrastructure).

The 1:20,000 base maps are used for planning purposes and data collection and provide the base to display data. Aerial imagery at a scale of 1:20,000 will be used to classify watercourses/waterbodies and delineate reaches, while the wetland classification from the desk-based refined ecosite dataset (see Section 2.1.2.3.1) will be used to classify wetlands. These data sets will be combined with overland trail and roads to determine access and plan field logistics. A helicopter reconnaissance survey will need to be conducted by Zoetica and potentially CanNorth to assess the accuracy of reach delineation, to determine locations of habitat that cannot be identified using desk-based maps and imagery interpretation (e.g., transitions from riffle/run to pool habitat), and to inform changes to survey design (e.g., potential reselection of survey sites). Refinements to field maps will be made soon after return such that the field

data collection contractor can be provided with more accurate maps for habitat data collection at each selected survey reach. Immediately before habitat data collection, the field data collection contractor should also conduct a reconnaissance survey to plan field access and choose representative survey locations within reaches. Seasonal changes in flow (e.g., ephemeral habitats), and recent potential barriers to fish passage (e.g., new beaver lodges) not previously identified, should also be noted.

2.3.2.3.1.1 Watercourse/Waterbody Identification

During initial desk-based mapping conducted by Zoetica, all water features within the LSA_{AQU} will be identified as streams, lakes/ponds, or wetlands. Waterbody identification is required for the basis of the development of stream reach and waterbody reach tables. Each waterbody (lakes, ponds, and connected wetlands) will be considered as a separate reach for survey designation. The following describes the defining characteristics of each waterbody type (RIC 2001a):

Stream reach: a relatively homogenous length of a stream, that flows on a perennial or seasonal basis, having a continuous channel bed and demonstrating fluvial processes (e.g., flowing water has scoured the channel bed or deposited any amount of mineral alluvium within the channel).

Lake: an open waterbody with a depth greater than 2 m and with less than 25% of its surface area covered with wetland vegetation.

Pond: is a small body of still water formed naturally or by hollowing or embanking. Ponds differ from lakes in that they do not have an atrophic zone (an area deep enough that sunlight can't reach the bottom).

Wetland: an area where the water table is at, near, or above the surface, or where soils are saturated with water for sufficient time such that the principal determinants of vegetation and soil development are excess water and low oxygen.

Waterbodies less than 2 m deep are considered wetlands and may not be distinguishable from lakes using air photos alone. Thus, all shallow open water wetlands are considered part of waterbody surveys unless there are distinct 'channels' flowing through them, in which case they are considered as part of the stream surveys.

2.3.2.3.1.2 Stream Reach Identification

Stream reach identification through desk-based mapping is required for the selection of field survey sites. Stream reaches are relatively homogenous lengths of a stream composed of repeating structural characteristics and fish habitat types (e.g., riffle, run).

Stream reaches should be defined by a minimum length of 100 m (0.5 cm on a 1:20,000 scale map or air photo). Stream reaches are delineated using all available sources including, at minimum, the most recent air photos and maps at a scale no smaller than 1:20,000. **Table 2-13** identifies the physical factors used to determine reaches based on their uniformity and reach boundaries are defined by changes to these attributes. Obstructions or potential barriers to fish passage are considered reach boundaries if they meet the following:

- Are less than 100 m or 10 times the bankfull width in length (if longer, they are defined as reaches); and,
- Are consistent with changes in physical criteria listed in **Table 2-13** (e.g., falls with characteristics that are different from both upstream or downstream reaches). Reach breaks mark the boundary between adjoining reaches.

Table 2-13. Stream reach identification classifiers.

Reach determinations (uniformity)	Reach boundaries defined by:
Channel pattern	Changes in the stream channel (e.g., changes from a single channel to braided or multiple channels, or significant tributary confluences)
Channel confinement	Changes in confinement pattern (e.g., a change from a wide floodplain to a confined canyon)
Gradient	Changes in gradient
Stream size	Stream order (using Strahler stream order; Strahler 1957)
Stream habitat attributes	For stream channels that run through wetlands, changes in wetland type or change in the size of the wetland surrounding the stream
Streambed and bank material	Changes in streambed and bank materials (e.g., a change from erodible to non-erodible materials)

Each reach on a stream is assigned a unique number, in an upstream-ascending order, with the first reach situated closest to the mouth of the stream. If reaches need to be broken into smaller reaches based on field observations (e.g., a feature is not identifiable on air photo), numbers are assigned following decimals (e.g., 1, 2, 2.1, 2.2, 2.3, 3, …), limiting the number of changed reach identifiers. Details on reach numbering can be found in *Standards for Fish and Fish Habitat Maps* (RIC 2001b) and **Figure 2-6**.

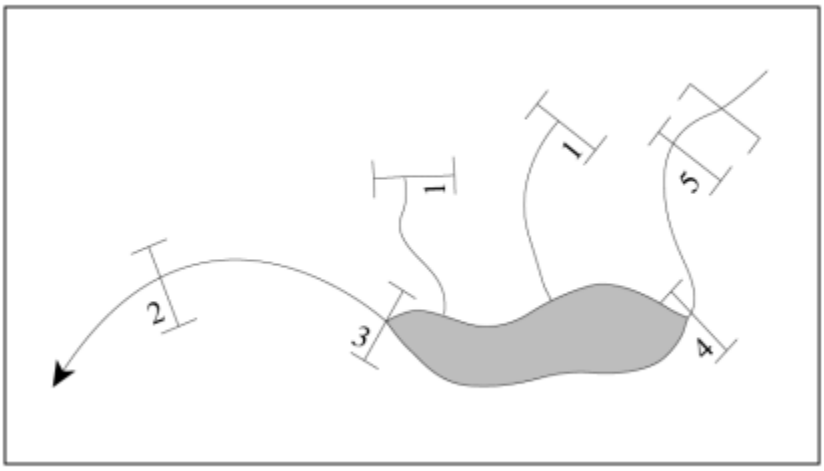


Figure 2-6. Reach numbering system. Bars represent reach breaks. Each tributary resets at a reach number of 1. From RIC (2001b).

A preliminary stream reach table including all stream reaches in the LSA_{AQU}, as well as reaches for control sites within the RSA_{AQU} (e.g., reaches along the Revell and Mennin Rivers upstream of the AOI and LSA_{AQU}) will be derived by Zoetica to record general physical data for each reach using desk-based information derived from aerial photography and refined ecosite mapping (see Section 2.1.2.3.1). For pre-field planning purposes, the information in the stream reach table will be used to determine sample size (i.e., the subset of stream reaches to be surveyed).

The following preliminary information will be gathered in the stream reach table:

a. Watercourse ID (a unique ID generated for each watercourse in the LSA_{AQU})
b. Watercourse name (gazetted name and/or alias)
c. Stream order
d. Drainage system
e. Reach number (determined through mapping)
f. GRTS number (assigned by desk-based stratified random reach selection)
g. UTM (zone, easting, northing, GPS unit make/model and uncertainty)
h. NID (a unique number for identification of features; e.g., reach breaks, survey site, obstacles)
i. Upstream elevation
j. Downstream elevation
k. Length
l. Gradient
m. Pattern
n. Confinement
o. Wetland and type

Referencing information (a to g) is consistent with that used on aquatic habitat mapping forms (see Aquatic Habitat Mapping SOP in **Appendix A**). The reference number (NID) is a user-defined number to aid in the identification of the reaches for surveying. Stream order and gradient are map derived data. The determination of stream order will include all identified channels as shown in **Figure 2-7**. Gradient is calculated using map-based measurements of upstream and downstream elevations of the reach and reach length. Pattern and confinement will be determined from maps and air photos. All connected wetlands will be identified in the stream reach table. Wetland type will be filled in based on descriptors in Section 2.3.2.3.1.3. Only shallow open water wetlands with distinct 'channels' flowing through them are considered as part of the stream surveys; those without 'channels' are considered as part of the waterbody surveys and recorded in the waterbody reach table (Section 2.3.2.3.1.3).

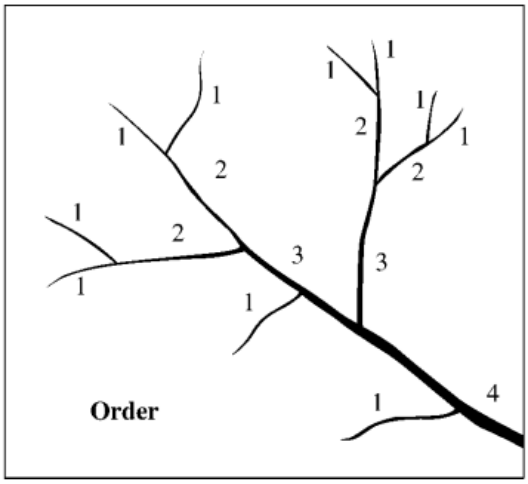


Figure 2-7. A visual graphic of the determination of stream order using Strahler Order (Strahler 1957). Adapted from RIC (2001a).

Determining Minimum Stream Reach Sample Size

The following example and criteria in **Table 2-14** will be followed by Zoetica after desk-based aquatic habitat mapping is completed to determine the appropriate sample size for reaches to be surveyed in the field. Desk-based aquatic habitat mapping is underway and will be completed in Year 1.

Table 2-14. Example of reach totals and sample size from RIC (2001a).

Reach Totals:					**Sample Size:**				
Gradient	Pattern	Size			Gradient	Pattern	Size		
		Small	Med.	Large			Small	Med.	Large
1	ST/SI	3	4	11	1	ST/SI	2	2	2
	IM/ME	9	7	5		IM/ME	2	2	2
	AN/BR	0	0	0		AN/BR	0	0	0
2	ST/SI	10	15	1	2	ST/SI	2	3	1
	IM/ME	4	10	0		IM/ME	2	2	0
	AN/BR	0	0	0		AN/BR	0	0	0
3	ST/SI	115	56	1	3	ST/SI	12	11	1
	IM/ME	0	6	0		IM/ME	0	2	2
	AN/BR	0	0	0		AN/BR	0	0	0
4	ST/SI	70	11	0	4	ST/SI	7	2	0
	IM/ME	0	0	0		IM/ME	0	0	0
	AN/BR	0	0	0		AN/BR	0	0	0
5	ST/SI	54	5	0	5	ST/SI	0	0	0
	IM/ME	0	0	0		IM/ME	0	0	0
	AN/BR	0	0	0		AN/BR	0	0	0
Total =	397				Sample =	57			
					% =	14.4			

Grad. Class	Gradient (%)	Size	Order	Pattern	Description
1	≤4	Small	1	ST/SI	Straight, sinuous and irregular wandering type reaches
2	>4 and ≤8	Medium	2 and 3		
3	>8 and ≤20	Large	≥4	IM/ME	Irregular meandering, meandering, and tortuous meandering type reaches
4	>20 and ≤30				
5	>30			AN/BR	Anastamosed or braided

- For lower gradient (<20%) and small or medium stream (third-order or lower), base the sample size on the equation y = 500 ($X^{-0.8}$), where X is the number of reaches of a certain group, and y is the survey site proportion.
- For higher gradient streams (20 – 30%) or large streams (fourth-order or higher), the sample size is the lower of the results of the equation listed above or 10%.
- For high gradient streams (>30%), survey when warranted (e.g., when fish are suspected to occur in a reach with a 32% gradient). Surveying in this group is based on professional judgement.

The following standards will be used to calculate the minimum and maximum sample size of stream reaches:

- For lower gradient or small/medium-sized streams, the minimum is the lower of the 25%, or the result of the equation. If this results in a value less than two, then two is used as the sample size. If there is only one reach of that type, then the sample size is one.
- For higher gradient streams or large streams, the minimum sample size is two, and the maximum sample size is 25.

Following sample size determination, to be completed by Zoetica in Year 1, reaches to be surveyed will be selected randomly (e.g., GRTS analysis; see Section 1.9.1) and identified on a working map. Additional reaches, including discretionary additions, may also be included to address the following:

- Above and below barriers (to determine fish presence);
- Adjacent to identified cutblocks;
- Major inlets and outlets to lakes; and
- Any identified areas potentially impacted by the APM Project.

Approximately 15 to 30% of lower gradient reaches (<20% gradient) should be identified for surveying with the lower end applied to larger project areas (RIC 2001a). For extremely large project areas, such as the RSA_{AQU}, survey rates may be below 5% and should be increased to 5 to 15%, where possible (RIC 2001a). Zoetica suggests surveying 100% of the stream reaches within areas potentially impacted directly by the APM Project (within the AOI, and including the Mennin and Revell Rivers), 30% within the LSA_{AQU}, and 5 to 15% within select areas used as control sites in the RSA_{AQU}.

Once the final reaches for field surveying have been selected by Zoetica, a modified stream reach table including only those reaches to be sampled as well as a list of contingency reaches, and a map to direct survey locations will be provided by Zoetica to the field data collection contractor (see Aquatic Habitat Mapping SOP in **Appendix A**). Additional information in the stream reach table will be filled in by the field data collection contractor at the time of field surveying (e.g., expected fishing gear required). The expected gear type can be filled out in the field once habitat characteristics are known to help guide the fisheries field program in Tier 2.

Once desk-based aquatic habitat mapping has been verified in the field (see Section 2.3.3), a re-assessment will be conducted to ensure that enough stream reaches have been surveyed in each habitat type to achieve statistical power to detect a small to medium change (see Section 1.9.2) and any adjustments required (e.g., additional surveying) will be made the following year.

2.3.2.3.1.3 Lake, Pond, and Wetland Identification

A lake is an open waterbody with a maximum depth greater than 2 m and with less than 25% of its surface area covered with wetland vegetation. Open waterbodies that do not fit these criteria are considered wetlands. Wetlands are classified into the following four classes/types based on the Ontario Wetland Ecosystem Classification System for Northern Ontario (from MNRF 2014a):

- *Marsh:* Marshes are wet areas periodically inundated with standing or slowly moving water, and/or permanently inundated areas characterized by robust emergent, and to a lesser extent, anchored floating and submergent plants. Marsh wetlands have mineral and sometimes well-decomposed peat soils. When peat soils are present, they are often enriched with mineral

materials. Waters are nutrient-rich with near neutral to basic pH. Surface water levels typically fluctuate seasonally with declining levels exposing matted vegetation or mudflats. Emergent vegetation includes grasses, cattails, sedges, rushes, and reeds which cover more than 25% of the wetland surface. Note: Shallow open water wetlands classified by the Canadian Wetland Classification System are lumped with marshes in the Ontario Wetland Evaluation System (MNRF 2014a).

- *Swamp*: Swamp wetlands are wooded wetlands with 25% cover or more trees or tall shrubs. In swamps standing or gently flowing water occurs seasonally or may persist for long periods, and have an abundance of pools and channels. Many are typically flooded in spring, and dry with relict pools later in the season. Swamps include forest swamps (having mature trees) and thicket swamps (having shrub-carrs characterized by thick growths of tall shrubs such as willow, red-osier dogwood, buttonbush and speckled alder). There is a pronounced internal water movement from adjacent mineral areas, making the water nutrient-rich. If peat is present, it is mainly well-decomposed wood and occasional sedges. The vegetation is typically dominated by coniferous or deciduous trees or dense shrubs and herbaceous species.
- *Fen*: Fens are peatlands characterized by surface layers of poorly to moderately decomposed peat, often with well-decomposed peat near the base and less than 25% live tree cover. Fen peats usually consist of mosses and sedges. Two main types of fens exist: nutrient-rich fens are fed by groundwater and have a high pH, and can be dominated by sedges and grasses; and nutrient-poor fens are fed by less groundwater and have lower pH, and may consist of low shrubs or ericaceous (belonging to or similar to heath family, genus *Erica*) species. Fens have a higher diversity of plants compared to bogs. Fen wetlands have organic soils and a water table at or above the surface. Soils are primarily moderately to well-decomposed sedge and non-sphagnum moss peats. Waters are mainly nutrient-rich with a near neutral to slightly acidic pH. The vegetation consists primarily of sedges, grasses, reeds, mosses, and some shrubs. Scattered trees may be present.
- *Bog*: Bogs are peat covered areas or peat-filled depressions with a high water table and a surface carpet of mosses, primarily sphagnum. Raised peat hummocks are present in bogs and the wetland is ombrotrophic (i.e., dependent on atmospheric moisture for its nutrients). Bogs have low plant diversity with less than 14 species and few to no fen indicator plant species and few or no tamaracks or eastern white cedar. Bog wetlands have organic soils with a water table at or near the surface. Soils are predominantly composed of poorly to moderately decomposed sphagnum moss peats. The bog surface is usually unaffected by groundwaters and thus waters are generally acidic and low in nutrients. Bogs are usually covered with sphagnum mosses and ericaceous shrubs, and may be treed or treeless but with less than 25% tree cover.

All reaches, including lakes, ponds, and wetlands, will be numbered by Zoetica during desk-based mapping. Lakes, ponds, and wetlands are considered reaches for the survey and are assigned a unique number in sequential, upstream-ascending order, consistent with the stream reach numbering system.

A preliminary waterbody reach table will be created by Zoetica to record all lake, pond, and wetland reaches in the LSA_{AQU} and select control sites within the RSA_{AQU}, and general physical data will be recorded for each reach using desk-based information derived from aerial photography and refined ecosite mapping (see Section 2.1.2.3.1). The following information will be gathered in the preliminary waterbody reach table:

a. Waterbody ID (a unique ID generated for each lake, pond, or wetland in the LSA_{AQU})

b. Waterbody name (gazetted name and/or alias)
c. Tertiary watershed name
d. Waterbody type (lake, pond, or wetland)
e. Reach number (determined through mapping)
f. GRTS number (assigned by desk-based stratified random reach selection)
g. Wetland type
h. NID (a unique number for identification of features; e.g., reach breaks, survey site, obstacles)
i. UTM (zone, easting, northing, GPS unit make/model)
j. Genesis
k. Number of inlets
l. Number of outlets
m. Inflow elevation (of the primary inflow)
n. Outflow elevation (of the primary outflow)
o. Aspect

The preliminary waterbody reach table is used to facilitate the selection of waterbodies to be surveyed in a watershed. For pre-field planning purposes, the information in the waterbody reach table is used to determine sample size (i.e., the subset of waterbody reaches to be surveyed).

Determining Minimum Waterbody Sample Size

All waterbodies will be surveyed within the AOI, as well as Mennin Lake within the LSA_{AQU}, and a selection of waterbodies in the RSA_{AQU} (including Revell Lake) will be surveyed to act as control site waterbodies and will be chosen based on a paired BACI design matching habitat types and conditions within the LSA_{AQU} to representative areas in the RSA_{AQU}. More than one control site will be used to ensure that control sites remain if others are impacted by activities such as forestry, forest fires, or development in the area over time). Justification will be provided by Zoetica in the comments in the waterbody reach table when a selected waterbody in the RSA_{AQU} is to be surveyed.

Once the final reaches have been selected for field surveying using a stratified sampling design (e.g., GRTS analysis; see Section 1.9.1), an updated waterbody reach table with more detailed information will be completed by Zoetica in Year 1 and distributed to the field data collection contractor along with a map of reaches that will be surveyed in the field (see Aquatic Habitat Mapping SOP in **Appendix A**). Additional information in the waterbody reach table will be filled in by the field data collection contractor at the time of field surveying (e.g., expected fishing gear required). The expected gear type can be filled out in the field once habitat characteristics are known to help guide the fisheries field program in Tier 2.

2.3.3 Field Program

This section describes the requirements for field verification of aquatic habitat mapping and fish habitat assessments that will be conducted by the field data collection contractor. Field methods are focused on checking the accuracy of the reach delineations derived via desk-based mapping products (outlined in Section 2.3.2.3.1) and include detailing habitat characteristics that cannot be sufficiently addressed using aerial imagery alone. Field methods in Tier 1 are also aimed at collecting fish habitat information that can inform fish habitat suitability for species presence and distribution during various life history stages, to inform additional future sampling requirements for fish presence and community surveys to be conducted in future assessments (i.e., Tier 2 studies), to identify where more detailed fish and fish habitat

assessments will likely be required (e.g., SAR habitat, the habitat that will be lost due to the APM Project), and to identify potential areas suitable for compensation (if required).

2.3.3.1 Survey Timing

The standard survey period for aquatic habitat mapping in the field is during the summer low flow periods (May – September; MTO 2009) to characterize stream habitat information. Conducting surveys during summer low flow periods ensures optimum visibility into the water and that seasonal aquatic and riparian vegetation is present (MTO 2009). Measurements of temperature regimes during low flow periods can be used to confirm or determine the potential use of waterbodies and watercourses by cold water species (MTO 2009). Surveying during low flow periods can also effectively determine flow permanence, baseflow conditions, and connectivity of refuge habitats (MTO 2009). Field visits conducted in the spring can also assist in mapping non-permanent waterbodies, and should be conducted where ephemeral streams and waterbodies are suspected, and where non-permanent waterbodies could act to connect other permanent waterbodies for part of the season. Survey timing should be conducted during weather conditions suitable to allow for good visibility through the water column. Limnological surveying (conducted by the EMBP) should be carried out as near to mid-day as possible.

Helicopter services will likely be required to cover the entire study area efficiently. It should be acknowledged that weather days may delay the field data collection schedule if general flight conditions are not satisfied (e.g., heavy fog or precipitation reducing visibility).

In light of current events (COVID-19 pandemic), field-based aquatic habitat mapping studies will likely not begin until spring of Year 2.

2.3.3.2 Survey Crew

Each survey team may consist of two experienced fisheries biologists familiar with fish habitat identification and survey methods in Ontario, including limnology and water chemistry measurements, bathymetry, and general knowledge of aquatic plant identification; and one local field assistant (if community interest and availability allow). Alternatively, one fisheries biologist familiar with limnology, water chemistry, and bathymetry; one vegetation specialist with expertise in identification of aquatic vegetation; and one local field assistant can comprise each field crew team. At each site, the field crew lead (fisheries biologist on the team with the most experience) will be responsible for directing the field activities. The field lead must be familiar with all requirements of field collection outlined in Section 2.3.3.4, including the identification of wetland types as per the *Ontario Wetland Evaluation System: Northern Manual* (MNRF 2014a), and knowledgeable in contingency planning where selected sites are inaccessible, or issues in data collection arise. One fisheries biologist will be responsible for recording data on field forms and mapping habitat (see Aquatic Habitat Mapping SOP in **Appendix A**), and one fisheries biologist or vegetation specialist and a field assistant will be responsible for conducting waterbody measurements. Since bathymetry and water chemistry will be recorded as part of the EMBP (CanNorth 2020), field crews for the fish habitat program may be the same as those identified for the EMBP. Alternatively, if bathymetry crews are not fisheries biologists, bathymetry and limnology measurements may be conducted separately from fish habitat measurements using two separate crews.

This field work will likely require contracted helicopter services. The pilot is not expected to be directly involved in fish habitat (watercourse/waterbody mapping) studies but will be relied upon to find appropriate landing spots when transporting the survey team and equipment from location to location.

2.3.3.3 Equipment and Materials

Equipment and materials required for bathymetry, limnology and water chemistry are described in the EMBP (CanNorth 2020). A water quality meter will be required to measure water (and air) temperature, pH, conductivity, and dissolved oxygen at locations where full bathymetry and limnology are not collected. If it is feasible, water quality instruments can be coordinated between the BIS Program and the surface water sampling program of the EMBP. Survey equipment specific to habitat data collection includes a clinometer, or instrumentation such as an Abney level or a more accurate measuring device capable of measuring low watercourse gradients, a metre stick for depth measurements, and measuring tape (roll). Standard field gear and data collection materials (e.g., binoculars, camera, notebook, pencil, clipboard) should also be carried to record incidental observations, information about site access, or other pertinent notes. For field portion of aquatic habitat mapping, the field crew will require general navigational equipment to travel from the helicopter landing spot to the pre-determined survey site, including GPS unit (also used to record geographic coordinates of reach locations and important features), compass, and tablet or smartphone with georeferenced digital maps as well as hard copy field maps as a backup.

As discussed, helicopter services are likely needed to enable cost-effective and efficient coverage of the LSA_{AQU} and select control sites in the RSA_{AQU} for aquatic habitat mapping studies. Ideally, the aircraft will be able to accommodate the aquatic habitat mapping survey crews; however, staggering of crew pick-up and drop-off may be needed and will be determined when additional details about logistics are available. Helicopters may also be required to move equipment (boats or field equipment) to survey locations. Alternatively, if helicopter services are not used – for example, if it is determined by the NWMO that ground travel to the pre-determined survey locations is feasible – then other modes of transportation will be required from home base to the study sites. In this case, a truck and/or ATVs will likely be required.

2.3.3.4 Field Protocol

The field protocol for aquatic habitat mapping surveys is divided into field survey methods for watercourses (including wetlands with distinct ‘channels’ flowing through them) and field inventory methods for waterbodies such as lakes, ponds, and wetlands (all except for those with distinct ‘channels’ flowing through them). However, the general process for field survey is the same and is outlined in **Figure 2-8**.

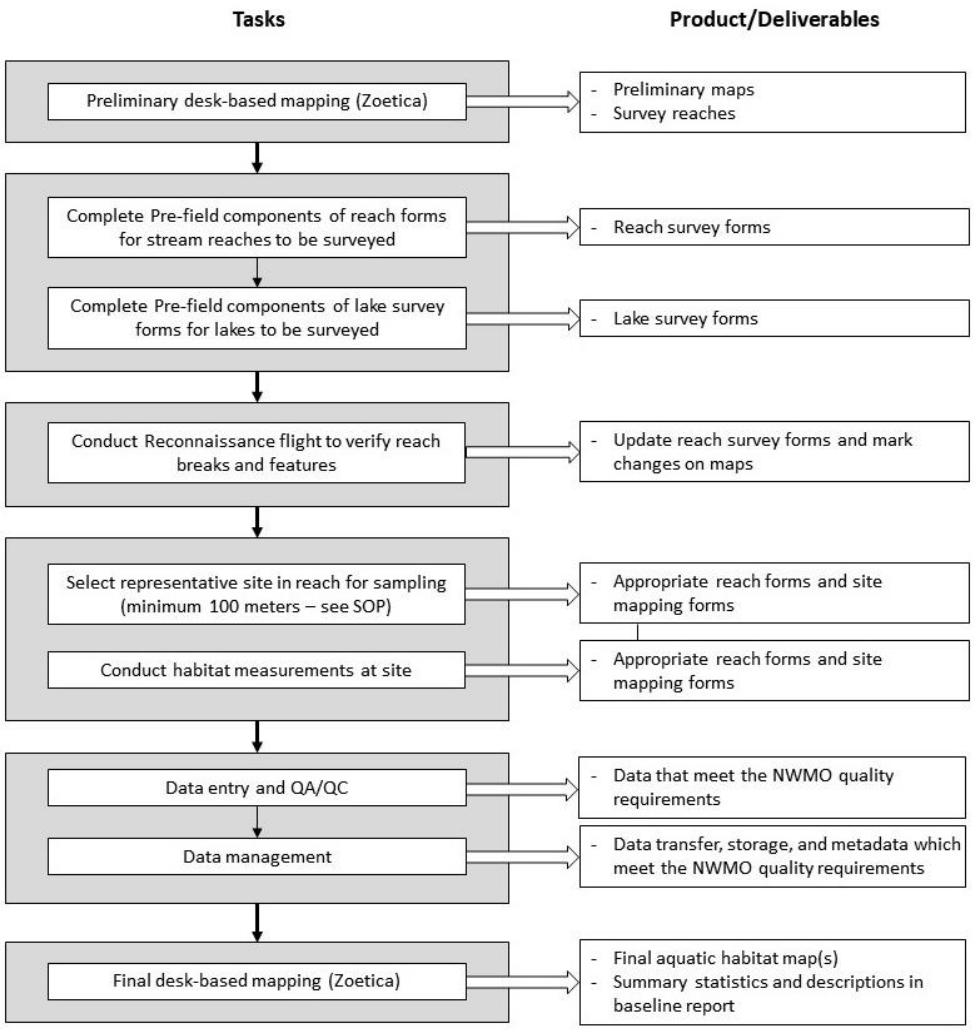


Figure 2-8. Flowchart of the tasks and products in the field-based aquatic habitat mapping.

2.3.3.4.1 Watercourse (Stream) Surveys

To maximize the efficiency of time in the field, a standardized field plan must be followed. Biophysical information will be collected at the lowest stream flows (typically between May and September). To minimize disturbance to fish and water quality, water sampling should be conducted before physical survey.

The field component of the watercourse (stream) surveys includes the following:

1. description of survey and water conditions;
2. survey site description (including surrounding land use);
3. watercourse morphology characterization;
4. channel measurements;
5. cover and habitat inventory;
6. features identification (e.g., migratory obstructions);
7. wildlife observations (recorded in comments section);

8. photography; and,
9. mapping reach information on the Aquatic Habitat Mapping Form (see Aquatic Habitat Mapping SOP in **Appendix A**).

For wetland reaches, the minimum information required is location (site referencing), cover estimates, water characteristics (temperature, pH, conductivity, turbidity), photos, and cover and habitat inventory. Details including data collection procedures and recording are summarized in Section 2.3.3.5.1 and SOPs, field forms, and instructions on filling forms detailed in **Appendix A**.

2.3.3.4.2 Waterbody (Lake/Pond/Wetland) Surveys

The field component of the waterbody (lake/pond/wetland) surveys includes the following:

1. Description of survey and water conditions;
2. survey site description (including the surrounding land use);
3. waterbody morphology characterization;
4. waterbody major inlet and outlet survey (to be conducted following watercourse survey protocol)
5. characterization of water quality and limnology (to be coordinated with the EMBP; CanNorth 2020);
6. bathymetric characterization (to be coordinated with the EMBP; CanNorth 2020);
7. bank and shoreline cover and habitat characterization
8. In-water cover and habitat characterization;
9. wildlife observations (recorded in comments section);
10. photography; and,
11. mapping reach information on the Aquatic Habitat Mapping Form (see Aquatic Habitat Mapping SOP in **Appendix A**).

2.3.3.4.2.1 Field Survey Organization for Waterbodies

The following is an example of a sequence of field activities for the waterbody surveys adapted from RIC (2001a) (Note: fish sampling will occur as part of Tier 2 activities):

1. Conduct a preliminary sounding track (an E-line) along the main axis of the waterbody (to be coordinated with the EMBP; CanNorth 2020). This will assist in the planning of transects for bathymetric mapping, in locating the best site for the limnological station, and in the placement of fish sampling gear during Tier 2 studies.
2. Conduct a shoreline cruise to record various descriptions (e.g., lakeshore features, surrounding terrain, bank and shoreline vegetation and aquatic plant distribution).
3. Complete the bathymetric sounding transects; establish, mark and record the survey benchmark (to be coordinated with the EMBP; CanNorth 2020).
4. Conduct the assessment/description of associated inlet and outlet streams (to be conducted as part of the watercourse surveys).
5. Complete the limnological survey. Temperature and oxygen profiles should be done before any water samples or Secchi disk measurements are taken in order not to disturb the water column (to be coordinated with the EMBP; CanNorth 2020). Limnological surveys should be carried out as near to mid-day as possible.
6. Throughout the survey, take photographs.
7. Review data forms and complete any other data collection required.

Bathymetric, water quality, and limnological survey procedures are described in the EMBP (CanNorth 2020). Data collection procedures for the above activities are discussed in Section 2.3.3.5.2, and in the Aquatic Habitat Mapping SOP (**Appendix A**), which includes field forms and instructions on filling forms.

2.3.3.5 Data Collection and Recording

This section describes the field data collection and recording procedures required for the aquatic habitat mapping studies. The Aquatic Habitat Mapping SOP, including field forms and instructions for filling out forms, is attached in **Appendix A**.

2.3.3.5.1 Watercourse (Stream) Surveys

Stream reach identification and referencing information collected during the desk-based program (Section 2.3.2.3.1.2) for the pre-field planning phase of aquatic habitat mapping are provided in the Watercourse (Stream) Survey Reach Table and Map (see Aquatic Habitat Mapping SOP in **Appendix A**) and carried through to the field stream and watercourse data forms. Stream reach survey field data are collected on the Watercourse (Stream) Survey Form and the Aquatic Habitat Mapping Form (see Aquatic Habitat Mapping SOP; **Appendix A**). Detailed guidelines for filling out the field forms are included in the Watercourse Reference Guide: Stream Reaches in the Aquatic Habitat Mapping SOP (in **Appendix A**). The Watercourse (Stream) Survey Form is filled out as completely as possible for each reach selected for field assessment. Each reach must be mapped separately on an Aquatic Habitat Mapping Form (included in the Aquatic Habitat Mapping SOP in **Appendix A**) and information from each reach is compiled to complete the collection record from for the station.

Any sections of the form that do not pertain to the location should be crossed out or marked with an "N/A" to indicate that the information was omitted on purpose and not forgotten.

Watercourse (Stream) Survey Form (included in Aquatic Habitat Mapping SOP in **Appendix A**): adapted from MTO (2009) and designed to capture data collected in the pre-field (desk-based) and field phases of the watercourse (stream) aquatic habitat mapping survey. Some data types collected during the pre-field mapping are verified in the field.

Aquatic Habitat Mapping Form (included in Aquatic Habitat Mapping SOP in **Appendix A**): used to record field observations during the watercourse survey. Record locations of photograph locations and direction, shoreline features, and significant stream side and aquatic plant communities on these maps. Substrate classes should be mapped and the transition of any changes to substrate composition denoted. Any connected wetlands should be marked on the map. Conditions of the banks showing erosional and depositional areas should be marked.

2.3.3.5.2 Waterbody (Lake/Pond/Wetland) Surveys

Waterbody identification and referencing information collected during the desk-based program (Section 2.3.2.3.1.3) for the pre-field planning phase of the aquatic habitat mapping are carried through to the field data forms. Waterbody (lake/pond/wetland) field data are collected on a Waterbody (Lake/Pond/Wetland) Survey Form and the Aquatic Habitat Mapping Form (see Aquatic Habitat Mapping SOP in **Appendix A**). Information on inlet and outlet streams (if surveyed) is collected on the Watercourse (Stream) Survey Form (included in Aquatic Habitat Mapping SOP in **Appendix A**).

Waterbody (Lake/Pond/Wetland) Survey Form (included in Aquatic Habitat Mapping SOP in **Appendix A**): adapted from MTO (2009) and designed to capture data collected in the pre-field (desk-based) and

field phases of the waterbody surveys. Some data types collected during the pre-field mapping are verified in the field. Refer to **Appendix A** or data collection and recording instructions.

Aquatic Habitat Mapping Form (included in Aquatic Habitat Mapping SOP in **Appendix A**): used to record field observations during the waterbody surveys. Record locations of the limnological station, photograph locations and direction, inlets/outlets, shoreline features, and significant aquatic plant communities on these maps. Details of bathymetric surveys (transect locations and direction, spot depth measurements) should be recorded on the Aquatic Habitat Mapping Form.

2.4 Drone Pilot Program

2.4.1 Overview

A drone pilot program is recommended to investigate the usefulness and viability of this rapidly improving technology that has promise for collecting imagery data. Utilization of drones could potentially reduce the amount of field effort required to verify mapping products and identify important ecological features now and into the future. The use of drones in biodiversity data collection is relatively new and is largely in an exploratory and experimental phase of research; the use of drones for data collection has not been thoroughly tested and compared to traditional methods. Solely relying on drones to collect data without comparison to conventional methods could introduce risks due to unknown accuracy and reliability. For this reason, Zoetica is proposing the use of drones as a pilot program, not an independent form of data collection.

2.4.1.1 Program Objectives

The drone pilot program is proposed to run alongside more traditional field-based methods within the terrestrial and aquatic LSAs. Drone surveys have the capability of producing meaningful spatial data that are accurate, reproducible, and comparable across time and other studies. The primary data objectives of the drone pilot program studies for the BIS are to act as supplementary data sources to evaluate the viability of the technology and methods for:

1) Testing the effectiveness and logistical potential (against more traditional methods) for the use of drones in the terrestrial and aquatic LSAs for objectives 2-6 that follow;
2) Vegetation and landscape feature inventory maps covering the LSA_{TER} and LSA_{AQU} to a high resolution;
3) Aquatic habitat mapping, including updates of barriers to fish and other features that change with time and affect hydrology;
4) Production of high-resolution imagery that will enable the counting of trees to estimate density, the identification of raptor stick nests in trees, and identification of features such as beaver dams and lodges;
5) Detection of vegetation disease and green-up using multispectral cameras where reflectance can be linked to vegetation health; and,
6) Detection of the presence of large mammals or congregations of birds via the use of imagery and infrared camera technology.

2.4.1.2 End Use

As best practices for drone use in ecological studies become better established and field-tested/verified, drone studies could become a valuable component of the BIS, which could be carried through the monitoring phase. The use of drone surveys would support the NWMO's goal of integrating new and

emerging technologies and providing opportunities for local people to meaningfully participate in the baseline program. Data imaging gathered can be utilized for multiple biodiversity values:

High-Resolution Imagery: Aerial imagery collected by drones will be available at a higher spatial resolution than the imagery that is available from satellites. Drones can be easily deployed to collect high-resolution aerial imagery and can accurately follow a pre-programmed path for frequent monitoring. Detailed imagery will enable Zoetica to estimate tree inventories and tree density, as well as calculate overall landscape cover and composition using the i-Tree software suite. The i-Tree software suite also includes tools that can estimate ecosystem services such as carbon sequestration, air quality, and hydrology characteristics that will change due to vegetation removal. Detailed images may also assist in mapping watercourses, lakes, wetlands, barriers to fish movement, ecosite classifications, landforms, and human-caused disturbances (e.g., roads, railways, logging). Updated data can then be used for habitat suitability modelling (see Section 2.2). Drone-collected imagery could also be used to survey for or opportunistically identify wildlife or wildlife features such as raptor nests, beaver dams, waterbird congregations (Hodgson *et al.* 2018) and large mammals.

Multispectral Imagery: Multispectral sensors can capture data within specific wavelengths utilizing multiple bands (i.e., each wavelength range falls within each band). The wavelengths of light that will be collected will likely include blue, green, red, and near-infrared. Gathering data across the light spectrum into multiple bands provides more information and allows for more advanced processing techniques than traditional aerial photography (e.g., supervised or unsupervised land cover classifications, vegetation mapping). Areas mapped using multispectral imagery will be explored for patterns of phenology (green-up), species identification and distribution, and existing vegetation health and diseases. These values can be confirmed during field work to provide insight into the utility of the technology for monitoring vegetation health, including changes in existing stressors such as spruce budworm or glyphosate spraying.

Infrared Imagery: Infrared imagery can very efficiently detect warm-bodied animals and could be used in targeted wildlife surveys. Depending on a combination of factors such as the target's radiation and emissivity, the temperature of the background behind the target, the resolution of the instrument sensor, and height from the ground, imagery is capable of detecting animals as small as mice (Bushaw *et al.* 2019). While this technology is capable of detecting such small warm-bodied animals, its use in the BIS will be focused on larger animals like ungulates and carnivores for the baseline data collection as these are more reliable targets for surveys.

2.4.1.3 Best Practice Guidance

Best practices for drone ecological surveys do not yet exist; therefore, Zoetica will be compiling methods in collaboration with the contracted drone operator based on requirements, existing methods, technological limitations, and aspects recognized by Zoetica as important for ecological studies. Generally, best practices for drone usage includes following manufacturer directions, piloting best practices (e.g., always fly with a full battery, conduct mechanical checks before flight, employ trained assistants for health and safety grounds), and proper pre-trip planning. These general best practices can reduce the likelihood of drone crashes; however, there can be undetectable mechanical issues or unexpected environmental issues that cannot be avoided. Post-flight, all activity details should be logged. The potential for the impacts on wildlife and sensitive species need to be considered when applying drone technology to ecological surveys. Drone noise and visual impacts could affect bird species, leading to altered behaviour and flight initiation. For example, raptors (e.g., bald eagles, hawks, osprey) have been observed to attack drones. If flying in areas where bird interactions are identified as a potential threat to both wildlife and

property, drone pilots must be accompanied by a biologist acting as a spotter to observe the behaviour of the birds. In comparison to traditional methods of aerial wildlife surveys that are predominately done by helicopter, drone technology introduces a much safer and cost-effective alternative to both pilots and surveyors.

Assmann *et al.* (2019) has reviewed drone practices used for vegetation monitoring, providing guidance on initial steps and aspects to consider, which can be generalized to other drone surveys. Their proposed workflow for scientific data collection using multispectral drone sensors includes the following 10 steps, after developing specific research questions and scientific objectives:

1. Identification of spatial and temporal scales (grain and extent)
2. Flight planning
3. Ground control points and radiometric in-flight targets
4. a) Metadata collection and pre-flight survey, and b) radiometric calibration imagery
5. Flight and data acquisition
6. Initial processing
7. Georeferencing
8. Radiometric calibration
9. Final processing
10. Geolocation and radiometric quality control

Steps 1 through 4 reflect pre-flight considerations both off and on site, Step 5 is the flight and data collection, Steps 6 through 10 are post-flight processing, control considerations, and output. This workflow will result in scientific data outputs (reflectance maps/vegetation index maps) used to answer the research questions and objectives. Data will then be analyzed and stored. Standardized best practices and workflow will help reduce key errors associated with solar angle, weather conditions, geolocation, and radiometric calibration, allowing multispectral sensors to generate meaningful, reproducible, and comparable spatial data (Assmann *et al.* 2019).

2.4.2 Desk-based Program

2.4.2.1 Study Area

The goal for the drone pilot program is to initially gather imagery data for the entire LSA_{TER} and LSA_{AQU} (see **Figure 2-2** in Section 2.1.2.1 and **Figure 2-4** in Section 2.3.2.1), followed by an assessment of success and viability to expand to biodiversity value-specific RSAs (study areas were defined in Zoetica's BPPA Report, Section 5.2).

2.4.2.2 Data Sets

In 2017, the NWMO collected aerial imagery using a fixed-wing aircraft within the Revell Batholith Area, which covers the AOI and the surrounding area. The data collected by the drone pilot program will supplement the existing imagery by allowing repeated measures to look at change in vegetation cover, vegetation health, and aquatic habitat cover over time; increasing the resolution for calculations of things like tree cover and raptor nests; and by increasing the spectral resolution by capturing a wider range of wavelengths over multiple bands.

2.4.2.3 Methods

Methods for specific biodiversity value surveys cannot be detailed at this time as they will be a Tier 2 activity that requires information from Tier 1 surveys. The selection of study methods will depend on the

target biodiversity value(s) and survey goals, as well as the drone and cameras that are commercially available. It is likely that some of the methods for achieving program objectives of the drone pilot program (see Section 2.4.1.1), beyond the higher level methodological guidance listed as Best Practice in Section 2.4.1.3, may need to be developed by the drone company as they will be more familiar with flight protocols that work best for their drones.

2.4.3 Field Program

2.4.3.1 Survey Timing

The seasonal timing of field work will depend on which objective (see Section 2.4.1.1) the drone program will be assisting in. All drone efforts will rely on the vegetation cover, snow cover, and potential seasonal presence and distribution of biodiversity values. Drone survey timing will be informed by Tier 1 studies (TEM, Section 2.1; Aquatic Habitat Mapping, Section 2.3) and Tier 2 studies focusing on specific biodiversity values (Section 3.0), especially for vegetation, ungulates, semi-aquatic mammals, and birds. Depending on the technical specifications of the drone, there will need to be considerations of constraints and limitations due to ambient weather, wind, and temperature.

2.4.3.2 Survey Crew

The success of the drone pilot program will depend on skilled drone pilots with previous experience working and flying in remote locations with the sensors required in the targeted survey. Legal requirements for the survey crew include are:

- all pilots must carry a valid Transport Canada issued drone pilot certificate;
- all drones must be marked and registered; and,
- the rules in the *Canadian Aviation Regulations* must be followed.

If these qualifications are met, there is potential for community involvement in the drone pilot program. Local companies can supply drones and operators to conduct the surveys. There may also be opportunities for local field assistants to help drone operators by providing their knowledge of the area.

2.4.3.3 Equipment and Materials

The capacity for drones to achieve the potential applications will depend greatly on the drone specifications (e.g., quality of sensors available to the drone operators, and quality, speed, and battery life of the drone itself). Great diversity in drones exists, and each type has certain advantages and disadvantages for research. Rotary-wing drones display advantages in terms of their ease of use, their ability to hover at specific locations and altitudes, and their ability to take off and land vertically (i.e., no runway is required). Fixed-wing drones have a simpler design and are less prone to mechanical failures; they also have increased aerodynamics, which allows for greater travel distances. Ultimately, the equipment used will need to have the proven ability to function in the expected ambient weather, use batteries or fuel to cover long distances, contain an auto-pilot system that can be programmed for repeatability, signal proximity from pilot and location, and cameras with the necessary resolution and quality (e.g., camera sensor size, lens characteristics, multispectral imaging systems, GPS unit). The success of the field work will also depend on the experience of the drone contracting company ultimately hired, including their training, knowledge and experience with relevant post-field work imagery processing and analyses. Demonstration of state-of-the-art equipment and highly skilled operating and processing personnel will greatly decrease the risk of such a program failing to produce useful results.

2.4.3.4 Field Protocol

No established protocols exist to date, and field protocols will largely be established to meet field goals via the contracted drone operators. As the drone program is a pilot program, Zoetica intends for the contracted drone company to compile best practices and methods, including optimal drone specifications, before initiating field work.

2.4.3.5 Data Collection and Recording

Drone surveys can produce large amounts of data, which can result in challenges for handling and storage. Recorded drone data will be digital, varying in format based on instruments utilized. The drone contractor will be responsible for the storage of pre-processed imagery, conducting post-survey image processing, and for evaluating the imagery for detections of key features identified by Zoetica in Section 2.4.1.1.

2.5 Environmental DNA (eDNA) Studies

2.5.1 Overview

Environmental DNA refers to extra-organismal genetic material that has been expelled into the environment, such as skin, feces, gametes, hair, and other cells. Instead of collecting samples directly from the organism, eDNA can be collected from the surrounding environment, such as water or soil, to assess habitat occupancy. Compared to traditional surveys, eDNA methods are non-invasive, highly sensitive and accurate, generally more cost-effective for taxa that are otherwise difficult to detect, have a low risk of pathogen transfer between sites, and typically do not require permits. Furthermore, eDNA metabarcoding uses high-throughput sequencing to enable the rapid detection of multiple species at the same time, and both samples and data can be archived for retroactive analysis in the future.

At the present time, there are some limitations for species detection using eDNA as a result of uncertainties in data interpretation, often due to an incomplete understanding of the ecology of eDNA and errors or issues in laboratory processes (e.g., PCR, sequencing, bioinformatics) (Cristescu and Hebert 2018, Zinger *et al.* 2019). For example, eDNA methods cannot necessarily tell us the abundance, density, or biomass of species; the duration, frequency, or temporal proximity of habitat use; or the precise physical location of a species. It is important to acknowledge the challenges of inferring species presence across time and space, or species presence versus viable population and confounding sources of eDNA (e.g., the feces from a predatory organism passing through the area) (Goldberg *et al.* 2016). Despite these limitations, eDNA metabarcoding is an exciting and promising new tool for biodiversity baseline data collection and monitoring studies. This technique is a powerful first-pass methodology to detect species (e.g., cryptic species that would require more effort for an initial detection using traditional methods), which can then be used for planning Tier 2 studies focusing on key population and community data collection methods that can provide more detail on abundance and relative abundance values.

2.5.1.1 Data Objectives

The primary data objectives of the eDNA metabarcoding studies for the BIS are to:

1) Provide community-level species composition data that can indicate areas that have more or fewer species and that contribute relatively more or less to the overall genetic diversity of the AOI and LSA_{AQU};
2) Locate cryptic species that are difficult to detect using traditional methods, some of which may be species-at-risk and/or important to stakeholders and rights-holders;

3) Characterize the presence and distribution of biodiversity values within the AOI and LSA_{AQU} under current conditions that may inform subsequent, more targeted studies that include measures of population abundance and relative abundance;
4) Locate suitable sampling stations appropriate for repeated sampling; and,
5) Provide baseline data for the development of species richness monitoring program(s) to address environmental, regulatory, and stakeholder/rights-holder concerns regarding potential losses of species and genetic diversity, to support the biodiversity impact assessment.

The purpose of collecting community-level species composition data (Objective 1) is to determine whether changes to the presence and distribution of a species (including loss) are due to the project. These baseline data can show that the species was not present to begin with, or that a range shift (natural or perhaps climate change-induced) is occurring regardless of the project. The consideration of genetic diversity is recommended as part of best practice guidelines for biodiversity-inclusive IAs (Secretariat of the Convention on Biological Diversity and Netherlands Commission for Environmental Assessment 2006) and is an important component of the Canadian Biodiversity Strategy in response to the Convention on Biological Diversity (Biodiversity Convention Office 1995). Preserving genetic diversity can help ensure the adaptation potential (i.e., natural selection) and long-term survival of a species or population, in light of natural or project-related changes to the environment.

For eDNA sampling from freshwater ecosystems (e.g., waterbodies, watercourses, wetlands), the data objectives within the AOI are primarily driven by the potential APM Project footprint and associated infrastructure, including roads, that could directly impact these habitats and the biodiversity values that depend on them to fulfill their life history requisites, such as fish, herpetofauna, and SAR.

Data objectives within the LSA_{AQU} are primarily driven by potential project interactions and localized cumulative effects on aquatic and semi-aquatic biodiversity values. The LSA_{AQU} encompasses the watercourses and waterbodies that could potentially be impacted by the APM Project, including the watershed drainages for the Mennin, Revell, and Wabigoon rivers downstream from the APM Project. Potential project-related and cumulative impacts on surface water quality and hydrology, such as accidental discharge of contaminated wastewater and increased water use/withdrawal, could result in direct and indirect habitat loss, mortality, and impacts to movement for aquatic and semi-aquatic biodiversity values.

To meet the data objectives, the sampling design for aquatic eDNA metabarcoding studies considered: spatial and temporal requirements, based on currently available recommended guidelines and subject matter expert advice for eDNA methods; areas of importance to local stakeholders/rights-holders; and, statistical validity and scientific rigour with respect to future analyses for informing the biodiversity IA (see Section 1.9). Aquatic eDNA studies for the BIS will be conducted in coordination with the EMBP's surface water program (CanNorth 2020).

2.5.1.2 End Use

Conducting eDNA metabarcoding studies from aquatic ecosystems will be a valuable component of the BIS. Use of eDNA metabarcoding would support the NWMO's goal of integrating new and emerging technologies, and as sampling methods are relatively simple, the opportunity for local community involvement during baseline data collection and subsequent monitoring studies is high.

eDNA metabarcoding analyses will provide community-level (species composition; i.e., number of species) data for an entire suite of biodiversity values in an area of northwestern Ontario that is relatively understudied. Detection of candidate biodiversity VCs, including at-risk and rare species, species of importance to local stakeholders and rights-holders, and selected indicator species (see BPPA Report) will help inform future baseline work using either traditional methods (e.g., population surveys to obtain abundance or relative abundance data) or targeted eDNA methods within the AOI, LSA_{AQU}, and RSA_{AQU}.

In addition, eDNA metabarcoding analyses can be employed throughout the duration of the BIS to collect robust baseline data on species composition and species associations throughout the study area for informing the biodiversity IA. These data can be used to help assess the range of natural variation in species composition and species associations (clusters) such that APM Project-specific significance thresholds and monitoring benchmarks for potential reductions in species or changes in species associations can be identified in conjunction with local stakeholders/rights-holders and regulatory agencies. By establishing baseline variability, eDNA metabarcoding can also be incorporated into a long-term monitoring program for the APM Project to help evaluate the accuracy of impact predictions that are uncertain or potentially significant, to inform whether mitigation is working, and to facilitate adaptive management to improve mitigation, where needed, to protect and conserve biodiversity.

Zoetica notes that it will be inadequate to simply predict impacts in terms of a reduction in species numbers; the abundance of species could decline by half due to theoretical impacts while the number of species detected through eDNA may remain unchanged. Thus, it is important to collect species composition data, but then use those data to direct targeted population level and community surveys that produce relative or absolute abundance estimates, where possible. These more focused studies can then employ BACI designs to assess project impacts on biodiversity.

2.5.1.3 Best Practice Guidance

The use of environmental DNA (eDNA) metabarcoding for assessing biodiversity is an emerging technology that has recently been applied to biodiversity monitoring studies around the world (reviewed in Ruppert *et al.* 2019). DNA barcoding is becoming recognized as an important tool for biodiversity baseline data collection (Gullison *et al.* 2015) and ecological assessment (Hering *et al.* 2018). Compared to traditional/conventional survey methods, eDNA methods are non-invasive; have low risk of pathogen transfer between sites (e.g., amphibian chytrid fungus); are highly sensitive and accurate; generally more cost-effective for species that are cryptic, secretive, or occur in low densities (e.g., SAR); and have little to no permitting requirements. Furthermore, eDNA metabarcoding along with next-generation sequencing can enable the rapid screening for multiple species (which can be measured in water and soil) simultaneously and retroactive analysis of previously collected data.

Some research has shown that eDNA metabarcoding performs equal to, or better than, traditional capture-based surveys for species detection, relative abundance estimates, or characterization of ecological fish communities (Civade *et al.* 2016, Hänfling *et al.* 2016, Li *et al.* 2019) and/or herpetofauna (Lacoursière-Roussel *et al.* 2016, Valentini *et al.* 2016, Lopes *et al.* 2017). In addition, terrestrial animals frequently travel to and from water sources and eDNA metabarcoding methods have been developed to identify mammals (Ushio *et al.* 2017, Harper *et al.* 2019b) and birds from water samples (Ushio *et al.* 2018). The use of eDNA metabarcoding has also been used for detection of rare and/or invasive aquatic species in the Great Lakes (Klymus *et al.* 2017, Balasingham *et al.* 2018).

Since eDNA survey methods are relatively new and continue to be optimized (especially eDNA metabarcoding), there are currently no formally accepted, standard methods; however, cohesive international guidelines are emerging, such as the *Environmental DNA Sampling and Experiment Manual Version 2.1* produced by the eDNA Methods Standardization Committee (The eDNA Society 2019). Within Canada, efforts are also being made through collaboration among academic researchers, government regulators, and industry experts to develop standards and guidelines for eDNA sample collection, analysis, and interpretation. For example, *Environmental DNA Protocol for Freshwater Aquatic Ecosystems Version 2.2* has been submitted to the BC Ministry of Environment for consideration as a new RISC Inventory Standard (Hobbs *et al.* 2017). Critical considerations for study design and results inference, and for incorporating appropriate QA/QC protocols, have been published as recommended guidelines (Goldberg *et al.* 2016). A recent paper presents recommendations for sampling protocols in lentic and lotic systems, including sample volumes and spatial intervals (Bedwell and Goldberg 2020). There are also recommendations specific to the eDNA metabarcoding workflow and increasing the transparency of bioinformatic data processing (Deiner *et al.* 2017, Zinger *et al.* 2019). The Canadian Standards Association (CSA Group) recently published a Standards Research report on eDNA standardization needs for fish and wildlife population assessments and monitoring, including minimum reporting requirements (Helbing and Hobbs 2019). The next Pathways to Increase Standards and Competency of Environmental DNA Surveys (PISCeS) International Conference, organized by Dr. Robert Hanner at the University of Guelph, is intended to explore and inform public policy, industry strategies, and future research.

Best practices and guidelines will continue to be informed by ongoing research aimed at overcoming uncertainties in data interpretation. These uncertainties can occur due to differences in eDNA persistence and fate within various environments (Harrison *et al.* 2019), accounting for potential biases introduced by the laboratory processes used to sequence DNA (Kelly *et al.* 2019), and differences in habitat-specific sampling needs (Harper *et al.* 2019). However, it is important to recognize that uncertainties in eDNA metabarcoding continue to exist, and misuses of the technology may be detrimental for biodiversity science and conservation (Cristescu and Hebert 2018). It is crucial that sources of uncertainty – most notably, the rates of false positives and false negatives – be understood, identified, and addressed. Table 1 in Cristescu and Hebert (2018) summarizes the problems associated with species detection using eDNA and presents possible solutions, which include optimization, calibration, and validation of eDNA field and laboratory methods; and comparison of results derived from eDNA methods with traditional assessments of community composition. The BIS aims to address these uncertainties through a rigorous study design that includes statistically valid survey site selection based on data objectives and anticipated analyses; field and laboratory quality controls and comprehensive data recording; repeated sampling between seasons and years; and an ongoing collaboration with eDNA metabarcoding experts (Dr. Hanner's Laboratory) who will assist with study design, field work, training of local field staff, and laboratory eDNA analyses.

2.5.2 Desk-based Program

2.5.2.1 Study Area

Freshwater eDNA studies are generally targeting aquatic and semi-aquatic biodiversity values, such as fish and amphibians. However, one of the major advantages of high-throughput metabarcoding analysis is that all of the detectable eDNA within a sample will be analyzed. Therefore, in addition to supporting baseline data collection for fish, amphibians, and primary and secondary aquatic producers (note: data for benthic invertebrates and zooplankton will also be analyzed as part of the EMBP; CanNorth 2020),

metabarcoding analyses can also detect the eDNA of other biodiversity values that have been in the area. Such additional values include turtles (e.g., snapping turtle, a SAR potentially occurring in the area), birds (e.g., waterfowl and wading birds), mammals (e.g., semi-aquatic mammals such as beaver and mink, as well as moose and black bear), and even aquatic vegetation. Aquatic eDNA studies in Years 1-2 are proposed to be focused within the AOI and the LSA_{AQU} (see **Figure 2-4**) as these areas are expected to be directly and indirectly impacted by the APM Project based on surface water flow and connections. However, it is important to note that knowledge of groundwater connections is needed to fully understand the areas of potential project impacts. Studies of groundwater are proposed as part of the EMBP (CanNorth 2020); Zoetica will use these results to refine eDNA sampling locations in Year 2 of the biodiversity baseline program, which may also include additional sampling and control sites within the RSA_{AQU}.

2.5.2.2 Data Sets

Design of aquatic eDNA studies will require analysis of existing geospatial data for water features in the AOI and LSA_{AQU}. Currently available data from Government of Ontario databases include watercourse and waterbody datasets from the Ontario Hydro Network, the Wetlands dataset from Ontario GeoHub, the eFRI dataset from MNRF. Although the MNRF recently updated their eFRI dataset in 2020, it used imagery from 2008-2011 and ecosites are coarsely defined, with a minimum polygon size of 8 ha. Zoetica's analysis of the Wetlands dataset from Ontario GeoHub indicated several large plots overlapping the AOI and LSA_{AQU} where the wetland type is labelled as 'Unknown'.

As the preferred alternative, new satellite imagery was acquired recently and ELC analysis by Sumac Geomatics for the BIS was completed for the terrestrial and aquatic LSAs in June 2020. As the biodiversity field studies are not expected to commence until the fall of Year 1 at the earliest, Zoetica recommends that study designs for eDNA and other biodiversity baseline program components (e.g., TEM, Aquatic Habitat Mapping) be developed using these updated ELC data for multiple reasons: 1) they will be the most up to date and accurate data available for the study area (with a minimum polygon size of 0.5 ha, these data are more refined than eFRI), 2) use of a common dataset for all applicable components of the BIS will promote consistency and ease of understanding, and 3) ecosite-based wetland classification is more detailed than the broader categories used in the Wetlands dataset (ELC Working Group 2009). Although wetland classification for the BIS baseline studies is recommended to follow that of the *Ontario Wetland Evaluation System: Northern Manual* (OWES; MNRF 2014a), having more detailed data is always valuable in case further analyses are warranted and/or desired. For the purposes of BIS study designs, wetland ecosites (i.e., Ecosites B126-156, B222-224) identified in the updated ELC dataset will be combined into the four wetland types classified in the OWES: marsh, swamp, bog, fen.

The Ontario MNRF and NHIC track important wildlife and fish habitat data that may be useful for informing eDNA sampling locations, especially for collecting field positive samples. Samples collected within areas known to host certain species should render positive identifications of those species. If there are failures to detect known species from these positive test sites, these failures will indicate failures in the protocols being used and can be used to help identify and mitigate against false negative results (i.e., it provides a QC check that protocols are working properly; see Section 2.5.3.4). Significant Wildlife Habitat most relevant for the focus of the eDNA studies includes Amphibian Breeding Habitat, Amphibian Movement Corridor, and Turtle Wintering Area (OMNRF 2017); and important fish habitat data documented in Ontario GeoHub including:

- Spawning Area – an area where a species of fish habitually spawns

- Nursery Area, Fish – an area where a fish species raises its newborn, if that area is different from the Spawning Area
- Feeding Area, Fish – an area where a fish species habitually feeds
- Staging Area, Fish – an area where a fish species rests during migration
- Travel Corridor, Fish – a route used by a fish species for migration; usually parts of streams or rivers, but could include pathways that connect wetlands to lakes or rivers, or pathways within or between lake basins that fish use seasonally as a migration route to carry out a component of their life cycle.

Life history requisites and habitat requirements for aquatic and semi-aquatic species of interest (e.g., see **Table 2-15**, **Table 2-16**, and **Table 2-17** below), as well as the information listed in the SWH Criteria Schedule for Ecoregion 3W for identifying candidate SWH, will also assist in site selection for sampling. Criteria schedules indicate ELC Ecosites and habitats within which the SWH is more likely to be found. For example, Amphibian Breeding Habitat may occur in swamps and thickets, vernal/seasonal pools, riparian areas and a variety of wetland interiors and margins within Ecosites B109-156 and B223-224 (OMNRF 2017).

2.5.2.3 Methods

As discussed in Data Objectives (Section 2.5.1.1) and Study Area (Section 2.5.2.1), Years 1-2 of the eDNA studies for biodiversity will focus on the AOI and LSA_{AQU}. To coordinate the BIS with the EMBP, Zoetica reviewed CanNorth's (2020) study design. Field data collection contractors that are collecting surface water for the EMBP are planning on collecting eDNA samples from Mennin Lake (n=2), Mennin River (n=1), and Revell River (n=1). Quarterly surface water sampling is proposed in AOI Ponds (n=7), Reference Ponds (n=3), Mennin Lake (n=3), Mennin River (n=3), North Inflow to Mennin Lake (n=1), Wabigoon River (n=3), Revell River (n=1), and Reference Lake (n=2) within the EMBP's LSA_{SW}. The EMBP also calls for the field data collection contractor to collect water samples from 10 locations in the RSA, but sampling lakes are to be determined following further engagement workshops with local communities (CanNorth 2019b, 2020). As shown in Figure 6.1 of CanNorth's *Environmental Media Baseline Program Design (Final Report)*, almost all ponds within the AOI are already planned for surface water sampling, which will allow for coordination with the BIS eDNA program. However, Zoetica also proposes to expand eDNA sampling within the AOI to include streams and wetlands and to cover more area and water features within the LSA_{AQU}, using a stratified random study design.

Zoetica's approach for eDNA study design is to first categorize water features into the following seven habitat groupings that are thought to be likely to host different species assemblages:

- Watercourses: rivers/streams in wetlands, rivers/streams not in wetlands
- Waterbodies: lakes/ponds ≤1 ha, lakes/ponds >1 ha
- Wetlands: marsh, swamp, peatlands (bogs and fens)

Bogs and fens will be grouped together as a single habitat grouping (peatlands) with respect to sample allocation. Bogs and fens tend to be drier and nutrient-poor compared to marshes and swamps; as such, less biodiversity, especially aquatic and semi-aquatic species, is expected in these peatlands.

The next step in Zoetica's approach is a stratified random, BACI sampling design. Within the AOI, the goal is to survey all waterbodies, watercourses, and wetlands that may be directly impacted by the APM Project. However, as the final project location has not yet been determined, the sampling design during

the first field campaign will consist of reduced effort over the entire AOI, along with paired sampling in the LSA_{AQU}. Additional surveying can be undertaken once more information about the APM Project is known (including location, components, activities, and the extent of potential impacts) and based on results from the initial eDNA studies. It will not be feasible to sample all watercourse segments or wetland polygons in the AOI in a single field season – according to the Ontario Hydro Network spatial data and the refined ecosite dataset, there are 93 watercourse segments and 125 wetlands fully or more than 50% contained in the AOI, respectively. Therefore, Zoetica proposes to focus on second-order or higher streams (n=26) and approximately 50% of wetlands (n=60) in the AOI in the first campaign of the eDNA studies. If species of interest (e.g., SAR or species of importance to stakeholders/rights-holders) are found in these streams and it is important to get more precise locations, then further sampling can be performed on the corresponding first-order streams during subsequent field campaigns. Zoetica's analysis of existing datasets also found 21 lakes/ponds in the AOI, all of which will be sampled. Thus, initial eDNA studies will consist of sampling at a total of 107 sites in the AOI.

To maximize statistical power, approximately equal sampling effort will be employed within the AOI and outside of the AOI. A stratified random study design (i.e., GRTS) will be used to select potential sampling sites in the LSA_{AQU}. Zoetica will attempt to create paired samples within and outside the AOI based on similar site characteristics, such as water quality and depth, vegetation cover, emergent aquatic vegetation, and other features, which would be assessed during Aquatic Habitat Mapping (see Section 2.3.3). Paired sampling would allow for more robust comparisons when using distance from the project as a covariate. However, existing datasets show that the representation of wetlands in the eDNA study areas is not the same; for example, there are 10 marshes within the AOI, whereas 37 marshes are present within the LSA_{AQU}. Zoetica recommends sampling multiple sites of each habitat grouping to capture the natural variability. To maintain equal sample sizes in total between the AOI and LSA_{AQU}, fewer sites in a common wetland type (e.g., fen or swamp) can be sampled in favour of rarer wetland types. Sample site selection will also consider stream flow direction because potential impacts may be expected to be greater downstream from the AOI than upstream. However, Zoetica notes that groundwater connections will also need to be considered (study of groundwater flow direction is part of the EMBP; CanNorth 2020).

After each season of field work and/or when the eDNA metabarcoding laboratory results are available, Zoetica will conduct a review of the survey effort within the LSA_{AQU} versus species data acquired. Zoetica will create a species accumulation curve to determine whether enough sampling was completed to detect all species in the area (i.e., when no new species would be expected to be found even if more sites are added). If it is determined that the slope of the curve is still increasing, then the study design will be amended to increase the sample size. Conversely, if it is determined that the slope reached zero well before the end of the campaign, then the sample size could potentially be reduced, such as the removal of sites that resulted in negative species detections.

2.5.3 Field Program

2.5.3.1 Survey Timing

Although eDNA methods for biodiversity are not as limited in their survey window as traditional methods that rely on live-capture or visual or audio observations, there are still optimal sampling times and conditions that should be employed for eDNA studies. It is best to collect samples during a time when biodiversity values are more likely to be present, which means that multiple, repeated seasonal samples may need to be collected from each target site to optimize the chances of detecting all or most of the species using each site. Some considerations of various species groups in this regard are presented below.

AMPHIBIANS: Many amphibian species will migrate to ponds and wetlands in the spring to breed, eggs will hatch after a few days or a few weeks, larval amphibians will require a few months to complete transformation (metamorphosis), and then young adults will either stay in the pond, migrate to nearby ponds, or migrate into woodland habitats in the late summer/fall (see **Table 2-15**). Freeze-intolerant amphibians typically overwinter by settling underwater in the deep areas of ponds that do not fully freeze, or by burrowing underground beneath the frost line. Freeze-tolerant species may instead overwinter above ground and beneath leaf litter. Some species aggregate in overwintering areas and tend to have high site fidelity. The overwintering locations of amphibians are generally not well understood; therefore, eDNA sampling in the winter may identify important overwintering areas and may be critical for developing mitigation measures for herpetofauna. The need for winter sampling will depend on the eDNA results from other seasons and the likelihood that the APM Project will impact amphibian winter habitat.

FISH: Fish species also undergo seasonal movements to and from suitable spawning areas. For example, northern pike (*Esox lucius*) and walleye (*Sander vitreus*) both spawn in the spring shortly after lake ice melts. While northern pike spawn in shallow (<30 cm) areas at the mouths of inflow streams or in marshes or sheltered bays with aquatic vegetation, walleye prefer to spawn over rocky areas of lakes or rivers with fast-moving water (Scott and Crossman 1973). In contrast to pike and walleye, coldwater species such as lake whitefish (*Coregonus clupeaformis*), and cisco (*Coregonus* spp.) inhabit deep (>8 m) areas of lakes during the summer months and spawn during the fall instead. These species move inshore to spawn in shallow water over a variety of substrates. Both adult and juvenile fish are particularly vulnerable when they form dense aggregations in these high-quality areas during the spawning period; Ontario has therefore developed restricted activity windows to protect fish during spawning migrations and other critical life history stages (**Table 2-16**). It is during the spring and fall windows that water sampling would be most likely to capture a wide variety of fish eDNA. Furthermore, since sample collection will likely occur at or just under the surface, coldwater species are more likely to be detected during the spawning period when fish are/have been present in shallower waters.

OTHER SPECIES (E.G., TURTLES, BIRDS, MAMMALS): Seasonal sampling designed for amphibians and fish will cover three key periods (or four, depending on results and potential for the APM Project to affect winter habitat) of life history schedules for many species in the area. Optimizing sampling for these biodiversity values should also coincide with habitat occupancy (and increased probability of eDNA capture) by various other aquatic organisms, turtles, birds, and mammals. In addition, for species with less well documented seasonal use schedules in the area, eDNA sampling and metabarcoding analyses may reveal seasons of use by species that would otherwise not be known.

Assuming that the APM Project will have year-round construction and operation activity schedules, characterization of species presence and community assemblages in different seasons will be important for the biodiversity IA and for developing appropriate spatial (e.g., avoidance) and temporal mitigation measures. Seasonal aquatic eDNA sampling at waterbodies and wetlands, in coordination with the surface water component of the EMBP, may capture eDNA of herpetofauna and fish (along with other biodiversity present) at the following stages of their life cycles (see **Table 2-15**, **Table 2-16**, and **Table 2-17**):

- Spring (early-mid April) – arrival of adult amphibians, spring and fall spawning fish;
- Summer (mid-late June) – late-breeding amphibians, spring/summer spawning fish, breeding snapping turtles; developing eggs, larvae, fry;

- Fall (mid-late September) – late-transforming amphibians and migration, fall spawning fish, snapping turtle hatchlings; and,
- Winter (December to February) – overwintering herpetofauna; fall spawning fish, eggs, larvae, fry. *Note: winter sampling will only be conducted if species of interest are present in the AOI in the summer or fall, and that would be expected to overwinter within certain habitat types in the AOI.*

Greater sampling effort can be undertaken during periods when the concentration of eDNA is expected to be higher, such as during the breeding season. Since pond-breeding amphibians relevant to the study area are not expected to undergo long-distance migrations (typically <1.0 km; Semlitsch and Bodie 2003, Semlitsch 2008), sampling throughout the LSA_{AQU} should capture amphibians that would also be overwintering within this study area. Therefore, Zoetica recommends a reduced sampling program in the fall of Year 1 and conducting higher intensity sampling (i.e., more sampling sites) during the spring and summer of Year 2. The need for winter sampling can be re-assessed after receiving the results of these initial studies and with consideration of whether the APM Project is expected to impact overwintering sites.

Survey conditions will also influence timing; it is recommended that sample collection be avoided during or immediately after heavy rains, as the resulting high-flow conditions are more likely to dilute or transport eDNA away from the source, or inhibit laboratory analyses by increasing suspended particulates, both of which may lead to false negative results (Hobbs *et al.* 2017). Conversely, these conditions may also stir up sediments that have trapped historic eDNA, leading to false positive results about current species presence (Turner *et al.* 2015). There are a number of other environmental factors that can affect eDNA persistence and degradation; as such, it will be important that the eDNA sampling program be well coordinated with the EMBP surface water sampling program to record water chemistry parameters and other site characteristics during sample collection, to enable appropriate data interpretation and transparent reporting (see Section 2.5.3.5).

Table 2-15. Breeding, hatching, transformation dates for at-risk and select indicator herpetofauna species in Revell Batholith Area (MNRF 2014b, Government of Canada 2019, Ontario Nature 2020). As appropriate, Indigenous Knowledge should be interwoven to better inform the life history summaries; local information should be gathered from the regional MNRF office and/or local stakeholders and rights-holders, if possible.

Species	Breeding Habitat	Breeding	Hatching	Transformation	Movement
Eastern (red-spotted) newt	Permanent ponds, usually with dense submergent vegetation, usually within or a short distance from forests. May also occur in swamps, roadside ditches, slow-flowing streams.	April to June	20-35 days	2-3 months	After transformation
Blue-spotted salamander	Wide variety of forest cover (deciduous, mixed, coniferous upland and lowland). Also breeds in ponds, marshes, roadside ditches.	First spring rains in March or early April, often before pond is completely ice free	3-4 weeks	Begin mid-July, occasionally late June, until early August	Adults leave shortly after breeding, young leave after transformation
Gray treefrog	Wide variety of habitat including swamps, marshes, farm ponds, flooded farmland, floodplains. Temporary and permanent pools.	Mid-May until early July	5 days	40-60 days; transformation in late July or early August	Adults arboreal
Wood frog	Ponds in swamps with many leaves on the bottom. May also breed in marshes and ponds a short distance from woodlands.	Late March, lasts about 3 weeks (information from S Ontario as none available in NW Ontario)	17-24 days	44-85 days	Adults usually remain in forest habitat
Mink frog	Edges of rivers, lakes, ponds, pools, puddles, ditches, streams, cold springs, open water with abundant lily pads. Prefers quiet bays and protected areas.	Late spring through mid-summer	unknown	1-2 years	unknown
Snapping turtle **(Special Concern)**[1]	Permanent, semi-permanent fresh water, marshes, swamps or bogs, rivers and streams with muddy banks or bottoms. Often uses soft soil or clean dry sand on south-facing slopes for nest sites. May nest some distance from water.	Early to mid-summer, late May or June	Early fall, mid to late September	n/a	Overwintering areas: often hibernate together in groups in mud under water. Home range size approx. 28 ha.

[1] As listed under the Species at Risk in Ontario (SARO) and Schedule 1 of the federal *Species at Risk Act*.

Table 2-16. Restricted activity timing windows for the protection of spawning fish and developing eggs and fry for the Northwest Region of Ontario (DFO 2013). DFO has developed restricted activity timing windows for various fish species; however, for the APM Project, only fish species of importance to local stakeholders/rights-holders and those protected by regulations are shown in this table.

Spring Spawning Species	**Spring Spawning Period**
Walleye	April 1 – June 20
Northern pike	April 1 – June 15
Lake sturgeon **(1 pop. of conservation concern)**[1]	May 1 – June 30
Other/Unknown spring spawning species	April 1 – June 15
Fall Spawning Species	**Fall Spawning Period**
Lake whitefish **(CWS)**[2]	September 15 – May 31
Lake herring/Cisco	October 1 – May 31
Other/Unknown fall spawning species	September 1 – June 15

[1] Saskatchewan-Nelson River pop. is listed as Threatened under SARO and assessed as Endangered by COSEWIC.

[2] 'Candidate Wildlife Species' selected by COSEWIC as high priority for assessment.

Table 2-17. Reproduction information for fish species not explicitly considered by DFO in Northwestern Ontario for restricted activity timing windows (Table 2-16) but proposed as biodiversity values for inclusion in the baseline program due to their importance to local stakeholders/rights-holders, legal protections, and potential inclusion for tissue sampling as part of the EMBP (CanNorth 2020). Data from Eakins (2020).

Species	**Spawning Season**
White sucker	Spring (April – June)
Longnose dace	Spring-summer (May – July)
Silver chub (Saskatchewan-Nelson River pop.; assessed as Not at Risk by COSEWIC)[1]	Summer (June – July)
Emerald shiner	Summer (June – August)
Blackchin shiner **(CWS)**[2]	Summer (June – August)
Shortjaw cisco **(Threatened)**[3]	Fall (October – December)

[1] Silver chub as a species is listed as Threatened under SARO; populations are currently not distinguished in Ontario under Provincial conservation listings. However, fish in the RSA_{AQU} are from the Saskatchewan-Nelson River population, which is assessed Federally as Not at Risk by COSEWIC.

[2] 'Candidate Wildlife Species' selected by COSEWIC as high priority for assessment.

[3] Listed as Threatened under SARO; also assessed as Threatened by COSEWIC.

Climatic conditions and seasonal water table fluctuations may also affect sampling in wetlands. For example, some wetlands may dry out in the summer, especially if there has been a prolonged drought. In these cases, it will not be possible to collect water samples for eDNA analyses. The presence of open/surface water during dry periods will likely need to be ascertained on the ground; assessment of ephemeral streams and waterbodies is a component of Aquatic Habitat Mapping (see Section 2.3.3).

In light of the current COVID-19 pandemic, eDNA studies will not begin until the fall of Year 1 at the earliest. Although the water and sediment eDNA sampling component of the EMBP program has been postponed until Year 2, the biodiversity eDNA studies schedule would coincide with the proposed fall campaign for the surface water sampling component of the EMBP (CanNorth 2019b, 2020).

In addition, it is likely that helicopter services will be required to cover the entire study area efficiently and to ensure that sites are not selected in a biased way (e.g., based on accessibility via roads and linear corridors). It should be acknowledged that weather days may delay the field schedule if general flight conditions are not satisfied (e.g., heavy fog or precipitation reducing visibility).

2.5.3.2 Survey Crew

Expertise and training in both aquatic and terrestrial eDNA sample collection will be provided by a field technician from Dr. Robert Hanner's research laboratory at the University of Guelph. Each survey team may consist of one Hanner Lab technician, one contracted field staff, and one local field assistant (if community interest and availability allow, see Section 1.4.2). At each site, one person will handle the eDNA sampling equipment, one person will take environmental measurements (e.g., water quality), and one person will be the record keeper. It is recommended that at least two survey teams be deployed simultaneously to cover the study areas as efficiently as possible to reduce the number and degree of potentially confounding environmental factors (see Section 2.5.3.1).

Zoetica will provide instruction on GRTS sampling (see Section 2.5.2.3), which involves sampling sites in a specific order and allows for site replacements (by assigning oversample points) if difficulties are encountered in the field. Although the waterbody, watercourse, or wetland to be sampled will be pre-determined (see Section 2.5.2.3), suitable microhabitats for sampling will need to be selected on the ground based on amphibian and fish habitat preferences (e.g., see Breeding Habitat column of **Table 2-15**), the professional/research experience and knowledge of the Hanner Lab technician and field data collection contractor, and local knowledge provided by the field assistant or stakeholders/rights-holders. For lotic systems, it is recommended that the sampling site consist of a single stream reach (i.e., a relatively homogenous length of stream; see Section 2.3.2.3.1.2). The survey team should verify that sample replicates will be collected within a discrete stream reach.

The required qualifications and experience for contracted field staff should be coordinated with the needs of the surface water component of the EMBP. the contracted field staff are required to be familiar with, at minimum:

- Local wildlife and fish biology and ecology – to make informed decisions about microhabitat selection.
- Wetland classification – while the updated ELC dataset will provide the most accurate wetland data (compared to the existing eFRI from MNRF and Wetlands dataset from Ontario GeoHub), wetland classification by air photo interpretation has inherent uncertainty and ground-truthing is recommended to verify the accuracy of wetland types and polygon boundaries (based on the OWES; MNRF 2014a).

Zoetica recommends that the Hanner Lab technician be retained for a full year of baseline data collection to provide continued guidance during seasonal sampling wherein different environmental conditions and logistical challenges are expected to be encountered. The Hanner laboratory's experience will be invaluable for developing any small-scale, on-the-ground contingency plans or modifications needed to ensure that the sampling design remain valid, including the collection of all applicable information to provide transparency in data interpretation. This approach will also allow for training of new contracted field staff and/or local field assistants (if the crew is expected to change between seasons) or refresher training for previous survey crew members.

There are no formal training requirements for contracted field staff nor local field assistants for eDNA studies; however, an understanding of best practices in eDNA sample collection with respect to the need for strict decontamination protocols and detailed record keeping prior to going into the field would be beneficial. The Hanner Lab technician will have an MSc degree and relevant eDNA experience, at minimum. It is expected that the Hanner Lab technician will train the field data collection contractor and local field assistant on eDNA sample collection, filtration, storage, and transport according to standard protocols for the Hanner laboratory and in such a way as to ensure that sample quality is maintained and preserved. It is also expected that the field data collection contractor and local field assistant will be sufficiently trained during Years 1 and 2 to enable them to complete subsequent years of baseline eDNA sample collection as well as for a potential future monitoring program. As such, all survey team members should directly participate in using the eDNA equipment and other instruments (see Section 2.5.3.3), recording information on data forms (see Section 2.5.3.5), and preparing samples for shipment to the Hanner laboratory.

The helicopter pilot is not expected to be directly involved in eDNA studies but will be relied upon to find appropriate landing spots for the survey team.

2.5.3.3 Equipment and Materials

The Hanner Lab technician will be providing/using the OSMOS eDNA sampling kit (Halltech Aquatic Research Inc., Guelph, ON), a backpack sampler that completes water filtration on-site and includes a telescopic pole with tripod and locking clamp to allow for hands-free sampling from a distance, which helps to prevent contamination by reducing the need to enter a waterbody, watercourse, or wetland. The OSMOS system has digital flow, pressure, and temperature sensors with programmable settings to enable standardization of these sample collection parameters. This eDNA sampler can efficiently filter 1-2 L of water in a few minutes if water turbidity is low. If there is high turbidity (e.g., tannins, suspended particulate matter), the Hanner Lab technician may install filters with a larger pore size to help increase the flow rate; all changes to standard protocol will be documented (see Section 2.5.3.4). One OSMOS eDNA sampler will be available per survey team.

Environmental data will be collected at each eDNA sampling site. A water quality meter will be required to measure water (and air) temperature, pH, conductivity, and dissolved oxygen. If it is feasible, water quality instruments can be shared between the eDNA program and the surface water sampling program of the EMBP. Canopy cover is a proxy for UV radiation and should be measured using a densiometer. Weather information such as cloud cover and precipitation can be estimated by eye.

Materials and consumables required for eDNA sampling include cellulose nitrate filters (or glass fibre filters, if cellulose nitrate filters cannot be acquired) with pore sizes ranging from 0.45-5 µm, disposable gloves, tweezers, storage containers, markers and labels, and field data forms printed on waterproof paper. Reagents required include bleach and liquid dish soap for decontamination, tap water and distilled water for rinsing equipment, and distilled water for the field negative (see Section 2.5.3.4). Submission of eDNA samples by the field data collection contractor to the Hanner laboratory should be accompanied by both hard copy and electronic data forms, and site photos. Standard field gear and data collection materials (e.g., binoculars, camera, notebook, pencil, clipboard) should be carried to record incidental observations, information about site access, or other pertinent notes.

Field work for eDNA studies will require general navigational equipment to travel from the helicopter landing spot to the pre-determined sampling area, including GPS units (also used to record the

coordinates of sampling locations), compasses, and tablets or smartphones with georeferenced digital maps as well as hard copy field maps as a backup. A comprehensive list of equipment and materials needed for eDNA field studies is available in the eDNA field SOP in **Appendix A**.

As discussed, helicopter services are likely needed to enable cost-effective and efficient coverage of the study area for eDNA studies. Ideally, the aircraft will be able to accommodate the eDNA survey crews (at least 6 passengers); however, staggering of crew pick-up and drop-off may be needed and will be determined when additional planning details are available from the NWMO. Alternatively, if helicopter services are not used – for example, if it is determined by the NWMO that ground travel to the selected water features is feasible or desirable due to safety reasons, taking into account that some bias in sampling locations would be introduced – then other modes of transportation will be required from home base to the study sites. In this case, a truck and/or ATVs will likely be required. However, it is Zoetica's opinion that use of biased sampling sites will threaten the scientific credibility of data collected and that all analyses and results will have to be framed with caveats around site selection and its potential influence on results.

2.5.3.4 Field Protocol

The eDNA survey team will travel to each pre-selected water feature, and suitable microhabitats will be identified in the field by skilled wildlife and fisheries field personnel within the field crew that is contracted for sampling. The OSMOS eDNA sampler will be set up according to the manufacturer's instructions. As much as practical, survey crew should avoid entering the watercourse, waterbody, or wetland. The geographic coordinates of the sampling location, along with other site descriptors and sampling information, will be recorded on the field data form (see Section 2.5.3.5) and marked as a waypoint on the GPS unit. The physical location can also be marked with flagging tape to help relocate the sampling site during the next seasonal campaign and subsequent years of study.

For sampling from **lotic systems** (watercourses), stationary point sampling will be used. It is recommended to sample in headwater streams and tributaries (rather than the mainstem), stream margins, and the thalweg (main concentration of flow, normally the deepest part of the channel) (Hobbs *et al.* 2017, Stanfield 2017). Sampling will occur at or just under the water surface to avoid stirring up sediment. Two litres of water will be collected through the OSMOS system per filter. Three replicates will be collected at each site, spaced 100 m apart (Bedwell and Goldberg 2020), for a total of 6 L of water sampled. Always collect replicates from downstream to upstream to prevent contamination.

For sampling from **lentic systems** (waterbodies and wetlands), mobile sampling is preferred to increase the area surveyed, wherein the surveyor will walk along the shoreline while running 2 L of water through the OSMOS system per filter. Sampling will occur at or just under the water surface to avoid stirring up sediment. Three replicates will be collected at each site for a total of 6 L water sampled. It is recommended to sample as much of the spatial extent of the shoreline as possible (Hobbs *et al.* 2017); therefore, replicates can be collected by continuing mobile sampling along the shoreline.

In addition, three types QA/QC 'samples' will be collected: one duplicate sample will be collected at every other site to assess reproducibility, one field negative sample (distilled water) will be collected every three sites to help assess the false positive rate, and where possible, a field positive sample will be collected at a site where the species of interest is known to occur. The field positive will help to confirm that the field protocols are working, establish rates of false negatives, and corroborate traditional surveying techniques.

eDNA sample collection, storage, and transport protocols will follow the standard operating procedures of the Hanner laboratory. For the biodiversity baseline program, Zoetica has developed a field SOP customized for the proposed eDNA studies – see **Appendix A**. After 2 L of water has passed through the OSMOS system, the filter will be carefully removed with clean tweezers and stored in a sample envelope in a Ziploc bag with silica bead desiccant packets. Preserved filters can be stored at room temperature or in the refrigerator (not frozen) until shipping to the Hanner laboratory for analysis. Although the shelf life of samples stored in this manner is thought to be 6-12 months (Hobbs *et al.* 2017), samples should be sent to the laboratory for DNA extraction as soon as possible.

Decontamination of sampling equipment and gear (e.g., reusable filter housing and extendable pole on the OSMOS system, rubber boots if it was necessary to enter the water feature) must be conducted between sites. This involves washing visible particulate matter from the equipment using soap, spraying/soaking the gear with a 10-50% bleach solution (the concentration depends on whether the equipment directly touches the sample), letting it sit for at least 1 minute, and then triple rinsing with water away from a watercourse or waterbody. Tap water may be used for boots and other gear; however, distilled water should be used to rinse the filter housing. Triple rinsing is essential to prevent the contamination of waterbodies with bleach, which can harm sensitive species such as amphibians. In addition to avoiding cross-contamination of eDNA samples, it is important to decontaminate field gear to avoid transferring pathogens such as chytrid fungus and ranaviruses to sensitive amphibians (CHHWG 2017).

Incidental observations of wildlife or wildlife sign, fish, and human use of the area will be recorded. Although the BIS eDNA studies do not include paired taxonomic analyses (as is being done for zooplankton and benthic invertebrates as part of the surface water component of the EMBP; CanNorth 2020), incidental wildlife observations can help corroborate the results of eDNA metabarcoding analyses if these species are detected.

2.5.3.5 Data Collection and Recording

Minimum recommended reporting for eDNA studies includes documentation at all stages of the process, including design, water collection, sample preservation, extraction process, and, in the case of metabarcoding analyses, high-throughput sequencing (Goldberg *et al.* 2016). The recommended reporting information relevant to the BIS field program includes:

Water collection

- Contamination precautions including negative controls
- Collection volume, container material, replicates, depth
- Site description (e.g., flow rate, area)

Sample preservation

- Method, temperature, duration
- Filter type (if applicable), filtering location (e.g., in field)

The inclusions of decontamination procedures, field negative samples, replicates/duplicates, collection of surface water (i.e., depth), and filter preservation/storage method were described in Section 2.5.3.4 above. Regarding container material and filtering location, this is more applicable to sampling approaches that use Nalgene collection bottles or Whirl-Pak bags and separate filtering equipment; the use of the

OSMOS eDNA sampler will be documented for the BIS. Information about watercourse flow rate and waterbody/wetland area can be used within an occupancy modelling framework to estimate detection probability for eDNA studies (e.g., Goldberg *et al.* 2018). The area of waterbodies and wetlands can be calculated by Zoetica from available spatial data (see Section 2.5.2.2). Flow rate measurements, which includes estimating flow in smaller streams and larger rivers, will be conducted by the field data collection contractor. Protocols for manual flow measurements will follow the guidelines of the Ontario Stream Assessment Protocol (Stanfield 2017) and MTO guidance (MTO 1997, 2016, 2019). The remaining recommended reporting guidelines are addressed below.

At each eDNA sampling site, the following information will be recorded on the data form (a copy can be found at the end of the eDNA field SOP; see **Appendix A**). Site photographs should also be taken as additional documentation of the habitat characteristics.

General Information

- Unique site code (pre-determined by Zoetica during eDNA study design and mapping)
- Replicate number
- Date and time
- Survey crew (initials of eDNA sample collector, environmental data collector, record keeper)
- Location (geographic coordinates in UTM NAD 83)

Environmental Conditions

- Weather (cloud cover, air temperature, precipitation)
- Canopy cover
- Water quality data (water temperature, pH, dissolved oxygen, conductivity)
- Site/habitat characteristics (e.g., presence of emergent vegetation, substrate type, riparian and upland vegetation, beaver modifications, human disturbance)
- Incidental wildlife and fish observations
- Photo IDs

Sampling Conditions

- Method (OSMOS eDNA sampler)
- Filter type and pore size
- Input parameters: sample volume and pump pressure (to be read from OSMOS digital sensor, but should be standardized at 2.0 L and -60 kPA, respectively)
- Output parameters: total volume pumped, average flow rate, runtime (to be read from OSMOS final results screen)

On each eDNA sample storage envelope, the following identifiers must be recorded:

- Unique site code
- Initials of eDNA sample collector
- Date and time
- Sample/replicate number (1, 2, 3) *or* duplicate, field neg, field pos

The OSMOS system has digital flow, pressure, and temperature sensors that will be monitored and adjustments may be made in the field if needed. For example, a pilot study using the Smith-Root eDNA backpack sampler found that peak filtration efficiency and eDNA capture occurred at a flow rate threshold of 1.0 L/min using 5 µm filters, and that high filtration pressures may reduce eDNA retention (Thomas *et al.* 2018). Thus, if flow rate is very low due to high levels of particulates, it would be better to switch to filters with increased pore size rather than turn up the pump pressure of the system. If any modifications are made to the initial (standard) parameters, however, these changes must be documented on the data form.

Documentation of sample/filter storage conditions (e.g., temperature, duration) is needed up to the point when samples are shipped to the Hanner laboratory for analysis. Once samples arrive at the University of Guelph, Hanner laboratory technicians will perform and document laboratory analyses according to their internal SOPs and QA/QC checks.

2.5.3.6 Laboratory Analyses

The Hanner laboratory has or will develop validated methods for DNA extraction, PCR amplification, library preparation, MiSeq sequencing, and bioinformatics analyses for detecting the biodiversity of the AOI and LSA_{AQU}. Their practices will consider the common critiques of eDNA metabarcoding and laboratory-based methods and will follow the most up-to-date best practices and guidelines available (Cristescu and Hebert 2018, Helbing and Hobbs 2019, Zinger *et al.* 2019) to ensure that data quality requirements of the NWMO are met.

The Hanner laboratory will present the eDNA metabarcoding results summarizing methods and results focused on species detected (and/or higher taxa as appropriate to taxonomic resolution of the marker gene and reference database used to infer analysis) in a summary report. In addition, because existing morphologically-based taxonomy can overlook cryptic species, a summary of "molecular operational taxonomic units" (MOTUs) will also be included to further facilitate site comparisons. Raw data will be appended (e.g., as an Excel spreadsheet) and archived. Data produced by the Hanner laboratory will need to meet the data delivery standards of the NWMO, including metadata, QA/QC, and transmittal requirements, at minimum (see Section 1.10).

There are some limitations to the BIS study design proposed above in comparison to the paired eDNA and taxonomic specimen collection planned for the EMBP, as CanNorth's parallel approach enables conventional Sanger sequence analyses to aid in building the barcode reference library (CanNorth 2020). Analyses for biodiversity, on the other hand, will be relying on existing sequences (e.g., in the Barcode of Life Database, GenBank and other marker-specific databases) unless tissue sample collection is also undertaken for the BIS. Zoetica presented options for building barcode reference sequence libraries for biodiversity in Table 5-1 of the BPPA Report. Zoetica's preferred approach is to start with available reference sequences and to reserve a small tissue sample from specimens already being collected for the EMBP. Additional input from stakeholders and rights-holders is needed prior to deciding on any invasive procedures. One of the major advantages of eDNA metabarcoding, however, is that all DNA present in the sample (that can be amplified with a given set of PCR primers) will be sequenced and all data can be stored indefinitely for re-analysis (e.g., against tissue samples that may be collected in future years of the baseline program).

2.6 Data Analysis and Reporting

This section outlines the data analysis and reporting that will be conducted for the Tier 1 studies. While statistical methods including GRTS analysis were used for the stratification of habitat data for survey site selection in the field program, the results of the Tier 1 studies are not data complex and do not require any statistical modelling for reporting purposes. Except for eDNA studies (detailed in Section 2.5) where seasonal and annual surveys may be conducted, most Tier 1 studies will involve only one survey site visit to collect data to map and verify habitat within the applicable study areas. With only one season of field data collected, trends cannot be determined but will be calculated after more field seasons (at least 2 required).

Assuming field data collection contractors submit their data according to the two-week timeline outlined in Section 1.10, the final baseline report will be completed by the end of Year 1. This completion timeframe will allow for adequate time to integrate the Year 1 baseline results into the planning process for the Year 2 field season. The following sections provide an overview of the data analyses and reporting that will be conducted for each study conducted in Tier 1.

2.6.1 Terrestrial Ecosystem Mapping

The objective of the TEM Tier 1 studies is to improve the ecosite data currently available in the eFRI dataset into smaller and more accurate polygon units. Data collected will be used to plan stratified random study locations for use in collecting more species-specific data during Tier 2 studies.

Data collected as part of Tier 1 studies will be presented as summary statistics for each ecosite classification to determine the vegetation and soil characteristics that are common and potentially unique to the study areas. The field verified data collected at a subset of randomized polygons (by ecosite) selected for ground-truthing during Tier 1 studies will be used to extrapolate data to similarly classified polygons that have not been field verified. Summary statistics will include:

- The proportion of ecosites ground-truthed that agree with the desk-based refined ecosite dataset;
- Areas (in hectares and percentage) comprised of each ecosite relative to all ecosites within the AOI and LSA_{AQU}; and,
- Basic comparative statistics to assess the ecosite uniqueness within the AOI compared to the LSA_{AQU}, and once mapping the RSA_{VEG} is completed, compared to the RSA_{VEG}.

Analyses of ecosite data will be presented as maps and summary tables showing the distribution and amounts of habitat within each study area level (i.e., AOI, LSA_{AQU}, and RSA_{VEG}). All desk-based maps will be updated to represent the ecosite classification changed during ground-truthing. The updated ecosite data will be used for the selection of Tier 2 study sites for various biodiversity values.

2.6.2 Habitat Suitability Modelling

The objectives of habitat suitability modelling include:

- Assigning wildlife suitability ratings to various polygons derived from TEM based on their relative importance to select wildlife;
- Determining the suitable habitat and SWH in the AOI compared to the LSA_{AQU}, and once mapping the RSA_{VEG} is completed, comparing it to RSA; and,
- Assisting in the selection of survey locations for future Tier 2 baseline studies.

Polygons in the refined ecosite dataset will be used to assign a preliminary wildlife-specific habitat suitability rating based on various life history requirements and seasonal requirements for that species. Preliminary mapping will be used in the field to ground-truth the ratings of the ecosites. Field data collected at a subset of randomized polygons as part of the Tier 1 TEM studies will also be ground-truthed for verification of wildlife habitat suitability ratings and results will be used to extrapolate data collected in these sampled plots to similarly classified polygons that have not been field verified. Summary statistics will include:

- The proportion of ecosites ground-truthed that agree with wildlife-specific habitat suitability ratings derived via desk-based methods;
- Amount in hectares and percentage of each rating level (Nil, Low, Moderate, High) of each ecosite relative to the rating levels of all ecosites within the study areas (AOI, LSA, and once mapping is complete the RSA); and,
- Basic comparative statistics to assess the uniqueness of high-quality habitat, and presence and distribution of candidate SWH within the AOI compared to the LSA_{AQU}.

The report will include updated ratings criteria based on evidence from the field and will present updated habitat suitability maps for each species and life requisite/season assessed to show the distribution of high-quality habitat across the study areas. Updated species accounts will also be presented in the baseline report. The reports will include a discussion of the important habitat features for each life history stage chosen for each selected species and will also indicate whether any potential candidate SWH was observed during field assessment.

2.6.3 Aquatic Habitat Mapping

The primary objectives of the aquatic habitat mapping for the BIS are to characterize the presence and distribution of fish habitat within the LSA_{AQU} and select control sites within the RSA_{AQU} under baseline conditions, detect any important fish areas, assess the potential for SAR habitat, and to evaluate the distribution of habitats within the LSA_{AQU} and select control sites in the RSA_{AQU} to choose suitable sampling sites for fish community characterization in Tier 2 studies.

As part of the desk-based program, watercourses and waterbodies will be mapped and segregated into reaches, with a selection of those reaches ground-truthed during the field-based program. Data collected as part of Tier 1 studies will be presented as summary statistics for each reach classification to determine the available habitat within the aquatic study areas. The field verified data collected at a subset of randomized reaches ground-truthed during Tier 1 studies will be used to update waterbody habitat classifications (e.g., wetland classifications) and will be used to extrapolate data to reaches that have not been field verified. Summary statistics will include:

- The proportion of reaches ground-truthed that agree with desk-based aquatic habitat mapping;
- The proportion of each reach type derived through desk-based aquatic habitat mapping relative to all reaches within the study areas (AOI, LSA_{AQU}, and in select areas of the RSA_{AQU}); and,
- Basic comparative statistics to assess the uniqueness of aquatic habitat within the AOI compared to the LSA_{AQU} and select control sites in the RSA_{AQU}.

Field truthing of watercourses and waterbodies will be used to update maps and will be presented in the baseline report to show the distribution of various aquatic habitat types within each study area (AOI, LSA_{AQU}, and RSA_{AQU}). The updated habitat data will be used to identify the potential use by various fish

species and will be used for the selection of Tier 2 study sites. Results of aquatic habitat mapping within the study areas will assist in the assessment of potential species presence, the identification of critical and sensitive aquatic and riparian habitat, and gear type required for future Tier 2 community composition studies. The results of the eDNA program can be used in parallel with aquatic habitat mapping to identify species presence and in planning for future studies. Lake characteristics (bathymetry and limnology/water chemistry) will be presented within the EMBP baseline report and may be summarized in the BIS baseline report as it relates to fish habitat. In addition to fish habitat, aquatic habitat mapping will identify stream reaches where suitable riffle habitat exists for future benthic invertebrate collections conducted as part of the EMBP (CanNorth 2020).

2.6.4 Drone Pilot Program

The objective of the drone pilot program is to assess the capability of using drone technology to collect detailed data in a reproducible manner. Once the data collected from drones on a smaller spatial scale (e.g., LSA_{TER} and LSA_{AQU}) can be verified as equal or better than traditional methods, the program can be expanded to the larger regional areas.

The data collected as part of the drone pilot program will include imagery which is much more detailed than freely available satellite imagery. The different types of imagery collected will each serve different purposes and will include detailed imagery, multispectral imagery, and infrared imagery. The raw drone imagery will require some initial processing (e.g., mosaicking, orthorectification), which will be completed by the contracted drone company. Detailed image analysis can then be targeted towards mapping watercourses, lakes, wetlands, barriers to fish movement, and other hydrological features; and ecosite classifications and other vegetation information (e.g., vegetation health as determined by using multispectral analysis). Imagery analysis of tree counts and tree density will allow for the calculation of overall landscape tree cover and composition; this can be done using programs such as the i-Tree software suite, which allows for estimates of tree density as well as contributions and potential effects on ecosystem services such as carbon sequestration and air quality.

The report will include imagery maps based on various data types (high-quality imagery, multispectral imagery, and/or infrared imagery). Drone imagery can be integrated into terrestrial ecosystem maps and/or habitat suitability maps, or imagery of significant landscape structures like beaver dams and raptor nests can be directly presented in the baseline report as part of photographic documentation of the study area.

2.6.5 Environmental DNA (eDNA) studies

The objective of the eDNA program in Year 1 is to provide community-level species composition (i.e., number of species) data across the AOI and LSA_{AQU}, giving indications as to which areas contribute more or less to the overall genetic diversity, and to detect species of interest (e.g., SAR) and cryptic species. These data will also provide the BIS with baseline information for the development of species and community baseline collection programs, many of which will seek to quantify abundance and relative abundance along with measures of diversity. Further, eDNA metabarcoding analyses can be employed throughout the duration of the BIS to collect robust baseline data on species composition and species associations throughout the LSA_{AQU} for informing the potential impacts of the project to genetic diversity (e.g., as would occur through the extirpation of species) for the biodiversity IA.

Data analysis can be used to help assess the range of natural variation in species composition and species associations (clusters) across habitat types and to set monitoring benchmarks (e.g., species expected

within various habitats) that can detect reductions in species numbers or changes in losses in species over time. The resulting species composition data, which will then be reviewed alongside TEM data and input from local stakeholders and rights-holders, will also be used to help refine the scope of biodiversity values for subsequent Tier 2 baseline data collection.

Data analysis will be conducted to determine the sources of uncertainty – most notably, the rates of false positives and false negatives, which must be understood, identified, and addressed. Besides creating a list of identified species within the area potentially affected by the APM Project, eDNA data collected within stratified random aquatic habitats will also enable cluster analyses, which can identify species clusters that tend to co-vary among habitat types; this will help to better understand species associations and communities, and the abiotic conditions that segregate them.

Reporting of eDNA data will be through species composition maps of the AOI and LSA_{AQU}, and statistical comparisons of species composition between study areas. Updated species presence accounts will also be presented in the baseline report. The reports will include a discussion of species composition and presence in relation to expectations found from habitat suitability modelling.

3.0 FUTURE TIER 2 AND 3 STUDIES

Tier 2 studies on community composition (which will provide measures of abundance and relative abundance of key fish and wildlife groups), population metrics, ecosystem services, and targeted biodiversity values will build on results from Tier 1 data collection conducted during the Year 1 and Year 2 field seasons. The data collected during Tier 1 field work will be used to update the desk-based TEM, habitat suitability modelling, and aquatic habitat mapping. The updated mapping products will allow for the generation of a defensible stratified random allocation of sampling/survey effort, completed using GRTS (see Section 1.9.1 for details on GRTS), for Tier 2 studies.

The Tier 2 protocols will be added to the BPD Report by Zoetica during the next iteration of the report scheduled to be issued in spring of Year 2. However, some Tier 2 survey protocols (e.g., snow track surveys and moose aerial surveys) may be issued prior to the final report to allow for surveys to be conducted during the late winter (i.e., in January or February of Year 2) as to not delay the overall schedule for the APM Project. Greater efficiencies will be achieved in waiting to write Tier 2 study design protocols until after assessing initial habitat mapping data and possibly eDNA results (depending on how long lab analysis takes) in the terrestrial and aquatic LSAs; receiving feedback on proposed methods from the ERG, communities, and regulators; and after identifying additional government protocols and potential collaborations that could be realized for these studies. The SOPs for Tier 2 studies, and maps detailing how survey efforts should be distributed over space and among ecosystem habitat groupings (informed by Tier 1 terrestrial and aquatic habitat mapping) will be generated starting in the fall of Year 1 and will enable the construction of a defensible GRTS design and survey effort allocation.

Baseline data from Tier 1 will also be used to inform whether the more “uncertain” Tier 2 study options should be triggered. Currently, Zoetica has presented in the BPPA Report many options for studies that may be triggered in Tiers 2 and 3, and not all will be required. Based on results of Tier 1 studies, some studies are likely to be no longer relevant. For example, if suitable habitat is not found for a species within an area that could be impacted by the APM Project (e.g., snapping turtles), and if eDNA results do not suggest presence, a study on such a species (or group, if relevant) may be removed from consideration.

While lists of species potentially present in the area have been developed to assist in determining biodiversity values to focus on, they will need to be refined by the Tier 1 studies to ensure that no species are missed or unnecessarily included. In some cases it may be appropriate to focus on key species of importance like a SAR, while in other cases, a guild approach, or another habitat-species grouping can be used, if the project is predicted to functionally alter the ecosystem such that a guild or grouping (e.g., water-dependent birds) could be impacted, and where dynamics between species (inter- and intra-specific competition due to changes in relative abundance) could also play a role in impacts. A review of Tier 1 study results may also indicate a need for subsequent Tier 1 effort, which can be indicated in BPD Report updates.

Baseline methods for subsequent years of the program will be updated through ongoing reviews of best practices, standards, guidelines, and emerging technologies, and through learning from the first year(s) of baseline data collection. The Tier 2 studies, as directed by results from the Tier 1 studies, will focus primarily on a refined selection of biodiversity values relative to Tier 1 studies (see Zoetica's BPPA Report, Section 5.2). The anticipated preferred methods to be used for Tier 2 studies are listed below:

- Vegetation:
    - Floristic inventory and intuitive meander to survey for rare plants, culturally significant plants, and weeds, introduced and invasive plants
- Ungulates:
    - Moose aerial inventory
- Carnivores:
    - Barbed wire hair traps with DNA analysis, paired with remote camera traps
    - Snow track surveys
- Small Terrestrial Mammals:
    - Snowshoe hare tracking program
    - Snow track surveys
- Semi-Aquatic Mammals:
    - Beaver lodge and/or food cache counts
    - Snow track surveys
- Bats:
    - Visual and/or acoustic monitoring for hibernacula (*if needed*)
    - Surveys to identify candidate maternity colony/roost sites
    - Exit surveys at candidate roost trees (*if needed*)
    - Stationary point acoustic surveys (automated bat detectors)
- Birds:
    - Helicopter nest surveys (raptors)
    - Point count surveys (upland breeding birds, game birds)
    - Call playback surveys (cryptic waterbirds, nocturnal raptors)
    - Lek surveys (sharp-tailed grouse; *if needed*)
    - Observation stations (waterbirds) – spring staging, breeding, fall staging
    - Nightjar acoustic surveys (SAR: eastern whip-poor-will, common nighthawk)
    - Ground sign and nest surveys (raptors) and transect surveys (open habitat raptors)
    - Autonomous song meters (upland breeding birds including SAR, owls)

- Herpetofauna
    - Ground visual encounter surveys (amphibians and reptiles)
    - Aquatic visual encounter survey and egg mass surveys (amphibians), supplemented with aquatic traps (amphibians) and drift fences (amphibians and reptiles) if needed
    - Song meters and auditory surveys (amphibians)
- Fish and Fish Habitat
    - Fish community surveys
- Primary and Secondary Aquatic Producers
    - Taxonomic surveys (to be completed as a collaboration between the BIS and EMBP)

Eventually, Tier 1 and 2 data collection efforts are expected to result in a select number of focused questions, which can be addressed using focused Tier 3 protocols. Some Tier 3 studies have been identified as possibilities in Zoetica's BPPA Report, Section 5.2, but they will have to be solidified and modified based on data from Tiers 1 and 2. The focus of Tier 3 studies will be answering specific and relevant questions that arise from undertaking Tier 1 and Tier 2 studies and will typically focus on target taxa.

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APPENDIX A – STANDARD OPERATING PROCEDURES

Standard Operating Procedures (SOPs), field data forms, work instructions, checklists, and other instructional documents needed for the BIS baseline field studies are presented in this appendix and distributed to the field data collection contractor. SOPs for Tier 1 studies include:

- Terrestrial Ecosystem Mapping & Habitat Suitability Modelling SOP
- Aquatic Habitat Mapping SOP
- Aquatic Environmental DNA Field Sampling SOP

TEM and Habitat Suitability Field SOP

THE NWMO BIODIVERSITY IMPACT STUDIES: STANDARD OPERATING PROCEDURE FOR TERRESTRIAL ECOSYSTEM MAPPING & HABITAT SUITABILITY MODELLING

August 14, 2020

PREPARED BY Zoetica Environmental Consulting Services

SUBMITTED TO Melissa Mayhew
Nuclear Waste Management Organization
22 St. Clair Avenue East
Sixth Floor, Toronto, ON
M4T 2S3, Canada

ZOETICA
ENVIRONMENTAL CONSULTING SERVICES

OFFICE 102-22351 St Anne Ave, Maple Ridge, BC, V2X 2E7
PHONE 604 467 1111
WEBSITE www.zoeticaenvironmental.com

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A000	10-Jul-2020	First submission to NWMO	D. MacKinnon	A. Buckman	H. Bears
B000	14-Aug-2020	BIS Year 1 final submission to NWMO	D. MacKinnon	C. Chui	H. Bears

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GLOSSARY OF TERMS

BAF	Basal Area Factor
CWD	Coarse Woody Debris
DBH	Diameter at Breast Height
ELC	Ecological Land Classification
GPS	Global Positioning System
GRTS	Generalized Random Tessellation Stratified
NWMO	Nuclear Waste Management Organization
OS	OverSample
P1	PanelOne
QA	Quality Assurance
QC	Quality Control
SOP	Standard Operating Procedure
TEM	Terrestrial Ecosystem Mapping
UTM	Universal Transverse Mercator
Full, Ground and Visual Survey Types	Each survey type explains the level of detail which will be collected at each site. A Full Survey is the highest level of detail; all forms are completed. Ground Surveys have a moderate amount of detail; the Vegetation Form, Wildlife Assessments, and only the required fields of the Site and Soil Description Form need to be completed. A Visual Survey is the lowest level of detail; only the required fields of the Site and Soil Description Form need to be completed.
PanelOne and OverSample points	PanelOne (P1) points are pre-selected points in a Generalized Random Tessellation Stratified study design. If a P1 site is found to be inaccessible after reasonable effort, then the surveyor can visit/consider the next OverSample (OS) point as a replacement.

1.0 INTRODUCTION

1.1 Purpose and Principle

The purpose of this Standard Operating Procedure (SOP) is to guide field-contractors in the collection of data to support the Nuclear Waste Management Organization's (NWMO) goal of building a Deep Geologic Repository for the storage of used nuclear waste.

There are currently insufficient data available for mapping the landscape to the level of detail or spatial resolution needed to support other studies and habitat suitability modelling products. The current SOP focuses on collecting baseline data for defining ecosystems, ecosystem attributes, and habitats that could host various species of interest in the area. The completion of Terrestrial Ecosystem Mapping (TEM) and Habitat Suitability Modelling for the project will act as a foundation of information that will be used in planning and focusing on future studies. The methods described in this Standard Operating Procedure (SOP) are based on the general methodology in *Standard for Terrestrial Ecosystem Mapping in British Columbia* (RIC 1998) and the detailed field methods in *Field Manual for Describing Terrestrial Ecosystems 2nd Edition* (BC MFR and BC MOE 2010). All figures and tables are from *Field Manual for Describing Terrestrial Ecosystems 2nd Edition* (BC MFR and BC MOE 2010) unless otherwise indicated.

1.2 General Precautions

Suggestions and precautions noted herein should not be interpreted as prescriptive or exhaustive. The field contractor is responsible for their own field safety protocols, which includes the planning of field safety gear.

The area in which the fieldwork is to be completed is remote, and hazards may arise while in the field. The field contractors should be experienced in the region, or a similar remote area, and should ensure that adequate safety protocols are developed for field crews. It is the responsibility of the field contractor to develop safety protocols and to ensure field crews are properly trained and skilled in the data collection methods being employed. The field contractor is responsible for their own field safety protocols, which includes the planning of field safety gear (including redundancies).

Where certain survey points are inaccessible due to safety concerns, the field crew will be able to skip the point and move to the next available point without compromising the statistical validity of the survey, following the design within this program. If a helicopter is available, it can be used to access points that are inaccessible by ground, to access locations close enough to facilitate ground-based entry to points. Where helicopters are used, it is the responsibility of the field contractor and contracted helicopter company to ensure that all helicopter related safety protocols are followed.

1.3 Quality Control

The field contractor is expected to develop their own Quality Assurance (QA) and Quality Control (QC) protocols to ensure that field-based activities (e.g., data collection and data entry) meet the expectations of the NWMO. QA/QC protocols must include a method to back up all field data collected and to prevent the loss of data stored in one location or method (e.g., a lost field form).

2.0 SITE AND SOIL DESCRIPTION

2.1 Equipment and Materials

Field forms for printing can be found in Appendix A; all hard copies brought into the field should be printed on waterproof paper. Where possible, electronic equipment should also be waterproof. The field contractor is responsible for their own field safety protocols, which includes the planning of field safety gear.

2.1.1 General Field Equipment

- Hard copies of field forms
- Hard copies of SOP
- Hard copies of survey location coordinates and field maps
- Hard copy of *Ecosites of Ontario* (ELC Working Group 2009)
- Pen/pencils
- Waterproof notebook
- Clipboard
- Binoculars
- Camera
- GPS unit
- Tablet or smartphone with georeferenced map
- Compass
- Large spool measuring tape
- Small measuring tape

2.1.2 Soil Description Specific Equipment

- Shovel
- Trowel
- Golf tees
- Soil pH testing kit
- Pruners
- Water bottle (for texturing)
- Grain size card
- Munsell colour charts
- Hand lens
- Hard copy of *Field Guide to the Substrates of Ontario* (OMNR 2015)

2.2 Field Procedure

1. Each day, a route should be determined using the *List of Sites and Site Information* in Appendix B and *Map of Field Sampling Locations* in Appendix C. Each daily route plan should ensure any sites not visited the previous day due to time limitations are included in the next day's route. The field contractor should note any required use of helicopters to assist access or speed up fieldwork.
2. When in the field, follow the day's route to navigate to the next site. Sites have been determined using a GRTS design to create stratified and randomized points. As part of this design method, some points are pre-selected as PanelOne (P1) for sampling or OverSample (OS). If a P1 site is determined after a reasonable effort to be inaccessible, an OS site can be used in place of the P1 site. If the OS site is also deemed inaccessible after reasonable effort, the next OS site can be visited/considered. The field contractor should take care not to skip points too quickly and without effort in favour of randomized points that happen to occur next to easily accessible areas (roads, cleared forest); this may impose a site selection bias meant to be diminished via the use of pre-selected, random points. While points should be visited in order wherever possible, logistical constraints due to difficulty accessing areas may render it necessary to deviate from the ordering from P1 points and OS points from time to time, (e.g., in a situation where there is only one way into a large, difficult to access area and points can be selected along a hiking route). In such cases, the replacement of any P1 sites with an OS site should be noted in the notes section of the ***Site and Soil Description Form***.

3. Using the *List of Sites and Site Information* in Appendix B, check what type of survey is meant to be conducted at each point visited (**Full, Ground, or Visual**; this survey type is predetermined for the field contractor by Zoetica).
4. Locate and mark plot boundaries (20m x 20m square) for Full plots or plot center for Ground plots (no marking required for visual plots).
5. Assemble required gear for survey type according to the gear lists in Section 3.1.

2.2.1 Full Site and Soil Description Procedure

1. Photograph the plot. It is a good idea to take 4 photos in each cardinal direction (begin looking north and turn clockwise), as well as one that shows canopy cover (up) per site. Photographs should also be taken of any notable features and the location the soil pit will be dug. Record the number of each photo in notes and photo number, along with a description of what the photo is showing.
2. Locate and excavate a soil pit to a depth of about 50 cm. Photograph pit profile.
3. On the ***Site and Soil Description Form***, enter the information in the header (date, plot number, crew ID, all surveyors' names and roles, weather, page number).
4. Using the *List of Sites and Site Information* in Appendix B, record the information in the predetermined site information section (UTM zone, easting/lat., northing/long., GRTS number, polygon number, ecosite code, and survey type)
5. Record latitude and longitude or UTM coordinates using field GPS. Record estimated location accuracy and elevation.
6. Determine the slope and aspect.
7. Traverse the entire plot systematically, observing the position of the plot relative to the surrounding landscape, microtopographic features, and the composition of surface substrates. Record the mesoslope position.
8. Assess the soils and determine humus form, soil drainage, rooting zone soil texture, and percent coarse fragments. Estimate the depth of soil and rooting depth. Note presence and depth of Ah or Ae horizons, gleying, and seepage. Record the depth and type of root restricting layer, if any, and the depth of the surface organic horizon.
9. Confirm the ecosite code. Integrate site, soil and vegetation factors and keys in *Ecosites of Ontario* (ELC Working Group 2009) to determine ecosite code. On the ***Site and Soil Description Form,*** note the new ecosite code and rationale for change if the ecosite determined in the field is different from the predetermined ecosite.
10. Describe the key site features under notes and photo number. Draw a site diagram if important features can be effectively depicted.
11. Check to be sure that all required fields have been completed.

2.2.2 Ground Site and Soil Description Procedure

1. Photograph the plot. It is a good idea to take 4 photos in each cardinal direction (begin looking north and turn clockwise), as well as one that shows canopy cover (up) per site. Photographs should also be taken of any notable features and the location the soil pit will be dug. Record the number of each photo in notes and photo number, along with a description of what the photo is showing.
2. Locate and excavate a soil pit to a depth of about 50 cm. Photograph pit profile
3. On the ***Site and Soil Description Form***, enter the information in the header (date, plot number, crew ID, all surveyors' names, weather, page number).

4. Using the *List of Sites and Site Information* in Appendix B, record the information in the predetermined site information section (UTM zone, easting/lat., northing/long., GRTS number, polygon number, ecosite code, and survey type).
5. Record latitude and longitude or UTM coordinates using field GPS. Record estimated location accuracy and elevation.
6. Confirm the ecosite code. Integrate site, soil, and vegetation factors and keys in *Ecosites of Ontario* (ELC Working Group 2009) to determine ecosite code. On the ***Site and Soil Description Form,*** note the new ecosite code and rationale for change if the ecosite determined in the field is different from the predetermined ecosite.
7. Describe the key site features under notes and photo number. Draw a site diagram if important features can be effectively depicted.
8. Check the form to ensure all the required information has been collected.

2.2.3 Visual Site and Soil Description Procedure

1. Photograph the plot. It is a good idea to take 4 photos in each cardinal direction (begin looking north and turn clockwise), as well as one that shows canopy cover (up) per site. Photographs should also be taken of any notable features. Record the number of each photo in notes and photo number, along with a description of what the photo is showing.
2. On the ***Site and Soil Description Form***, enter the information in the header (date, plot number, crew ID, all surveyors' names, weather, page number).
3. Using the *List of Sites and Site Information* in Appendix B, record the information in the predetermined site information section (UTM zone, easting/lat., northing/long., GRTS number, polygon number, ecosite code, and survey type).
4. Record latitude and longitude or UTM coordinates using field GPS. Record estimated location accuracy and elevation.
5. On the ***Site and Soil Description Form***, record what ecosite code the plot is in. Use the keys in *Ecosites of Ontario* (ELC Working Group 2009) to identify the ecosite code. If different from the pre-typed ecosite, detail why it was changed.
6. Describe the key site features under notes and photo number. Draw a site diagram if important features can be effectively depicted.
7. Check the form to ensure all the required information has been collected.

2.3 Field Form

Date (DD/MM/YY) (1)	Plot Number (2)	Crew ID (3)	All Surveyors (4)	Weather (5)	Page Of (6)

Predetermined Site Information

UTM Zone	Easting/Lat. (7)	Northing/Long.	GRTS Number (8)	Polygon Number (9)	Ecosite (10)	Survey Type Full ☐ Gr. (11) ☐ Vis ☐

Information Determined at Site

UTM Zone	Easting/Lat. (12)	Northing/Long.	GPS Accur. (m)	Elevation (m)	Ecosite (32)	Rational for Ecosite Change (33)

Site Description

Slope (13) % | **Aspect** (14) ° | **Surface Shape** CCV CVX STR ☐ (15) ☐ ☐ | **Microtop Size** (16)

Meso Slope Pos. Crest ☐ Upper ☐ Mid (17) ☐ Lower ☐ Toe ☐ Level ☐ Dep. ☐ Gully ☐ | **Floodplain?** (18) ☐ | **Drainage** (1-7) (19)

Site dist. ☐ n/a ☐ fire ☐ harvest ☐ site prep ☐ planted ☐ terrain ☐ biotic ☐ soil dist ☐ other _____ (20) _____

Humus/ Organic Form ☐ Mull (21) ☐ Fibrimor ☐ Peatymor ☐ Moder ☐ Humimor ☐ Anmoor

Gleying or Mottling ☐n/a (22) _____cm | **Seepage** ☐n/a (23) _____cm

Humus Thickness (24) _____cm | Ah? ☐ Ae? ☐ (25) _____cm | **Estimated Soil Depth** (26) _____cm | **Estimated Rooting Depth** (27) _____cm | **R.Z. Coarse Frag** (28) % | **R.Z. Text.** (29)

Site Diagram (35)

Substrate Series (30) | **Restricting Layer** ☐n/a ____cm (31) | **Restricting Layer Type** ☐ Cement ☐ Compact ☐ Water ☐ Pan ☐ Lithic ☐ Chem

Notes and Photo Numbers: (34)

Date entered into spreadsheet: (36) Entered by: QA/QC by:

Site and Soil Description Form

2.4 Completing the Form

Field Label	Description and Instructions
1	Enter the **date** in DD-MM-YY format. (e.g., 01-01-20 for January 1st, 2020)
2	Enter the **plot number**, which is composed of the crew number and a sequential 3-digit number determined in the field. For example, If the crew number is 1 and it is their first plot, then the plot number is 1001, with the second plot being 1002.
3	Enter the **crew ID**.
4	Enter all **surveyor(s)** first initial and last name, followed by their role.
5	Record the **weather** at the beginning of the survey and update with any major changes. If more room is needed, use the notes section.
6	Enter digit for the **page number**. Complete the total number of pages once the survey is complete in case multiple pages are used.
7	Copy the predetermined site coordinates from the *List of Sites and Site Information* in Appendix B. If using UTM coordinates, enter the **UTM zone, easting**, and **northing**. Otherwise, enter the **latitude** and **longitude**.
8	Copy the **GRTS number** from the *List of Sites and Site Information* in Appendix B.
9	Copy the **polygon number** from the *List of Sites and Site Information* in Appendix B.
10	Copy the **ecosite** code that was previously determined from the *List of Sites and Site Information* in Appendix B.
11	Check the box for the **survey type** being completed, either full, ground, or visual. This information will be on the *List of Sites and Site Information* in Appendix B.
12	Using a GPS unit, write the **coordinates** of the center of the plot, the **accuracy** of the GPS, and the **elevation**.
13	Record the percent **slope** gradient, measured with a clinometer or similar instrument.
14	Record the **aspect**, which is the orientation of the slope relative to true north, measured by compass, in degrees. Enter due north as 360°, and for level ground enter 0.
15	Note the general **surface shape** and mark the appropriate surface shape box. **CCV** **Concave** – surface profile is mainly "hollow" in one or several directions **CVX** **Convex** – surface profile is mainly "rounded" like the exterior of a sphere **STR** **Straight** – surface profile is linear, either flat or sloping in one direction
16	Record the type, size, and frequency of **microtopographic** features. **Size and frequency of microtopographic features:** **mc.** **micro** – low relief features (< 0.3 m high) with minimal effect on vegetation **sl.** **slightly** – prominent features (0.3–1m high) spaced > 7 m apart **md.** **moderately** – prominent features (0.3–1m high) spaced 3–7 m apart **st.** **strongly** – prominent features (0.3–1m high) spaced 1–3 m apart **sv.** **severely** – prominent features (0.3–1m high) spaced < 1 m apart **ex.** **extremely** – very prominent features (> 1 m high) spaced > 3 m apart **ul.** **ultra** – very prominent features (> 1 m high) spaced < 3 m apart

Types of microtopographic features:

cha **channelled** – incised water tracks or channels
dom **domed** – raised bogs
gul **gullied** – geomorphic ridge and ravine patterns
hmk **hummocked** – mounds composed of organic materials
lob **lobed** – solifluction lobes
mnd **mounded** – mounds composed of mineral materials
net **netted** – net vegetation patterns from freeze-thaw action in alpine or subarctic terrain
pol **polygonal** – polygonal patterns associated with permafrost
rib **ribbed** – wetland pattern with raised ridges perpendicular to the direction of water flow
dmo **smooth** – surface relatively flat
tus **tussocked** – associated with tussock-forming graminoids
und **undulating** – controlled by bedrock

17 Indicate the **mesoslope position** of the plot relative to the localized catchment area (see **Figure 2-1**).

Crest The generally convex uppermost portion of a hill; usually convex in all directions with no distinct aspect.
Upper Slope The generally convex upper portion of the slope immediately below the crest of a hill; has a specific aspect.
Middle Slope Area between the upper and lower slope; the surface profile is generally neither distinctly concave nor convex; it has a straight or somewhat sigmoid surface profile with a specific aspect.
Lower Slope The area toward the base of a slope; generally has a concave surface profile with a specific aspect.
Toe The area demarcated from the lower slope by an abrupt decrease in slope gradient; seepage is typically present.
Depression Any area concave in all directions; may be at the base of a mesoscale slope or in a generally level area.
Level Any level meso-scale area not immediately adjacent to a meso-scale slope; the surface profile is generally horizontal and straight with no significant aspect.

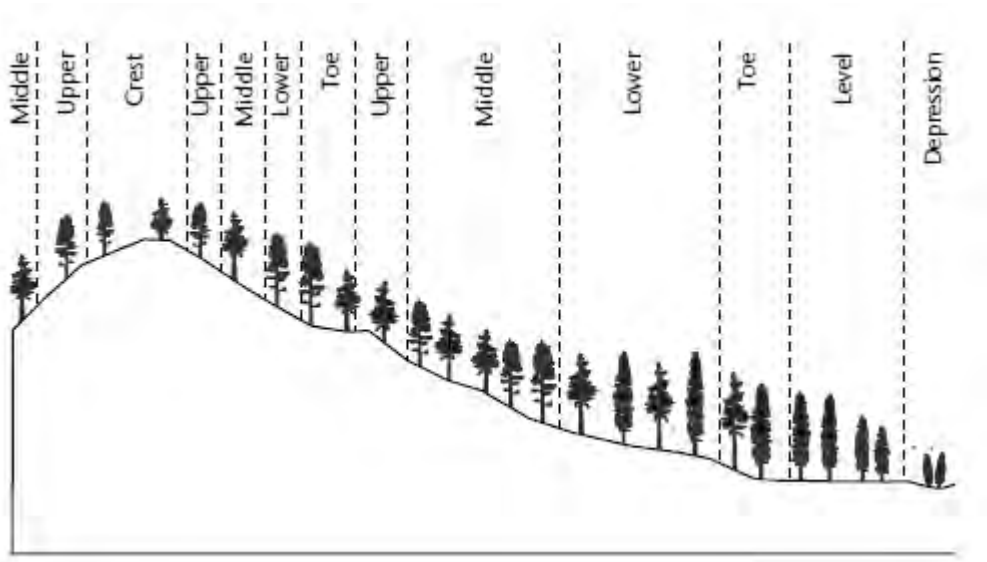

Figure 2-1. Mesoslope position.

18 Indicate whether the site is within a **floodplain**.

19 **Drainage class** describes the speed and extent to which water is removed from a mineral soil in relation to additions. Enter the code according to the chart in **Figure 2-2** or the definitions in **Table 2-1.**

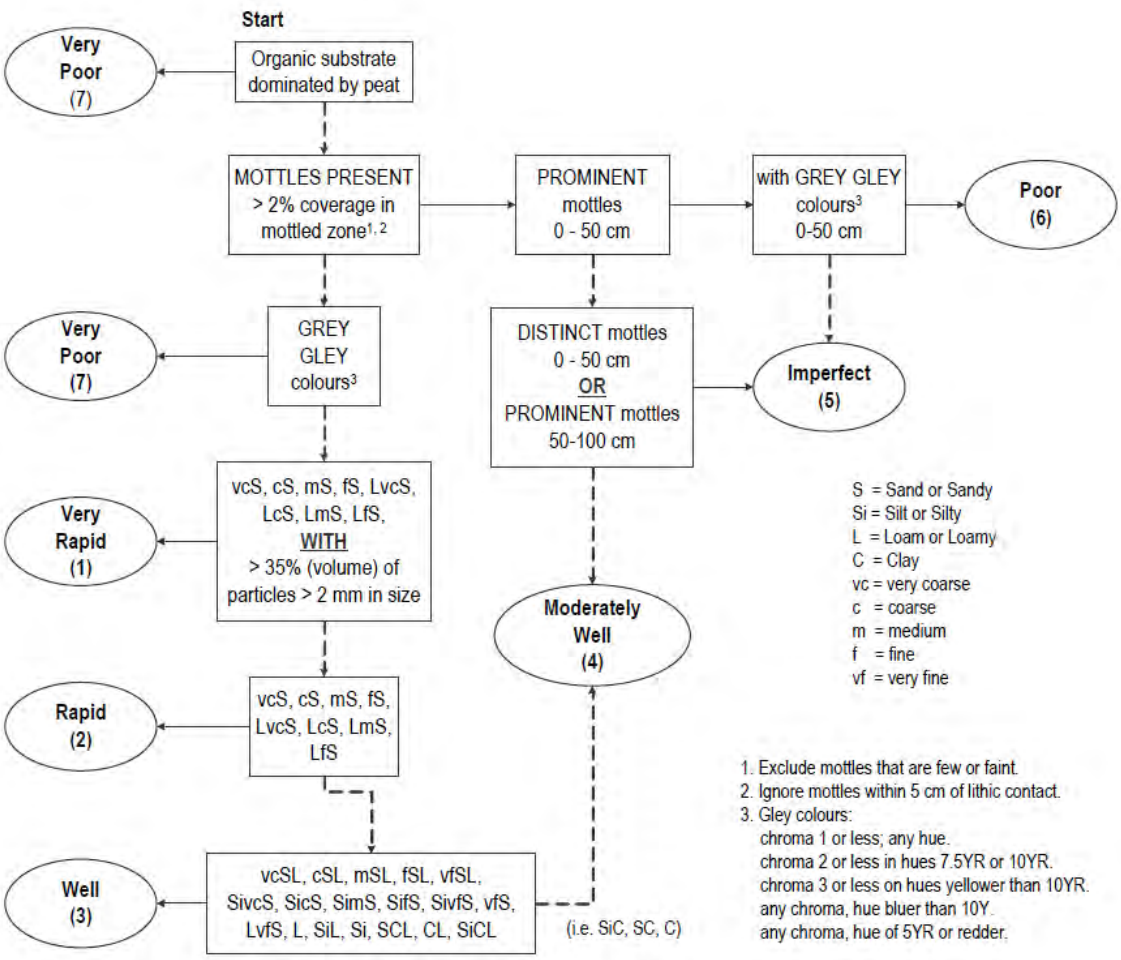

Figure 2-2. Drainage classes (OMNR 2015).

Table 2-1. Drainage classes and codes.

Code	Name	Description
1	**Very Rapid**	Water is removed from the soil very rapidly in relation to supply. Water source is precipitation and available water storage capacity following precipitation is essentially nil. Soils are typically fragmental or skeletal, shallow, or both.
2	**Rapid**	Water is removed from the soil rapidly in relation to supply. Excess water flows downward if the underlying material is pervious. Subsurface flow may occur on steep gradients during heavy rainfall. Water source is precipitation. Soils are generally coarse-textured.
3	**Well**	Water is removed from the soil readily, but not rapidly. Excess water flows downward readily into underlying pervious material or laterally as subsurface flow. Water source is precipitation. On slopes, subsurface flow may occur for short durations, but additions are equalled by losses. Soils are generally intermediate in texture and lack restricting layers.

4	**Moderately Well**	Water is removed from the soil somewhat slowly in relation to supply because of imperviousness or lack of gradient. Precipitation is the dominant water source in medium- to fine-textured soils; precipitation and significant additions by subsurface flow are necessary in coarse-textured soils.
5	**Imperfect**	Water is removed from the soil sufficiently slowly in relation to supply to keep the soil wet for a significant part of the growing season. Excess water moves slowly downward if precipitation is the major source. If subsurface water or groundwater (or both) is the main source, the flow rate may vary but the soil remains wet for a significant part of the growing season. Precipitation is the main source if available water storage capacity is high; contribution by subsurface or groundwater flow (or both) increases as available water storage capacity decreases. Soils generally have a wide range of texture, and some mottling is common.
6	**Poor**	Water is removed so slowly in relation to supply that the soil remains wet for much of the time that it is not frozen. Excess water is evident in the soil for a large part of the time. Subsurface or groundwater flow (or both), in addition to precipitation, are the main water sources. A perched water table may be present. Soils are generally mottled and/or gleyed.
7	**Very Poor**	Water is removed from the soil so slowly that the water table remains at or near the surface for most of the time the soil is not frozen. Groundwater flow and subsurface flow are the major water sources. Precipitation is less important, except where there is a perched water table with precipitation exceeding evapotranspiration. Typically associated with wetlands. For organic wetlands, also evaluate the soil moisture subclass, and when entering on the form, separate from drainage by a slash. For example, v/ac.

20 Note any **site disturbance** events that have caused vegetation and soil characteristics to differ from those expected at climax for the site. Provide details in the **Notes** section.

21 Examine the **humus form profile** and tick the appropriate box according to **Figure 2-3**.

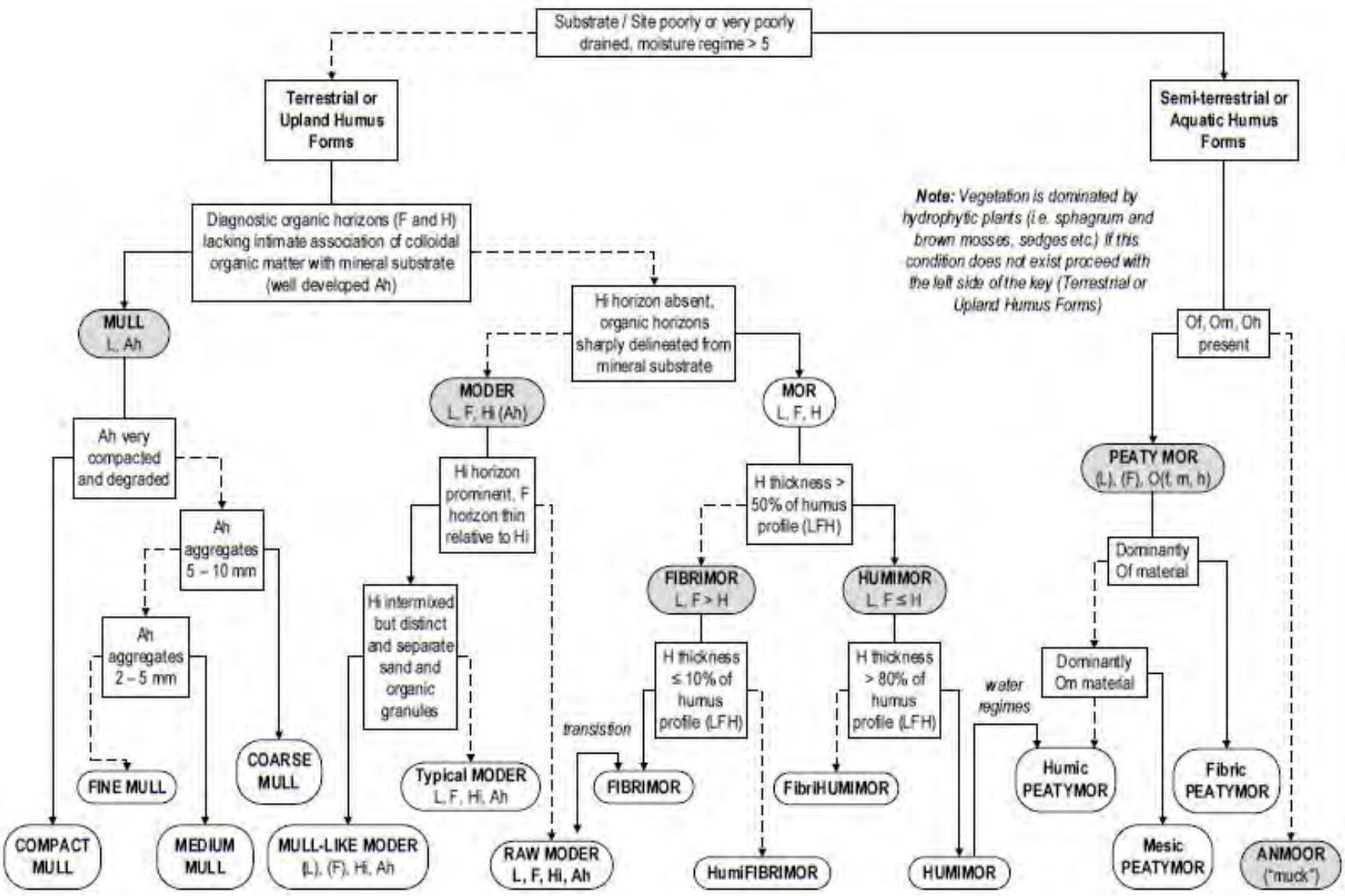

Figure 2-3. Key to humus form classification (MNR, 2015).

22 Record the depth (from the ground surface) at which **mottles or gleying** appear. If no mottling is present, mark the n/a box.

23 Record the depth (from the ground surface) of active **seepage** or water table. If no seepage is present, mark the n/a box.

24 Measure the **thickness of the surface organic horizon**, in centimetres, from the top of the ground surface to the top of the first mineral horizon. Check the appropriate box.

25 If an **A horizon** is present, mark the appropriate type box and record the thickness of the horizon in centimetres.

26 Record the **thickness of the entire soil profile** from the ground surface to a root restricting layer. If no restricting layer is noted in the soil pit, estimate the depth of soil based on nearby road cuts or other indicators of active seepage or water table.

27 Record the **rooting zone depth.** It is measured from the ground surface to the point at which the majority of roots stop.

28 Estimate the **percent coarse fragment** (>2 mm diameter) volume in the rooting zone of the soil profile rounded to the nearest 10%.

29 After determining the rooting depth, estimate the **rooting zone texture** class as a weighted average of the mineral horizons within the rooting zone (**Figure 2-4**, **Table 2-2**). Where rooting is restricted to the organic horizons, use the organic material codes in **Table 2-2**. Rooting zone classes are greatly simplified and use only percent coarse fragments (> 2 mm) by volume, and texture class sizes by percent weight for sand (.05 to < 2 mm), silt (< .05 to .002 mm), and clay (< .002).

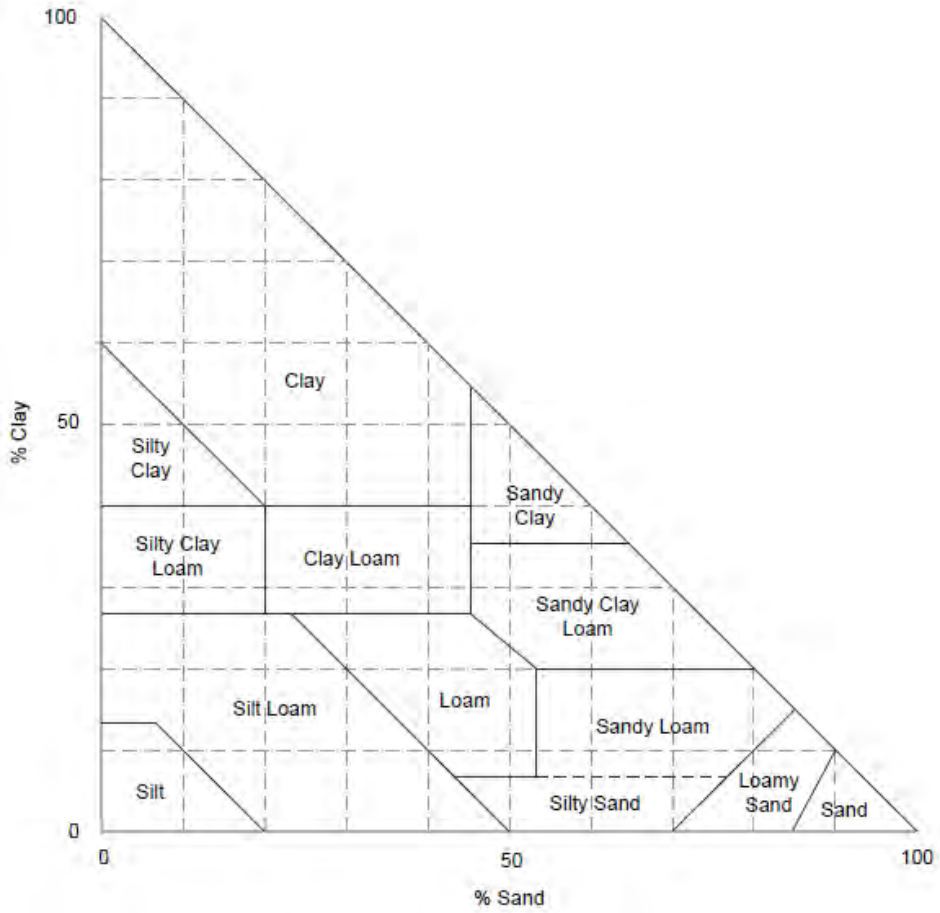

Figure 2-4. Rooting zone particle size classes (OMNR 2015).

Table 2-2. Rooting zone particle sizes classes.

Code	Class[a]	Definitions	
Coarse fragments ≥ 70%			
F	Fragmental	Particles <2 mm of various textures	
Coarse fragments ≥ and less than 70%			
SS	Sandy-skeletal	Particles <2 mm sandy	
CLS	Coarse-loamy-skeletal	Particles <2 mm coarse-loamy	
FLS	Fine-loamy-skeletal	Particles <2mm fine-loamy	
SIS	Silty-skeletal	Particles <2 mm fine-silty or coarse-silty	
CS	Clayey-skeletal	Particles <2 mm clayey	
Code	**Class**	**Code**	**Class**
Coarse fragments < 35%			
S	Sandy	Organic Material Codes	
CL	Coarse-loamy	**FI**	Fibric
FL	Fine-loamy	**ME**	Mesic
CSI	Coarse-silty	**HU**	Humic
FSI	Fine-silty	**WO**	Woody
FC	Fine-clayey		
VFC	Very-fine-clayey		

30 Determine the **substrate series u**sing the keys found in *Field Guide to the Substrates of* Ontario (OMNR 2015).

31 Identify and record the **type of root restricting** layer, if present. Mark the n/a box if no restricting layer is observed. Measure and record the **depth** in centimetres. From the ground surface (top of the humus layer). Mark the appropriate type code.

32 Discuss with the vegetation specialist and decide on the **ecosite** code which best describes the site.

33 Describe the **rational for ecosite change** by detailing what about the plot does not align with the previously assigned ecosite code and/or why the new ecosite code fits better. If more room is needed, use the notes section.

34 Use the **notes and photo number** space to record important site or soil features not described elsewhere, or for explanatory notes keyed to other entries on the form. All photos taken for this form need the photo number recorded, and a brief description of what is shown in the photo. If more space is needed, use the back of the field sheet and indicate with a large and bold arrow that more information is recorded on the back of the form.

35 Provide a sketch of the site if there are any features of note.

36 Not to be completed in the field. Data should be entered into a spreadsheet as soon as possible, ideally completed daily. Once data are entered into the spreadsheet, fill in the date entered and who entered it. Data entry should be checked by a second person and any discrepancies discussed with the original surveyor. Enter the name of the QA/QC checker once completed.

3.0 VEGETATION SURVEY

3.1 Equipment and Materials

Field forms for printing can be found in Appendix A; all hard copies brought into the field should be printed on waterproof paper. Where possible, electronic equipment should also be waterproof. The field contractor is responsible for their own field safety protocols, which includes the planning of field safety gear.

3.1.1 General Field Equipment

- Hard copies of field forms
- Hard copies of SOP
- Hard copies of survey location coordinates and field maps
- Hard copy of *Ecosites of Ontario* (ELC Working Group 2009)
- Pen/pencils
- Waterproof notebook
- Clipboard
- Binoculars
- Camera
- GPS unit
- Tablet or smartphone with georeferenced map
- Compass
- Large spool measuring tape
- Small measuring tape

3.1.2 Vegetation Specific Equipment

- Plant identification guides
- Hand lens
- Diameter tape
- Releskop or prism set
- Increment borer
- Straws
- Tape
- Flagging tape
- Flagging stakes
- Hard copies of critical distance tables
- Calculator
- Callipers

3.2 Field Procedure

1. Each day, a route should be determined using the List of Sites and Site Information in Appendix B and Map of Field Sampling Locations in Appendix C. Each daily route plan should ensure any sites not visited the previous day due to time limitations are included in the next day's route. The field contractor should note any required use of helicopters to assist access or speed up fieldwork.
2. When in the field, follow the day's route to navigate to the next site. Sites have been determined using a GRTS design to create stratified and randomized points. As part of this design method, some points are pre-selected as PanelOne (P1) for sampling or OverSample (OS). If a P1 site is determined after a reasonable effort to be inaccessible, an OS site can be used in place of the P1 site. If the OS site is also deemed inaccessible after reasonable effort, the next OS site can be visited/considered. The field contractor should take care not to skip points too quickly and without effort in favour of randomized points that happen to occur next to easily accessible areas (roads, cleared forest); this may impose a site selection bias meant to be diminished via the use of pre-selected, random points. While points should be visited in order wherever possible, logistical constraints due to difficulty accessing areas may render it necessary to deviate from the ordering from P1 points and OS points from time to time, such that points can be planned to be visited along hiking routes (e.g., in a situation where there is only one way into a large, difficult to access area). In such cases, the replacement of any P1 sites with an OS site should be noted in the notes section of the ***Site and Soil Description Form***.

3. Using the *List of Sites and Site Information* in Appendix B, determine survey type (Full, Ground, or Visual).
4. Locate and mark plot boundaries (20m x 20m square) for Full plots or plot center for Ground plots (no marking required for visual plots).
5. Assemble required gear for survey type according to the gear lists in Section 3.1.

3.2.1 Full Vegetation Survey Procedure

1. Photograph the plot. It is a good idea to take 4 photos in each cardinal direction (begin looking north and turn clockwise), as well as one that shows canopy cover (up) per site. Photographs should also be taken of any notable features. Record the number of each photo in notes and photo number, along with a description of what the photo is showing.
2. On the ***Vegetation Survey Form***, enter the information in the header (date, plot number, polygon number, vegetation surveyors' names, weather, survey type, page number).
3. Standing at one point in the plot, list all species observed in each layer.
4. Traverse the entire plot (or one quadrant at a time) in an increasing spiral or zigzag pattern, noting each new species.
5. Collect unknown species, recording each by a temporary name and plot collection number on the form (e.g., moss 01, hairy grass 02, herb 03, etc.). Mark sample bags and pressing sheets with plot and collection numbers.
6. When the list seems complete, begin estimating percent cover. For each layer:
    - estimate total layer cover and enter at top of the form;
    - estimate individual species covers for the entire layer and sublayers, if present (i.e., first A, then A1, A2, A3);
    - add up species covers and compare to total species cover and total layer cover; reconcile any discrepancies, remembering that overlap can occur between species and layers.
7. Check that all required fields have been completed on the form.
8. On the ***Tree Attributes for Wildlife Survey Form***, enter the information in the header (date, plot number, polygon number, vegetation surveyors' names, BAF, Plot Size, Minimum DBH, page number).
9. For each sample tree, record the species and classify them as standing or fallen.
10. Determine dbh and percentage of bark remaining at breast height.
11. Record data required to calculate the length of each sample tree or estimate the length.
12. For each standing live tree, assign a crown class and determine the height to live crown.
13. For each sample tree, assess the appearance, crown condition, bark retention, wood condition, lichen loading, and wildlife use.
14. Check that all the required information has been collected and noted on the form. Strikethrough any fields that were not assessed.
15. On the ***Coarse Woody Debris Survey Form***, enter the information in the header (date, plot number, polygon number, vegetation surveyors' names, page number).
16. Establish the first coarse woody debris sampling line by following a random azimuth for that plot.
    - Measure out a 24-m line from the plot centre, correcting the slope distance to horizontal by using slope tables or trigonometry.
    - Anchor the tape at both ends of the line.
17. Establish the second sampling line at plus 90° from the first line by following the same procedures in (16) above.
18. Record the azimuth of each line.

19. Note the length of each line sampled out of the total. The full length of one or both lines may not be sampled because of unsafe conditions or heavy accumulations of CWD. Otherwise, they will be 24 out of 24 m.
20. Walk out along the first sampling line and select the pieces of CWD to be measured according to the sampling rules. Take care not to trample and crush the CWD as you walk along the line.

    As each piece that fits the definition of CWD is encountered, note the following:
    - tree species to the level that is reliable,
    - diameter,
    - decay class, based on the entire piece, by using the table of decay class indicators,
    - tilt angle of each piece, and
    - length of each piece.
21. Where CWD pieces are suspended above the sampling line it may be necessary to estimate certain attributes (diameter and/or length).
22. If odd-shaped pieces are encountered, use the rectangular method by record their diagonal length at intersection and width (ground to height of piece) (Marshall *et al.* 2000).
23. Repeat steps 20, 21, and 22 for the second transect line.
24. Check the form to ensure all the required information has been collected.
25. Confirm the ecosite code. Integrate site, soil and vegetation factors and keys in *Ecosites of Ontario* (ELC Working Group 2009) to determine ecosite code. On the ***Site and Soil Description Form,*** note the new ecosite code and rationale for change if the ecosite determined in the field is deferent to the predetermined ecosite.

3.2.2 Ground Vegetation Survey Procedure

1. Photograph the plot. It is a good idea to take 4 photos in each cardinal direction (begin looking north and turn clockwise), as well as one that shows canopy cover (up) per site. Photographs should also be taken of any notable features. Record the number of each photo in notes and photo number, along with a description of what the photo is showing.
2. On the ***Vegetation Survey Form***, enter the information in the header (date, plot number, polygon number, vegetation surveyors' names, weather, survey type, page number).
3. Record the dominant and indicator plant species, noting the layer. Evaluate the percent cover by species and total for each layer.
4. Determine the structural and successional stages.
5. Measure or estimate stand age, average stand height, and canopy composition.
6. Confirm the ecosite code. Integrate site, soil and vegetation factors and keys in *Ecosites of Ontario* (ELC Working Group 2009) to determine ecosite code. On the ***Site and Soil Description Form,*** note the new ecosite code and rationale for change if the ecosite determined in the field is deferent to the predetermined ecosite.
7. Check the form to ensure all the required information has been collected.

3.2.3 Visual Vegetation Survey Procedure

1. Photograph the plot. It is a good idea to take 4 photos in each cardinal direction (begin looking north and turn clockwise), as well as one that shows canopy cover (up) per site. Photographs should also be taken of any notable features. Record the number of each photo in notes and photo number, along with a description of what the photo is showing.

2. On the ***Site and Soil Description Form***, record what ecosite code the plot is in. Use the keys in *Ecosites of Ontario* (ELC Working Group 2009) to identify the ecosite code. If different from the pre-typed ecosite, detail why it was changed.
3. Check the form to ensure all the required information has been collected.

3.3 Field Forms

Date (DD/MM/YY) (1)	Plot Number (2)	Polygon Number (3)	Surveyor(s) (4)	Weather (5)	Survey Typ (6) Full ☐ Grd. ☐	Page (7)	Of

COL.	TREES	A1	A2	A3	A	B1	B2	B
(8)	(9)				(10)			

COL.	SHRUBS	B1	B2	B
(8)	(9)			
			(10)	

COL.	HERB LAYER (C)	%
(8)	(9)	(10)

COL.	MOSS/LICHEN/SEEDLING (D)	%
(8)	(9)	(10)

COL.	ADDITIONAL SPECIES	LAYER	%
(8)	(9)		(10)

Vegetation Survey Form

Notes and Photo Numbers: (11)

% Cover by Layer	TREE (A) (12)	SHRUB (B)	HERB (C)	MOSS/LICHEN (D)

Date entered into spreadsheet: (13) Entered by: QA/QC by:

Date (DD/MM/YY) (1)	Plot Number (2)	Polygon Number (3)	Surveyor(s) (4)	BAF (m^2/ha) (5)	Minimum DBH (cm) (6)	Page (7)	Of

Tree no.	Species	Stand/Fall	DBH (cm)	M or E	Rem. Bark (%)	Length				Estimated Length (m)	Cr. Class	Height to Live Crown	Wildlife Codes						Notes and Photo Numbers
						Top (%)	Bot. (%)	Bot. Pos. (m)	Slope Distance (m)				Appear	Crown	Bark	Wood	Lichen	Wildlife Use	(18)
01	(8)	(9)	(10)	(11)	(12)			(13)		(14)	(15)	(16)			(17)				
02																			
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17																			
18																			

Date entered into spreadsheet: (19) Entered by: QA/QC by:

Tree Attributes for Wildlife Survey Form

Date (DD/MM/YY) (1)	Plot Number (2)	Polygon Number (3)	Surveyor(s) (4)	Page	Of (5)

Tree no.	Transect #1							Transect #2							Degree and Type of Piling (in plot boundaries)					
	Azimuth (6)			(0-359)	Sampled (7)		of 24m	Azimuth (6)			(0-359)	Sampled (7)		of 24m						
	Species (8)	Diameter (cm) (9)	Class (10)	Tilt Angle (11)	Length (m) (12)	Height of end (cm) (13)	Angle grnd. (14)	Species	Diameter (cm)	Class	Tilt Angle	Length (m)	Height of end (cm)	Angle grnd.	Pile #	Size of pile (m) Length	Size of pile (m) Width (15)	Size of pile (m) Height	Diam (cm) (16)	Inter. space (17)
01															1					
02															2					
03															3					
04															4					
05															5					
06															6					
07															7					
08															8					
09															Notes and Photo Numbers					
10															(18)					
11																				
12																				
13																				
14																				
15																				
16																				
17																				

Coarse Woody Debris Survey Form

Date entered into spreadsheet: (19) Entered by: QA/QC by:

3.4 Completing the Form

3.4.1 Completing the Vegetation Survey Form

Field Label	Description and Instructions
1	Enter the **date** in DD-MM-YY format. (e.g., 01-01-20 for January 1st, 2020)
2	Enter the **plot number**, which is composed of the crew number and a sequential 3-digit number determined in the field. For example, If the crew number is 1 and it is their first plot, then the plot number is 1001, with the second plot being 1002.
3	Enter the **polygon number** where the plot is taking place.
4	Enter vegetation **surveyor(s)** first initial and last name.
5	Record the **weather** at the beginning of the survey and update it with any major changes. If more room needed, use the notes section.
6	Check the box for **survey type** being completed, either full or ground.
7	Enter digit for the **page number**. Complete the total number of pages once the survey is complete in case multiple pages are used.
8	Check this column if the species is **collected**.
9	Enter the appropriate **species code** for each observed species in the appropriate layer section. Species codes can be found in Chapter 5 – Life Forms in *Ecosites of Ontario* (ELC Working Group 2009); if uncertain of the correct code or a code has not been assigned, write the entire scientific name. Use the "Additional Species" section to list species from the E layer (epiphytes), additional species from any other layer if there has been insufficient space elsewhere on the form, and for species growing on subdominant substrates. Use a second page if needed.
10	Estimate **percent cover** for each species. Trees and Shrub species covers must be estimated by both strata and layer (A1, A2, A3, A, B1, B2, B). If trees in A1 are veterans, record this under "Notes." For each of the A and B layers, the total percent coverage for a species may be less than the sum of the covers for each of the sub-layers, due to crown overlap. **Vegetation Layers** All vegetation is assigned to one of the following layers. Criteria for A and B layers and sub-layers are depicted in **Figure 3-1**. **A**. The *tree layer* includes all woody plants greater than 10 m tall. Three sub-layers are recognized: **A1 Dominant trees** - includes the dominant (tallest) trees of the main canopy, which may be veterans of one or more fires (previously classed as A0), or the tallest trees of the same age class as the main canopy; usually a minor portion of the stand composition. **A2 Main tree canopy (codominant trees)** - the main layer of tree cover, composed of trees whose crowns form the upper layer of foliage; typically the major portion of the stand composition. **A3 Sub-canopy trees** - includes trees greater than 10 m high that do not reach the main canopy; may form a distinct secondary canopy; often a mixture of trees of various heights younger than those in the main canopy or may be suppressed trees of the same age; includes "intermediate" and "overtopped" trees.

B. The *shrub layer* includes all woody plants less than 10 m tall, except low (usually < 15 cm tall) woody or trailing plants which are considered part of the herb layer. Established tree regeneration more than two years of age and less than 10 m in height is considered part of the shrub layer. Two sublayers are recognized:

B1 Tall shrub layer - includes all woody plants 2–10 m tall, including shrubs and advance tree regeneration and trees in poorly growing stands where the canopy is less than 10 m high.

B2 Low shrub layer - includes all woody plants less than 2 m high, except low (< 15 cm) woody or trailing plants; includes shrubs and established tree regeneration more than two years old and dwarfed or immature specimens of species normally considered in the shrub category.

C. The *herb layer* includes all herbaceous species, regardless of height, and some low woody plants less than 15 cm tall.

D. The *moss, lichen, liverwort and seedling layer* includes all bryophytes, terrestrial lichens, and liverworts, and tree seedlings less than two years old that occur on mineral soil and humus.

Dr - *Mosses, lichens, liverworts* that occur on rock.

Dw - *Mosses, lichens, liverworts* that occur on wood.

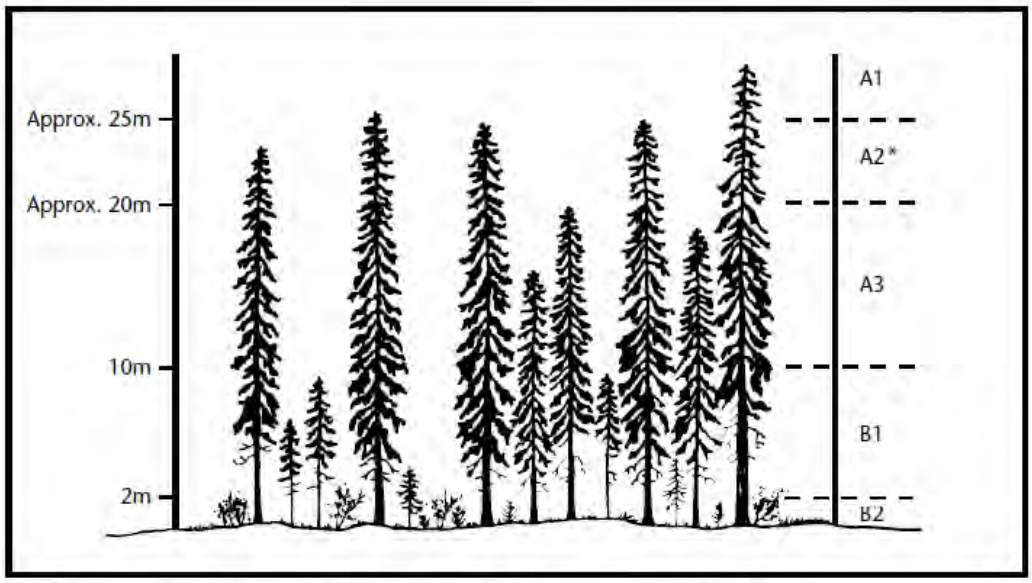


Figure 3-1. Stratification of forest stands, shrubs, and trees.

E. The *epiphyte layer* includes all species which grow on other living plants. Enter epiphytes in the additional species block.

11 Use **notes and photo number** space to record important features not described elsewhere, or for explanatory notes keyed to other entries on the vegetation form. All photos taken for this form need the photo number recorded, and a brief description of what is shown in the photo. If more space is needed, use the back of the field sheet and indicate with a large and bold arrow that more information is recorded on the back of the form.

12 After all species have been listed, enter the **total percent** cover by layer. Note that because of overlaps the sum of the percent cover values for all species within each layer may be greater than the total layer coverage.

13 Not to be completed in the field. Data should be entered into a spreadsheet as soon as possible, ideally completed daily. Once data are entered into the spreadsheet, fill in the date entered and who entered it. Data entry should be checked by a second person and any discrepancies discussed with the original surveyor. Enter the name of the QA/QC checker once completed.

3.4.2 Completing the Tree Attributes for Wildlife Survey Form

Field Label	Description and Instructions
1	Enter the **date** in DD-MM-YY format. (e.g., 01-01-20 for January 1st, 2020)
2	Enter the **plot number**, which is composed of the crew number and a sequential 3-digit number determined in the field. For example, If the crew number is 1 and it is their first plot, then the plot number is 1001, with the second plot being 1002.
3	Enter the **polygon number** where the plot is taking place.
4	Enter vegetation **surveyor(s)** first initial and last name.
5	Enter the standard metric (m^2/ha) **Basal Area Factor (BAF)** prism used.
6	Enter the **minimum diameter (dbh)** being used.
7	Enter digit for the **page number**. Complete the total number of pages once the survey is complete in case multiple pages are used.
8	Enter the appropriate **species code**. Species codes can be found in Chapter 5 – Life Forms in *Ecosites of Ontario* (ELC Working Group 2009); if uncertain of the correct code or a code has not been assigned, write the entire scientific name.
9	Classify the tree as **standing (S) or fallen (F)** using the following criteria: **S Standing** Trees or portions of trees with the root attached and self-supporting (i.e., the tree would remain standing if all supporting materials were removed). **F Fallen** Trees or portions of trees with the root attached and not self-supporting, greater than 1.3 m in length.
10	Measure the **diameter at breast height (dbh)**, i.e., 1.3 m, of all live, dead, standing, and fallen trees. • On slopes, breast height is measured from the high side of the tree. • Measure diameter to the nearest 0.1 cm. • Hold the diameter tape tight, making no allowance for missing bark. • If it is not possible to measure dbh accurately because of an obstruction or unsafe conditions, enter an estimate.
11	Record if dbh was **estimated or measured**. If it was necessary to estimate dbh, enter E; otherwise, enter M.
12	Record, to the nearest percent, the percentage of **bark remaining** at breast height. Use the diameter tape to measure the total circumference and the portion of the circumference with bark remaining. The ratio of the two numbers multiplied by 100 equals the percent remaining bark. For example, if a tree with a 60 cm circumference has bark remaining on 16 cm, the percent remaining is 16 ÷ 60 × 100 = 27%. If the field surveyor does not have a calculator in the field, then record both numbers as a fraction and complete the math once a calculator can be used.

13 Determine the total **length** of all trees greater than 1.3 m high by collecting all the information required to complete the fields on the form. Measure length from the ground surface on the high side of the stem, along the stem, to the top.

- If the tree is broken, record the length of the stem to the point of breakage.
- On fallen trees, measure from the root collar to the top of the last attached portion of the stem.
- Length may be estimated if it is not possible to measure accurately because of obstructions, unsafe conditions.

Slope to top of tree (Top):
Enter the percent slope to the top of the tree; the sign must be shown (usually '+'). The maximum acceptable reading is 99%. If a reading greater than 99 is obtained, move further from the tree, or upslope.
Slope to DBH or bottom of tree (Bot):
Enter the percent reading to dbh, or the base of the tree, or to the lowest visible point; the sign must be shown ('+' or '-'). The maximum allowed reading is 99%.
Bottom position (Bot pos):
Enter, to the nearest 0.1 m, the height at which the Bot % reading was taken.
Slope distance (SD):
Enter the distance, to nearest 0.1 m, from the observer's eye to the centre of the tree trunk at Bot pos.

The above information is will be used to calculate the length of each tree. The equation used is: Height = Bot pos + (Top - Bot)/100 × (SD× cos(arctan(abs(Bot/100)))).

14 If the information to calculate tree length cannot be collected, then an **estimate of length** can be entered.

15 Assign a **crown class** designation to all standing live trees as follows:

D **Dominant Trees** with crown extending above the general level of the layer; somewhat taller than the codominant trees, and have well-developed crowns, which may be somewhat crowded on the sides.

C **Codominant Trees** with crowns forming the general level of the crown canopy; the crown is generally smaller than those of the dominant trees and usually more crowded on the sides.

I **Intermediate Trees** with crowns below, but extending into the general level of the crown canopy; crowns usually small and quite crowded on the sides.

S **Suppressed Trees** with crowns entirely below the general level of the crown canopy.

16 For each live tree, measure **height to live crown** (effective portion of the live crown for growth) in metres. This is normally the height on the stem at which live branches occupy about three-quarters of the stem circumference. Enter negative one (-1) for trees with no "effective" crown (e.g., only a few green branches).

17 Each tree sampled is classified according to the following criteria and the appropriate wildlife code is entered on the field form.

Appearance (Appear):
For each tree, enter a code (1–9) which corresponds to the illustration in **Figure 3-2** that best represents the appearance of the tree, using the shape of the tree stem as the dominant characteristic.

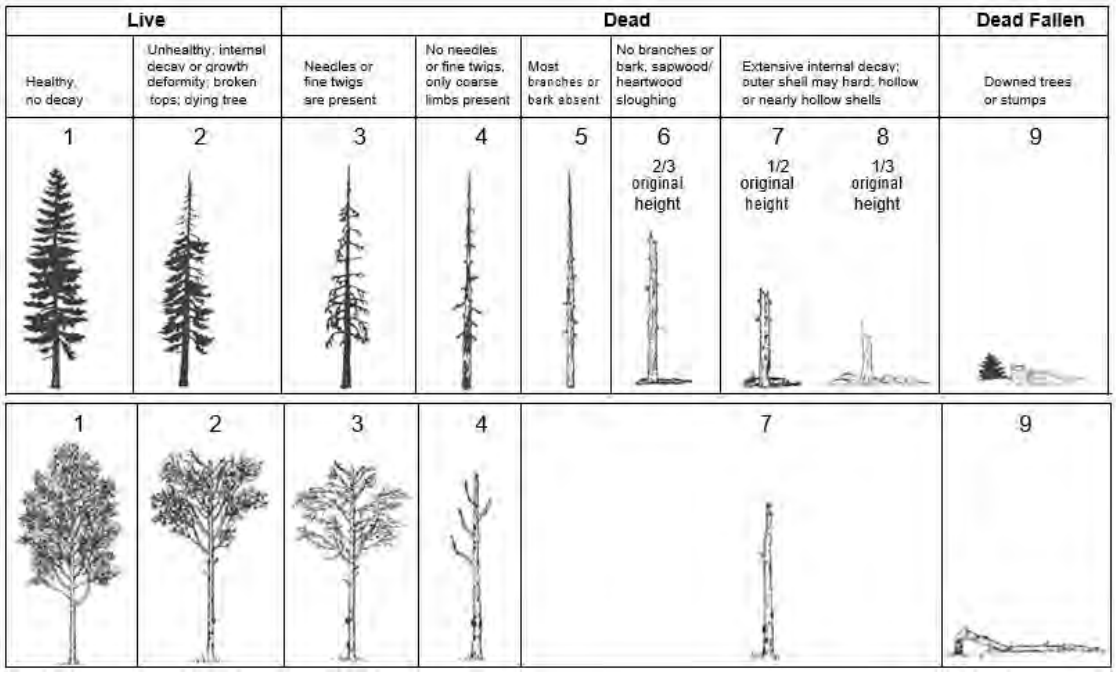


Figure 3-2. Visual appearance codes for wildlife trees.

Crown condition (Crown):
Using one of the classes in **Table 3-1**, rate the condition of the crown in relation to a normal live crown. Note: lower crown loss due to self-pruning is not counted as foliage or branch loss.

Table 3-1. Crown condition codes.

Code	Description
1	All foliage, twigs, and branches present
2	Some or all foliage lost; possibly some twigs lost; all branches usually present; possible broken top
3	No foliage present; up to 50% of twigs lost; most branches present; possible broken top
4	No foliage or twigs present; up to 50% of branches lost; top usually broken
5	Most branches have gone; some sound branch stubs remain; top broken
6	No branches present; some sound and rotting branch stubs, top broken

Bark retention (Bark):
Indicate the proportion of bark remaining on each tree, using the codes in **Table 3-2**.

Table 3-2. Bark retention codes.

Code	Description
1	All bark present
2	Bark lost on damaged areas only (< 5% lost)
3	Most bark present; bare patches; some bark may be loose (5–25% lost)
4	Bare sections; firm and loose bark remains (26–50% lost)
5	Most bark is gone; firm and loose bark remains (51–75% lost)
6	Trace of bark remains (76–99% lost)

7	No bark (100% lost)

Wood condition (Wood):
Classify the texture (soundness) of the wood for each tree, using the codes in **Table 3-3**.

Table 3-3. Wood condition codes.

Code	Description
1	No decay
2	Probable limited internal decay and/or deformities
3	Wood essentially hard; limited decay
4	Wood mostly hard, but decay spreading; softwood present
5	Balance of hard and softwood; spongy sections
6	More soft and spongy wood than hardwood
7	No more hardwood; all soft or spongy; powdery sections
8	Hollow shell; outer wood mostly hard or firm

Lichen loading (Lichen):
Assess all standing live or dead trees for lichen loading on branches that are within 4.5 m of the ground or root collar. Assign a rating (0–5) based on the approximate number of 5g clumps in **Table 3-4**. A value of 0 indicates no lichens, whether it is a live tree with branches and foliage or a dead tree. If a tree has lichens but none are below the 4.5 m mark, rate as zero.

Table 3-4. Lichen loading classes.

Class	Quantity of Lichen (5g clumps)
0	0
1	1
2	2-10
3	11-50
4	51-125
5	>125

Wildlife use:
If wildlife are observed using sample trees or if there is evidence of use, record a code for the type of use (activity) in the first column and the user in the second column (e.g., a feeding bird [FB], nesting amphibian [NA], denning mammal [DM]). If only the activity can be determined, leave the second column blank. If no evidence of wildlife use is observed, indicate with dashes (--).

Activity Code:

C Cavity nest
May be difficult to detect, but locations are somewhat predictable, and in season, the begging calls of nestlings are easy to detect; test a tree with a cavity nest by carefully striking it to determine if the nest is occupied; if possible, note species in the Comments section using the specific species code.

- Many woodpeckers prefer nesting in live hardwoods, often underneath branches.

- Nuthatches and chickadees nest in broken-off standing dead trees, or in broken branch holes, often directly below the breakage point where stem rots have entered the tree and softened the heartwood.
- Cavity nesters have perfectly round or oval nest holes.
- The Pileated Woodpecker and the Common Flicker have oval nest holes.
- Downy Woodpeckers, Chickadees, and Nuthatches have small round nest holes.
- Brown Creepers have hammock nests under the loose bark.
- Some ducks, owls, and squirrels nest in abandoned woodpecker holes.

O Open nest

Nests of eagles, hawks, owls, and herons are usually situated in the upper part or crown of live and dead trees; raptors and herons build large platform-style stick nests.

D Denning/resting

May be used by bears, squirrels, bats, marten, fisher, weasels, skunks, and raccoons.

- Bears often hibernate in the hollow trunks of large standing trees.
- Entrances to tree dens can be basal or arboreal.

F Feeding

Some examples of indicators are:

- Pileated Woodpeckers excavate large rectangular feeding holes.
- Yellow-bellied Sapsuckers drill horizontal patterns of sap wells.
- Three-toed and Black-backed Woodpeckers scale off bark to feed on insects.
- Porcupines gnaw on large sections of bark (diagonal tooth marks are often apparent).
- Rabbits, hares, and squirrels feed on the base of young trees (squarish “windows” or girdling at the base).
- Squirrels cache cones or leave basal accumulations of cone bracts.

T Travel

Some animals may leave signs of travel. This includes both daily travel and migration routes.

M Mark tree

Trees used mostly for communication of territorial boundaries and during courtship; examples of indicators include claw marks by black bears and antler rubbing by deer.

P Perching/roosting

Some examples of indicators are:

- Perch trees of aerial foraging and hawking birds are typically tall, with prominent dead branches which provide a good view of the surrounding area; such perches are especially common near riparian edges.
- Plucking spots where raptors feed can be identified by “whitewash” and remains of prey in the vicinity.

- Roost trees are often in sheltered locations with natural or excavated cavities; roosting sites include cavities, hollows, beneath bark, and in foliage.

S Squirrel cache

User Code:

M Mammal
B Bird
R Reptile
A Amphibian

If a wildlife species using a sample tree can be positively identified, record the species code on the ***Wildlife Habitat Assessment Form*** or record it in the Notes section of the ***Site and Soil Description Form***.

18 Note any additional features and the photo number for each tree in the **notes and photo numbers** section. All photos taken for this form need the photo number recorded, and a brief description of what is shown in the photo. If more space is needed, use the back of the field sheet and indicate with a large and bold arrow that more information is recorded on the back of the form.

19 Not to be completed in the field. Data should be entered into a spreadsheet as soon as possible, ideally completed daily. Once data are entered into the spreadsheet, fill in the date entered and who entered it. Data entry should be checked by a second person and any discrepancies discussed with the original surveyor. Enter the name of the QA/QC checker once completed.

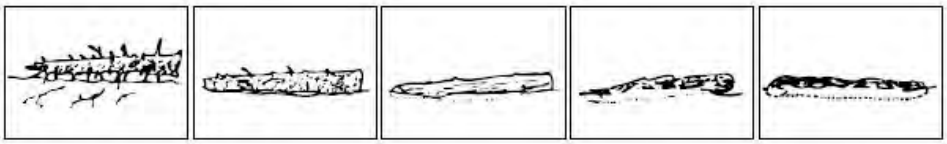
3.4.3 Completing the Coarse Woody Debris Survey Form

Field Label	Description and Instructions
1	Enter the **date** in DD-MM-YY format. (e.g., 01-01-20 for January 1st, 2020)
2	Enter the **plot number**, which is composed of the crew number and a sequential 3-digit number determined in the field. For example, If the crew number is 1 and it is their first plot, then the plot number is 1001, with the second plot being 1002.
3	Enter the **polygon number** where the plot is taking place.
4	Enter vegetation **surveyor(s)** first initial and last name.
5	Enter digit for the **page number**. Complete the total number of pages once the survey is complete in case multiple pages are used.
6	Record the first **azimuth** (randomly selected) for Transect No. 1, and the second at plus 90° to Transect No. 2.
7	Record the distance that was actually sampled (**Sampled ___ of 24 m**) out of the total distance, in the spaces provided.
8	Record **species** code for each piece. Species codes can be found in Chapter 5 – Life Forms in *Ecosites of Ontario* (ELC Working Group 2009); if uncertain of the correct code or a code has not

been assigned, write the entire scientific name. If the species can not be determined put "X" for unknown, "Xh" for unknown hardwood, or "Xc" for unknown conifer.

9 Record the **diameter** of the piece perpendicular to the bole at the point where the sampling line is considered to intersect the central axis of the piece. Wrap a diameter tape around the bole, when possible, or use the reverse side of the tape to estimate the diameter. Callipers may also be used and are often easier when coarse woody debris is in several layers. Measure diameter to the closest 0.1 cm. If the CWD is hollow, estimate the diameter equivalent required to approximate the volume of the remaining wood.

10 Assign a **decay class** (1 to 5) based on the majority condition of the entire piece. The five classes used to describe the condition of coarse woody debris are based primarily upon wood texture, and secondarily on other characteristics. See **Figure 3-3** for descriptions of classes.



	Class 1	Class 2	Class 3	Class 4	Class 5
Wood Texture	Hard	Sap rot (but still hard, thumbnail penetrates)	Advanced decay (spongy/large peices)	Extensive decay (crumbly-mushy)	Small pieces, soft portions
Portion on Ground	Elevated on support points	Elevated but sagging slightly	Sagging or broken	Fully settled on ground	Partly sunken
Branches	Hard branches with twigs	Soft branches	Branches/stubs absent	Absent	Absent
Bark	Firm	Loose	Trace	Absent	Absent
Wood Appearance	Fresh/recent	Colour fading	Fading colour	Light or brown	Reddish brown
Wood strength	Supports person	May not support person	Breaks easily. Pieces snap	Collapses with weight. Pieces do not snap	Feels firm like ground
Invading Roots	None	None	In sapwood	In heartwood	In heartwood

Figure 3-3. Decay classes for coarse woody debris.

11 Record the **tilt angle** of the individual log away from the horizontal, regardless of the slope of the ground. A clinometer is placed on the surface of the piece at the point of the intercept measurement and the angle from the horizontal (in degrees) is recorded (see **Figure 3-4**).

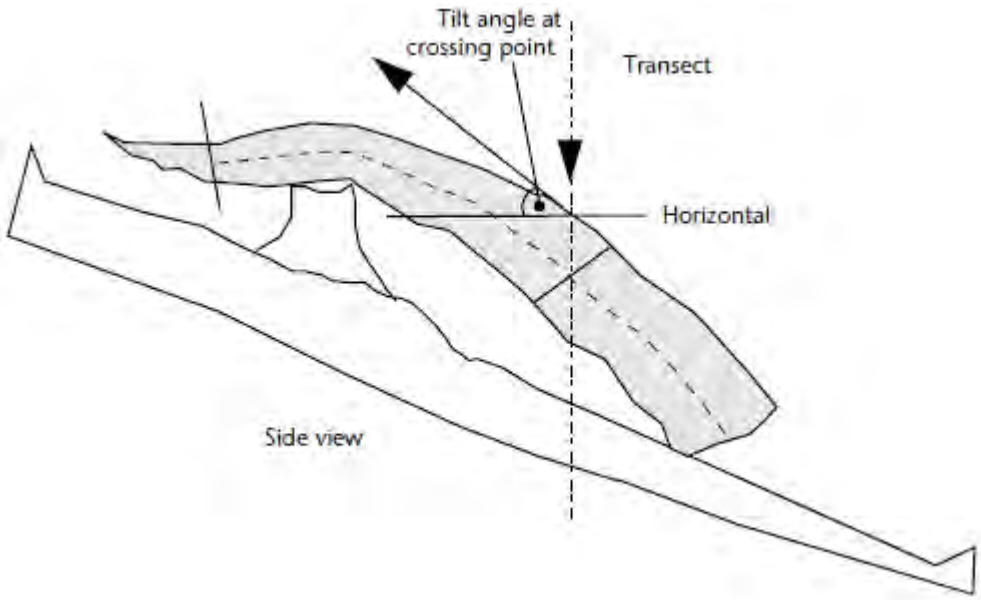


Figure 3-4. Recording the tilt angle of coarse woody debris.

12 Record the **length** of each piece to the nearest 0.1 m (see **Figure 3-5a**).

- If a log has broken lengthwise but is still partially held together, record the equivalent length as if the piece were whole.
- If the end(s) of the piece are broken, visually fold in the broken sections to compensate for the missing parts.
- Piece length is from the largest end down to the 7.5 cm diameter limit.

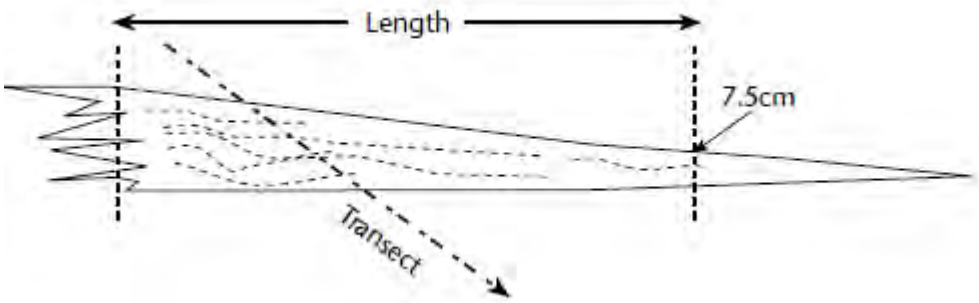


Figure 3-5a.

Measurement of stems from attached roots:

- For main boles with exposed roots, piece length is measured only down to the root collar (see **Figure 3-5b**).

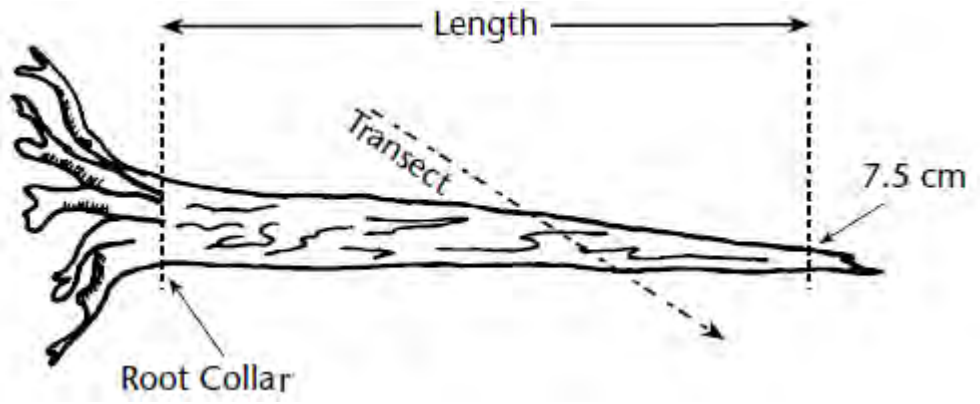


Figure 3-5b.

- If a root mass is transected, piece length for individual roots (larger than the minimum diameter) is measured only up to the root collar (see **Figure 3-5c**).

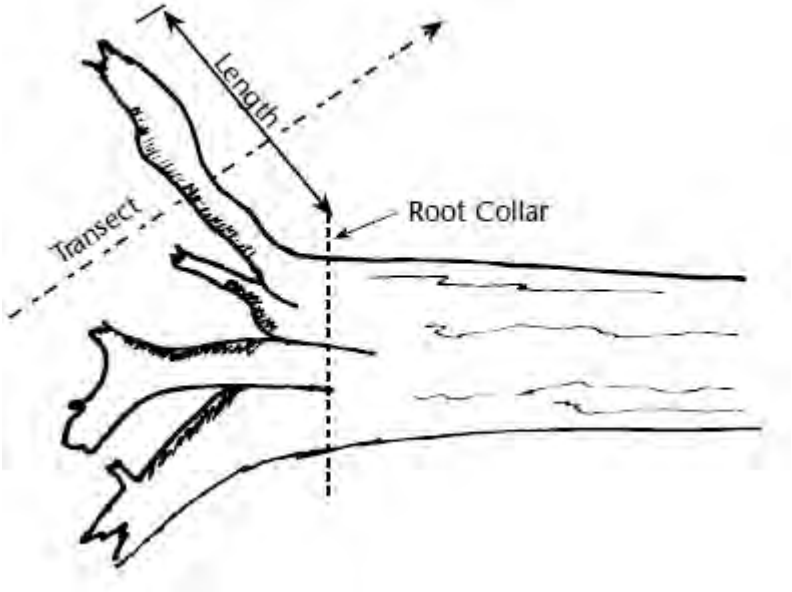


Figure 3-5c.

Measurement of forked stems:

- Where one of the forks transected is determined (by largest diameter) to be a continuation of the main bole then the length will be measured to the ends of the main piece (see **Figure 3-5d**).

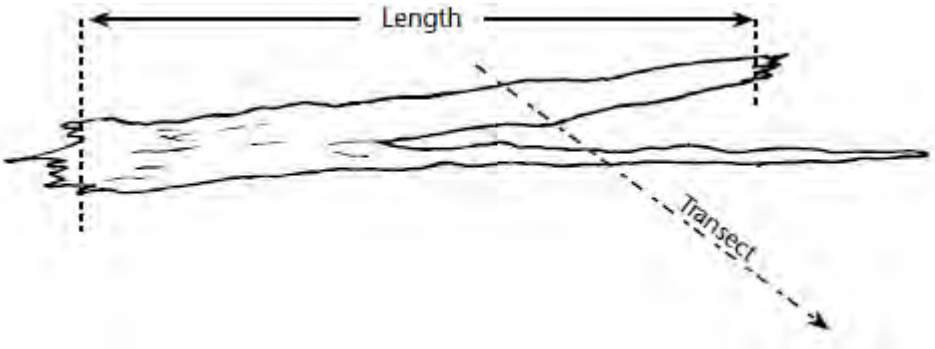


Figure 3-5d.

- The piece length of the smaller stem(s) (smaller diameter) will be measured only to the junction with the main bole (see **Figure 3-5e**).

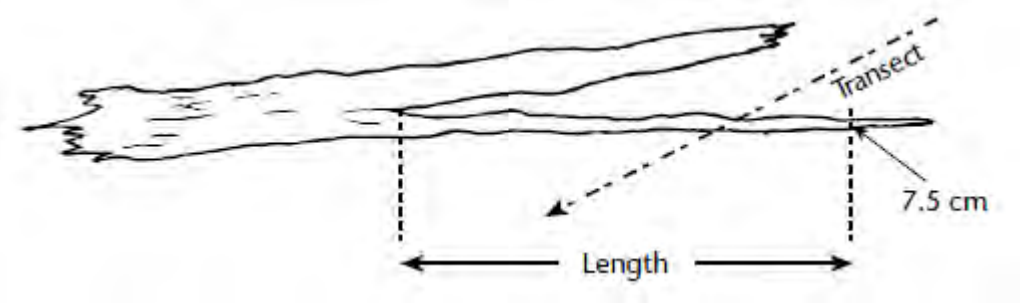


Figure 3-5e.

- For forks of nearly equal stature make a determination as above and measure accordingly.

Measurement of pieces that are crossed more than once on the transect:

- Pieces broken but still physically attached are measured as one piece at each transect point. The length measurement is taken along the central axis of the piece (see **Figure 3-5f**).

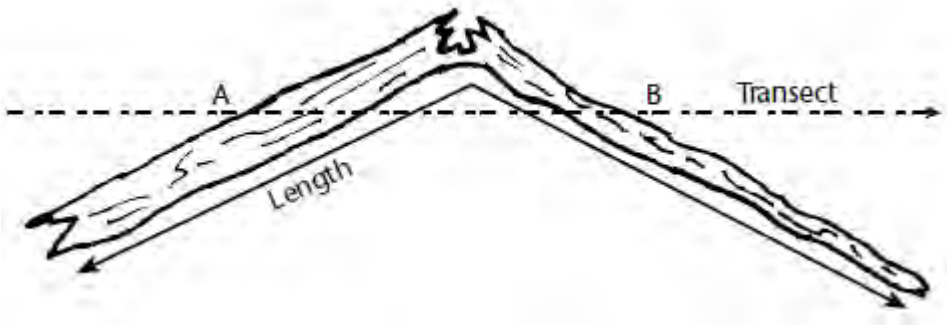


Figure 3-5f.

- The full piece length of curved/crooked pieces is measured at both crossings (see **Figure 3-5g**).

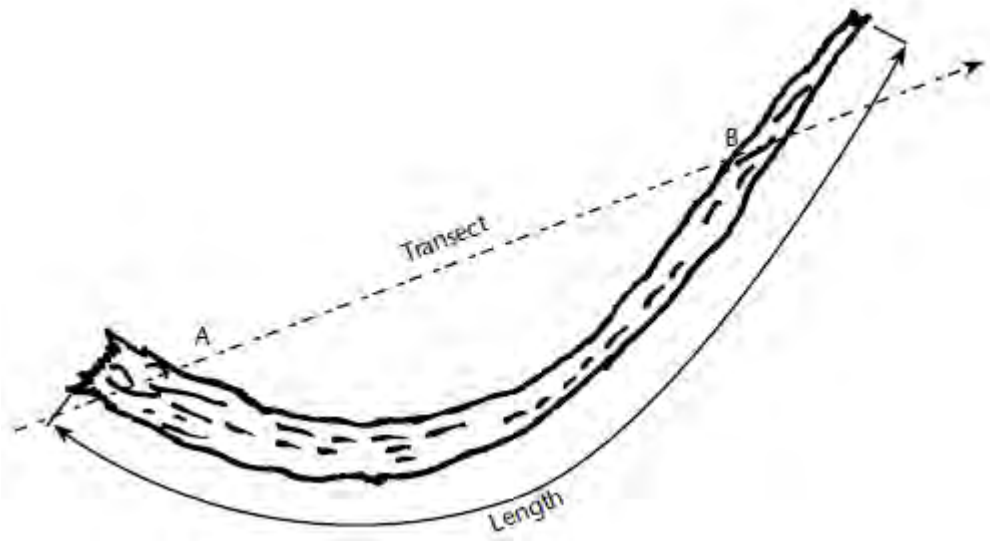


Figure 3-5g.

Figure 3-5. Rules for measuring the length of coarse woody debris.

In the same manner as above, record the full piece length twice where the same piece is crossed by two transects at right angles to each other.

13 This is the **height** above ground of the central axis of the lowest end of each piece of CWD, measured to the nearest cm. The lowest end is defined as the end of the piece that is in closest contact with the ground, not necessarily the end that is at the lowest altitude.

14 At the transect crossing measure and record the angle of the ground, following the same procedure for determining CWD piece tilt. It may be necessary to measure the ground angle over a 1 to 2 m (or more) distance if the surface is irregular.

Record this angle to the nearest degree and indicate whether it is positive or negative (e.g. -07, +12). When measuring the angle of the ground, face in the direction that gives a positive tilt angle for the piece of CWD. The angle of ground measured by sighting in that direction may be positive or negative.

15 Record the **size of the pile** by measuring the **length**, **width**, and **height** to the nearest 0.1 m.

16 Record, to the nearest cm, the average **diameter** of pieces of CWD composing the pile.

17 Using the codes in **Table 3-5**, estimate the size of interstitial spaces. This not intended to indicate which species will use the pile.

Table 3-5. Codes for the size of interstitial spaces.

Code	Class	Description
S	Small	Most interstitial spaces are the size of, or smaller than, a squirrel
M	Medium	Most interstitial spaces are the size of a lynx
L	Large	Most interstitial spaces are the size of, or larger than, the average black bear

18 Note any additional features and the photo number for each tree in the **notes and photo numbers** section. All photos taken for this form need the photo number recorded, and a brief description of what is shown in the photo. If more space is needed, use the back of the field sheet and indicate with a large and bold arrow that more information is recorded on the back of the form.

19 Not to be completed in the field. Data should be entered into a spreadsheet as soon as possible, ideally completed daily. Once data are entered into the spreadsheet, fill in the date entered and who entered it. Data entry should be checked by a second person and any discrepancies discussed with the original surveyor. Enter the name of the QA/QC checker once completed.

4.0 WILDLIFE HABITAT ASSESSMENT

4.1 Equipment and Materials

Field forms for printing can be found in Appendix A; all hard copies brought into the field should be printed on waterproof paper. Where possible, electronic equipment should also be waterproof. The field contractor is responsible for their own field safety protocols, which includes the planning of field safety gear.

4.1.1 General Field Equipment

- Hard copies of field forms
- Hard copies of SOP
- Hard copies of survey location coordinates and field maps
- Hard copy of *Ecosites of Ontario* (ELC Working Group 2009)
- Pen/pencils
- Waterproof notebook
- Clipboard
- Binoculars
- Camera
- GPS unit
- Tablet or smartphone with georeferenced map
- Compass
- Large spool measuring tape
- Small measuring tape

4.1.2 Habitat Assessment Specific Equipment

- Wildlife and wildlife signs identification book(s)

4.2 Field Procedure

1. Each day, a route should be determined using the List of Sites and Site Information in Appendix B and Map of Field Sampling Locations in Appendix C. Each daily route plan should ensure any sites not visited the previous day due to time limitations are included in the next day's route. The field contractor should note any required use of helicopters to assist access or speed up fieldwork.
2. When in the field, follow the day's route to navigate to the next site. Sites have been determined using a GRTS design to create stratified and randomized points. As part of this design method, some points are pre-selected as PanelOne (P1) for sampling or OverSample (OS). If a P1 site is determined after a reasonable effort to be inaccessible, an OS site can be used in place of the P1 site. If the OS site is also deemed inaccessible after reasonable effort, the next OS site can be visited/considered. The field contractor should take care not to skip points too quickly and without effort in favour of randomized points that happen to occur next to easily accessible areas (roads, cleared forest); this may impose a site selection bias meant to be diminished via the use of pre-selected, random points. While points should be visited in order wherever possible, logistical constraints due to difficulty accessing areas may render it necessary to deviate from the ordering from P1 points and OS points from time to time, such that points can be planned to be visited along hiking routes (e.g., in a situation where there is only one way into a large, difficult to access area). In such cases, the replacement of any P1 sites with an OS site should be noted in the notes section of the ***Site and Soil Description Form***.
3. Using the *List of Sites and Site Information* in Appendix B, determine survey type (Full, Ground, or Visual).
4. Locate and mark plot boundaries (20m x 20m square) for Full plots or plot center for Ground plots (no marking required for visual plots).
5. Become familiar with the character of the terrain, soil, and vegetation by traversing the plot and consulting with plant ecologist and soil scientist.

4.2.1 Full/Ground/Visual Wildlife Habitat Assessment Procedure

1. Photograph the plot. It is a good idea to take 4 photos in each cardinal direction (begin looking north and turn clockwise), as well as one that shows canopy cover (up) per site. Photographs should also be taken of any notable features, wildlife signs or observations, and any examples of exceptional wildlife habitat.
2. On the ***Wildlife Habitat Assessment Form***, enter the information in the header (date, plot number, polygon number, wildlife surveyors' names, weather, survey type, page number).
3. Record evidence of use in plot and in ecosystem unit represented by the plot.
4. List project species and additional species noted during the visit.
5. Record the habitat use and the season for each species.
6. Confer with plant ecologist and soil scientist about ecosite classification, values, and site management concerns.
7. Ensure that relevant wildlife habitat data are filled out on the site description and vegetation forms.
8. Assess the value of the plot-type for each species (not necessary for incidentally recorded species). Be sure that the plot-type assessment is completed before the plot-in-context assessment.
9. Assess the value of the plot-in-context for each species based on the spatial context of the plot.
10. Record comments at the bottom of the form, cross-referencing to species.
11. Photograph the plot to illustrate important wildlife habitat features or evidence of animal use.
12. Check that all the required information has been collected and noted on the form.
13. On the ***Significant Wildlife Habitat Survey Form,*** enter the information in the header on both pages (date, plot number, polygon number, wildlife surveyors' names, predetermined ecosite code, and final ecosite code).
14. Using the *Field Summary of Significant Wildlife Habitat Criteria Schedules for Ecoregion 3W* found in Appendix D, check the yes or no box if the ecosite codes listed for the Wildlife Habitat matches the plot's final ecosite code.
15. Using the *Field Summary of Significant Wildlife Habitat Criteria Schedules for Ecoregion 3W* found in Appendix D, check the yes, no, or unknown box if the habitat criteria listed for the Wildlife Habitat matches what is found in or around the plot. The unknown box should only be ticked if the criteria cannot be determined at the time of the survey to be present or not, likely due to seasonal timing (e.g., the survey is conducted in the summer so spring phenomenon cannot be observed).
16. Take a photo that shows each habitat criteria and record the photo reference number.
17. Fill in any details about the habitat criteria and the rationale for the decision. If multiple criteria are listed, specify which criteria is met. If the unknown box was checked, the reason why the habitat criteria could not be observed must be noted.
18. Check that all the required information has been collected and noted on the form.
19. If other surveyors are still working once the forms are completed, spend remaining time looking and listening for wildlife signs or observations.

4.3 Field Form

Date (DD/MM/YY) (1)	Plot Number (2)	Polygon Number (3)	Surveyor(s) (4)	Survey Type Full ☐ Grd. (5)	Non-Hab. Feature: Type / Dist. (6)	Page Of (7)

Wildlife Habitat Assessment Form

ASSESSMENT	Hab Use/Sen		Plot Type				Plot in Context													
Species	Sp. L.R.	Ssn	FD	SH	TH	Note #	Habitat feature	Conf.	Distance (km)	F/C L.R.	Imp.	Habitat Feature	Conf.	Distance (km)	F/C L.R.	Imp.	FD	SH	TH	Suit.	Note #
(8)	(9)			(10)								(11)								(12)	(13)

EVIDENCE OF USE	Inside plot						Outside plot and inside ecosite													
Species	Sex	Life Stage	Activity	Des.	No.	Note #	Sex	Life Stage	Activity	Des.	No.	Sex	Life Stage	Activity	Des.	No.	Sex	Life Stage	Activity	Des.	No.	Note #
(14)	(15)	(16)	(17)	(18)	(19)	(20)	(15)	(16)	(17)	(18)	(19)											(20)

Notes and Photo Numbers (20)

Date entered into spreadsheet: (21) Entered by: QA/QC by:

Significant Wildlife Habitat Survey Form					
Date (DD/MM/YY) 1	**Plot Number** 2	**Polygon Number** 3	**Surveyor(s)** 4	**Predetermined Ecosite** 5	**Final Ecosite** 6

Seasonal Concentration Areas for Wildlife Species			
Wildlife Habitat	**Ecosite Codes**	**Habitat Criteria**	**Habitat Details and Rationale**
Waterfowl Stopover and Staging Area (Terrestrial)	☐Yes ☐No 7	☐Yes ☐No 8 ☐ Unknown	9
Waterfowl Stopover and Staging Area (Aquatic)	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Shorebird Migratory Stopover Area	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Colonially - Nesting Bird Breeding Habitat (Bank and Cliff)	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Colonially - Nesting Bird Breeding Habitat (Tree/Shrubs)	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Colonially - Nesting Bird Breeding Habitat (Ground)	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Eagle and Osprey Concentration Area	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Sharp-tailed Grouse Lek	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Bat Hibernaculum	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Bat Maternity Colony	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Amphibian Breeding Habitat	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Turtle Wintering Area	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Snake Hibernaculum	☐Yes ☐No	☐Yes ☐No ☐ Unknown	

Animal Movement Corridors			
Habitat	**Ecosite Codes**	**Habitat Criteria**	**Habitat Details and Rationale**
Cervid Movement Corridor	☐Yes ☐No 7	☐Yes ☐No 8 ☐ Unknown	9
Amphibian Movement Corridor	☐Yes ☐No	☐Yes ☐No ☐ Unknown	

Significant Wildlife Habitat Survey Form

Plot Number (2)	Polygon Number (3)	Predetermined Ecosite (5)	Final Ecosite (6)

Specialized Habitat for Wildlife			
Specialized Wildlife Habitat	**Ecosite Codes**	**Habitat Criteria**	**Habitat Details and Rationale**
Waterfowl Nesting Area	☐Yes ☐No (7)	☐Yes ☐No ☐ Unknown (8)	(9)
Wild Rice Stand	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Milkweed Patch	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Bald Eagle and Osprey Nesting Habitat	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Woodland Raptor Nesting Habitat	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Turtle Nesting Area	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Aquatic Feeding Habitat	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Seeps and Springs	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Mineral Lick	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Mammal Denning Site	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Marsh Bird Breeding Habitat	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Open Country Bird Breeding Habitat	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Notes and Photo Numbers: (10)			
Date entered into spreadsheet: (11)	Entered by:	QA/QC by:	

4.4 Completing the Form

4.4.1 Wildlife Habitat Assessment Form

Field Label	Description and Instructions
	Assessment
1	Enter the **date** in DD-MM-YY format. (e.g., 01-01-20 for January 1st, 2020)
2	Enter the **plot number**, which is composed of the crew number and a sequential 3-digit number determined in the field. For example, If the crew number is 1 and it is their first plot, then the plot number is 1001, with the second plot being 1002.
3	Enter the **polygon number** where the plot is taking place.
4	Enter vegetation **surveyor(s)** first initial and last name.
5	Check the box for **survey type** being completed, either full/ground or visual plot.
6	Enter up to two types of human activity or other **non-habitat feature** (N-hab. feat.) near the plot that may affect usage by wildlife. A non-habitat feature is a feature of the environment that influences the amount of use of the plot by wildlife. A nonhabitat feature can be distinguished from a habitat feature because non-habitat features do not affect habitat attributes (i.e., something measurable to describe habitat) and therefore do not affect suitability.

Type:
Identify the type of prolonged human activity or other non-habitat feature near the plot using the codes in **Table 4-1**.

Table 4-1. Codes for types of non-habitat features.

Code	Type	Code	Type
FA	Farming	**OT**	Other (Specify in Notes)
FE	Fence	**RF**	Road traffic, four lanes
GD	Garbage dump	**RO**	Road traffic, one lane
LO	Logging activity	**RN**	Railroad
LR	Logging road	**RT**	Road traffic, two lanes
MI	Mining activity	**RR**	Rural

Distance:
Enter a code (1–5) indicating the approximate distance (dst.) from the plot to the nearest sites of prolonged human activity or other non-habitat features that may affect wildlife. See **Table 4-2** for distance codes.

Table 4-2. Code for distances to nearest non-habitat features.

Code	Distance
1	0-100m
2	100-250m
3	250-1000m
4	1-5 km
5	>5 km

7 Enter digit for the **page number**. Complete the total number of pages once the survey is complete in case multiple pages are used.

8 Indicate the **species** for which the habitat is being assessed.

9 For each **habitat use** (Hab use) to be assessed, use one row on the form.

Specified Life Requisite:
Specify the life requisite (SpLR) for which the habitat will be used with a two-letter code from **Table 4-3**.

Table 4-3. Specified life requisite codes.

Code	Specified life requisite	Description
AP*	Avoiding pests	Habitat used for avoiding pests
CO	Courting	Habitat used for courting; involves enticing a conspecific of the opposite sex into copulation, courtship feeding, and defence of mates. e.g., Rutting areas for ungulates.
DE	Denning/Roosting	Habitat used for sleeping or hiding in a cavity, cave, or burrow; does not include hibernating nor reproducing-birthing
FS	Feeding seasonal (e.g. berry patch)	Habitat used for feeding on a particular abundant/dominant food source at the site that is only present or ripe at a particular time of year
HI	Hibernating	Habitat used for hibernating
LI*	Living	Habitat used for activities other than; denning, birthing, courting etc.
MD*	Migrating daily	Habitat used for regular, daily travelling, including travelling away from or towards a communal habitat; e.g., habitat used by a bat for daily flights to and from a roosting site
MS*	Migrating seasonally	Habitat used for regular, annual travelling; habitat used for travelling away from or towards a communal habitat such as a hibernaculum
RB	Reproducing – birthing	Habitat used specifically for giving birth to live young; habitat used by amphibians, birds, and reptiles for the hatching of eggs is recorded as habitat used for reproduction by eggs (RE)
RE	Reproducing – eggs	Habitat used for building a nest, laying eggs, incubation, hatching, and feeding non-mobile young; reserved for amphibians, birds, and reptiles; specialized habitat used by some mammals to give birth to young is recorded as reproducing-birthing (RB) habitat
SG*	Staging	Habitat used for staging during spring or fall migrations

*Activities for which a season needs to be indicated (see below). The season is implied for all other activities.

Season:
If required, indicate the season (Ssn.) for which the habitat is being assessed. Use the codes listed in **Table 4-4**. Seasons are species specific; definitions for the seasons and dates will be provided in the species accounts for each species.

Table 4-4. Codes for the season of use.

Code	Season	Code	Season
A	All seasons	WE*	Early Winter
G	Growing	WL*	Late Winter
W	Winter	PE*	Early Spring
P	Spring	PL*	Late Spring
S	Summer		
F	Fall		

*Early Spring, Late Spring, Early Winter and Late Winter seasons should only be used for black bear. Also, Early Spring can be used to distinguish Early Spring feeding habitat from Spring feeding habitat for moose.

10 Assess the food (FD), security habitat (SH) and thermal habitat (TH) provided by the plot type for the species, life requisite use, and season being considered. For these assessments, **disregard plot size and shape, and position relative to other habitats**. Instead, imagine that the plot type covers a sufficiently large area to maximize its value for the species, use and season being considered. These data will be used to establish suitability ratings for the ecosystem unit represented by the plot. For assessments of relative quality and suitability use codes in **Table 4-5**.

Food (FD):
For species that require food (FD) for the use and season being considered, rate the ability of the plot type to fulfill food requirements.

Security habitat (SH):
For species that require security habitat (SH) for the use and season being considered, rate the ability of the plot type to fulfill security requirements. Security habitat is used for protection or hiding from predators.

Thermal habitat (TH):
For species that require thermal habitat (TH) for the use and season being considered, rate the ability of the plot type to fulfill thermal requirements.

For species where it is known that thermal habitat plays a significant role in overall suitability, but for which thermal qualities of the plot type cannot be assessed separately from the security qualities, do not attempt to enter a rating in the TH column—only use the SH column to enter a rating.

Table 4-5. Relative quality classes for assessing the plot type quality relative to the best in Ontario. The lower and upper limit ranges indicate how similar the plot is to the best habitat example in Ontario. For example, 100 would mean the site is at the highest limit of the best habitat in Ontario.

Class Quality	Suitability/ capability	Lower limit (%)	Upper limit (%)	
1	High	>75	≤100	Equivalent or slightly less
2	Moderate	>25	≤75	Moderately less
3	Low	>0	≤25	Substantially less
4	Nil	0	0	Habitat or attribute is absent

Notes:
To provide additional information about the plot-type assessment, or to clarify an entry made on this line, enter a numeric code (Com.). Enter the same code in the Notes section of the form, followed by the pertinent information.

11 **Given the location of the plot,** assess the quality and accessibility of food (FD), security habitat (SH) and thermal habitat (TH) for the species, use, and season being considered. This assessment includes the adjacent habitat features that are accessible to the species, for the specified use and season. These data will be used to develop a suitability rating for the specific plot in the area. For assessments of quality and suitability use coding from **Table 4-5**.

Habitat features:
A habitat feature is a feature of the environment that influences the amount of use of the plot by providing food, security or thermal habitat and thereby affects suitability (e.g., a nearby agricultural field may provide food and influence plot usage). Enter up to two habitat features (**Table 4-6**) that may affect the suitability of the plot.

Table 4-6. Habitat features codes for plot-in-context assessment [a].

Code	Habitat feature	Definition
AL [b]	Alkaline pond	Body of freshwater with a pH greater than 7 and a depth less than 2 m
AS	Aspect	Area which has an aspect associated with it, in which the aspect is the attribute important to the species, use, and season being considered
BE [b]	Beach	Area of sorted sediments reworked in recent time by wave action; at the edge of fresh or saltwater bodies
BF [b]	Blockfields, Blockslopes, Blockstreams	Level or gently sloping areas covered with moderately, sized or large, angular blocks of rock derived from the underlying bedrock or drift by weathering and/or frost heave
BU	Building	
BY	Berry Patch	Cluster of several berry bushes
CA [b]	Canal	Artificial watercourse created for transport, drainage, and/or irrigation purposes

CB [b]	Cutbank	Part of a road corridor or river course situated upslope of the road or river; created by excavation and/or erosion of the hillside
CF [b]	Cultivated field	Flat or gently rolling, non-forested, open area subject to human agricultural practices
CH	Clearcut, herbaceous	
CL [b]	Cliff	Steep, vertical or overhanging rock face
CO [b]	Cultivated Orchard	Agricultural area of fruit trees planted in rows
CS	Clearcut, shrubby	
CU	Clearcut, unvegetated	
DU	Dune	Mound or ridge of wind-blown, loose sediment
EK	Esker	A long, narrow, winding ridge composed of stratified sand and gravel deposited first by a stream in or on a glacier, then on the ground once the glacier rapidly melts
ES [b]	Exposed soil	Area of exposed soil; not included in any of the other definitions
ET	Electrical transmission line	
EY	Estuary	
FC	Forest, commercially thinned	
FE	Fence	
FM [f]	Forest, mature	
FO [f]	Forest, old	
FY [f]	Forest, young	
GB [b]	Gravel bar	Elongated landform generated by waves and currents; a mix of cobbles, pebbles, stones, and/or sand
GC [b]	Golf course	Grass-covered fairways and open areas for the playing of golf
GP [b]	Gravel pit	Area exposed for the removal of sand and gravel
GR	Grassland	
LA [b]	Lake	Naturally occurring, static body of water > 2 m deep (> 50 ha)
MI [b]	Mine	Unvegetated area for the extraction of mineral ore and other materials
ML	Mineral Lick	A natural source of salt and other minerals for some wildlife
MO [b]	Moraine	Unvegetated landform of unstratified glacial drift
MU [b]	Mudflat sediment	Flat plain-like areas of fine-textured sediment

NB	Nest boxes	
OT	Other	
OW [b]	Shallow open water	Wetland of permanent shallow open water (< 2 m deep); lacking extensive emergent plant cover
PA	Pasture	
PD [b]	Pond	A small body of water > 2 m deep (< 50 ha)
PI	Pipeline right-of-way	
PS [b]	Permanent snow	Snow or ice, not part of a glacier, but found during summer months
RD	Ridge	Area which has a ridge associated with it; the ridge is the feature important to the species, use, and season being considered
RE [b]	Reservoir	Artificial basin created by the impoundment of water behind a human-made structure such as a dam, berm, dike, or wall
RI [b]	River	Watercourse formed when water flows between continuous, definable banks
RN [b]	Railway surface	Roadbed with fixed rails for possibly single or multiple rail lines
RO [b]	Rock outcrop	Gentle to steep, bedrock escarpment or outcropping, with little soil development and sparse vegetation
RP [b]	Road surface	Area cleared and compacted for vehicle trans port
RR [b]	Rural	Area of residences and other human developments scattered and intermingled with forest, range, farmland, and native vegetation or cultivated crops
RU [b]	Rubble	Small angular rock fragments (between 2 and 256 mm) deposited by gravity or ice

a This is not a comprehensive list of habitat features. Other habitat features can be recorded by using the OT (other) code and adding a comment.

b Habitat features derived from Table 3.1 Symbology and definitions for non-vegetated, sparsely vegetated, and anthropogenic units in Standards for Terrestrial Ecosystem Mapping in B.C.

Confidence:

Use the codes in **Table 4-7** to identify a level of confidence (Conf.) in the assessment of habitat features (i.e., how confident you are that the habitat feature affects the species, use, and season being considered). Base this on your knowledge of the species' habitat requirements and on your knowledge of the quality and quantity of habitat present in the habitat feature.

Table 4-7. Confidence level codes for assessment of habitat features.

Code	Level of confidence	Description
1	Confident	Excellent knowledge of habitat attributes available in the habitat feature **and** of species' habitat requirements
2	Moderately confident	Excellent knowledge of habitat attributes available in the habitat feature **and** moderate knowledge of species' habitat requirements; **or**, moderate knowledge of habitat attributes available in the habitat feature **and** excellent knowledge of species' habitat requirements
3	Not confident	Moderate knowledge of habitat attributes available in the habitat **and** of species' habitat requirements

Distance:
Indicate, in kilometres, the distance from plot centre to the habitat feature.

Food/Cover life requisite:
Identify the life requisite (F/C L.R.) (**Table 4-8**) that the described habitat feature provides. If the habitat feature provides more than one life requisite, then use a combination of codes (e.g., FS indicates that both food and security are provided by the habitat feature).

Table 4-8. Food/cover life requisite codes provided by the plot-in-context habitat feature(s) noted from Table 4-6.

Code	Food /cover life requisite	Description
FD	Food	Provides habitat used for consuming food items, including searching for and consuming food simultaneously such as is done by grazers, browsers, flying insectivores, ducks, and other species with similar feeding habits; includes habitat used for searching for, pursuing and killing prey
SH	Security	Provides habitat used for protection or hiding from predators
TH	Thermal	Provides habitat used for protection from heat, cold, or precipitation

Impact:
Assess the impact (Imp.) of the habitat feature using codes from

Table 4-9. Given the presence of the habitat feature, the impact is a measurement of the increase or decrease in the quality and accessibility of the food/cover life requisite(s) relative to quality and accessibility if the plot type extended indefinitely.

Table 4-9. Impact of habitat feature on suitability rating.

Code	Description
1	Large increase

2	Moderate increase
3	Low increase
4	No effect
5	Low decrease
6	Moderate decrease
7	Large decrease

Food:
Considering the context of the plot, for species that require food (FD) for the use and season being considered, rate the overall quality and accessibility of food. Use coding from **Table 4-5**.

Security habitat:
Considering the context of the plot, for species that require security habitat (SH) for the use and season being considered, rate the overall quality and accessibility of security habitat. Use coding from **Table 4-5**.

Thermal habitat:
Considering the context of the plot, for species that require thermal habitat (TH) for the use and season being considered, rate the overall quality and accessibility of thermal habitat. Use coding from **Table 4-5**.

12 Assign a **suitability** rating (Suit.), using the codes in Table 5.5, for the plot-in-context, for the species, use, and season being considered. Base the suitability on the ratings entered in the food (FD), security habitat (SH), and thermal habitat (TH) columns. The suitability rating should be an average or weighted average of the three food/cover life requisite ratings.

13 To provide additional information about the habitat assessment, or to clarify an entry on this line on the form, enter a numeric code (Com). Enter the same code in the **Notes** section of the form, followed by the pertinent information.

Evidence of Use

14 Indicate the **species** for which the evidence of use is being recorded.

15 Note the **sex** of the animal. Code as M (male), F (female), or U (unknown).

16 Record the **life stage** of the animal using the codes in **Table 4-10**.

Table 4-10. Codes for life stages for wildlife evidence of use.

Code	Life stage	Description
E	Egg	Amphibian, bird, insect, and reptile eggs
N	Nestling	Nestling birds and newly hatched or newborn or neonate newborn amphibians, birds, insects, mammals, and reptiles; only used when it is apparent that the nest site is within the plot type
J	Juvenile	Amphibian larvae, fledged birds before their first winter, insect larvae, and mammals older than neonates, but still requiring parental care; reptiles do not have a juvenile stage
S	Subadult	Animal that is older than the juvenile stage, does not require parental care, and has not reached sexual maturity; includes amphibians and

		reptiles which have not reached adult size, but have adult form; insects have no subadult stage
A	Adult	Old enough to breed
U	Undetermined	

17 Code up to three different types or signs of **activity** relevant to the identified species (**Table 4-11**). If an animal is present in the plot, or in the ecosystem unit represented by the plot, record the type of activity it is engaged in on the appropriate section of the form. If there are signs that an animal was present, record the type of activity which caused the signs.

Table 4-11. Codes for activities and signs of activity.

Code	Activity	Description
AL [a]	Alert	Activity with the purpose of detecting predators; e.g., guard or sentry duty or a heads-up rigid stance
AN [b]	Antler	A cast, solid, annually deciduous horn of a cervid
AP	Avoiding pests	Avoiding pests
BA	Basking	Behaviour for the purpose of gathering warmth; e.g., a marmot or snake lying on warm rocks, or marmot hair and soiling stains on flat rocks
BE	Bedding	Bedding, sleeping, or resting above ground, including bedding for the purpose of cud-chewing, and roosting and resting of birds
BP[b]	Body	Incidental portions of an animal's body which are parts left behind, but do not indicate the animal is dead; e.g., feathers, hairs, and shed skins; shed antlers are recorded as "AN"
BU	Building	Building a nest, bed, burrow, den, lodge, or other dwelling
CA	Casting	Discharging bodily waste from the mouth; e.g., an owl or snake casting pellets
CO	Courtship	Behaviour for the purpose of enticing a conspecific of the opposite sex into copulation, including copulation, courtship feeding, and defence of mates
CR[b]	Carcass	A carcass, or portions of a carcass, that indicates the animal is dead
DE	Denning	Sleeping or hiding in a cavity, cave, or burrow; does not include hibernating; if the same den is used for hibernating and general denning, record as hibernating
DI[a]	Disturbed	Behaviour for the purpose of avoiding the observer; use only if the activity before disturbance is not known
DR[a]	Drinking	Drinking
EX	Excreting	Discharging waste through the anus

FD	Feeding	Consuming food items, including feeding by animals that search for food and eat simultaneously; e.g., grazers, browsers, flying insectivores, and filter feeders; does not include hunting
FL	Fleeing	Hurried movement to avoid conspecifics or other animals; does not include fleeing to avoid the observer
GR	Grooming	Behaviour for the purpose of arranging and protecting the fur, feathers, skin, etc., including scratching and rubbing of antler velvet
HI	Hibernating	If the same den is used for hibernating and general denning, record as hibernating
HU	Hunting	Searching for, pursuing, and killing prey
IN	Incubation	Incubating, protecting, or laying eggs
LI	Living	Activity could not be specified due to ignorance or the activity was too diverse
MD	Migrating daily	Travelling that is a regular daily activity, including travelling to or away from a communal habitat; e.g., a bat on its daily flight to or from a roosting site
MS	Migrating seasonally	Travelling that is a regular annual activity; e.g., a snake travelling away from a communal habitat such as a hibernaculum
RB	Reproducing, birthing	Giving birth to live young; preparing a birthing reproduction site, such as a den
RE	Reproducing, eggs	Laying eggs (amphibians, reptiles and birds), building a nest, and feeding non-mobile young
RR	Rearing	Adults feeding neonates and juveniles
SH	Security habitat	Using habitat for protection or hiding from predators
ST	Security and/or thermal	Using habitat for its security and/or thermal values; used when differentiating between the two values is difficult or impossible
TE	Territoriality	Behaviour for the purpose of marking or defending a territory; e.g., singing, drumming, winnowing, howling, antler rubbing, wallowing, or scraping the ground
TF [a]	Travelling, flying	Used when the purpose of flying is not known; if known, use a more specific description such as hunting
TH	Thermal habitat	Using habitat for protection from heat, cold, or precipitation
TP	Travelling on a path	Walking on a trail that is embedded in the ground due to animals walking the same route for many years

TR	Travelling	Travelling by a method other than flying, swimming, and walking; usually used for animals that do not normally fly, swim, or walk; includes seeing an isolated track; does not include running if the purpose for running is known
TS [a]	Travelling, swimming	Used when the specific purpose of swimming is not known; if known, use a more specific description such as fleeing
TW	Travelling, walking	Used when the purpose of walking is not known; if known, use a more specific description such as migrating; does not include travelling on a path (see "TP")
UR	Urinating	Urinating

a Code is only associated with seeing or hearing an animal

b Code is only associated with sign of an animal

18 Enter a coded **descriptor** (Des) that indicates whether the animal was observed or heard in the plot or ecosystem unit or gives the probable age or season of the sign (**Table 4-12**).

Table 4-12. Codes for descriptors of wildlife evidence of use.

Code	Meaning
S	The animal was seen
H	The animal was heard
F	Fresh sign (<1 week old)
Y	Sign is <1 year old but >1 week old
O	Old (> 1-year-old)
U	Undetermined (age of sign is unknown)
W	Sign is from the winter season
G	Sign is from the growing season

19 Record the **number** (No.) of animals present or the number of sign elements. Codes for relative abundance can be used for sign elements instead of numbers (i.e., H [high], M [moderate], L [low], or T [trace]).

20 To provide additional information about the evidence of use, or to clarify an entry on this line on the form, enter a numeric code (**Note #**). Enter the same code in the **notes and photo numbers** section of the form, followed by the pertinent information. All photos taken for this form need the photo number recorded, and a brief description of what is shown in the photo. If more space is needed, use the back of the field sheet and indicate with a large and bold arrow that more information is recorded on the back of the form.

21 Not to be completed in the field. Data should be entered into a spreadsheet as soon as possible, ideally completed daily. Once data are entered into the spreadsheet, fill in the date entered and who entered it. Data entry should be checked by a second person and any discrepancies discussed with the original surveyor. Enter the name of the QA/QC checker once completed.

4.4.2 Significant Wildlife Habitat Survey Form

Field Label	Description and Instructions
1	Enter the **date** in DD-MM-YY format. (e.g., 01-01-20 for January 1st, 2020)
2	Enter the **plot number**, which is composed of the crew number and a sequential 3-digit number determined in the field. For example, If the crew number is 1 and it is their first plot, then the plot number is 1001, with the second plot being 1002.
3	Enter the **polygon number** where the plot is taking place.
4	Enter vegetation **surveyor(s)** first initial and last name.
5	Using the *List of Sites and Site Information* in Appendix B and record the **predetermined ecosite code.**
6	Consult with the vegetation and soil specialist and record the **final ecosite code**.
7	Using the *Field Summary of Significant Wildlife Habitat Criteria Schedules for Ecoregion 3W* found in Appendix C, check the yes or no if the **ecosite codes** listed for the Wildlife Habitat matches the plot's final ecosite code.
8	Using the *Field Summary of Significant Wildlife Habitat Criteria Schedules for Ecoregion 3W* found in Appendix C, check the yes, no, or unknown box if the **habitat criteria** listed for the Wildlife Habitat matches what is found in or around the plot. The unknown box should only be ticked if the criteria cannot be determined at the time of the survey to be present or not, likely due to seasonal timing (e.g., the survey is conducted in the summer so spring phenomenon cannot be observed).
9	Fill in the **habitat details & rationale, photo reference, UTM**. Include a brief description of the habitat criteria that is present. If multiple criteria are listed, specify which criteria is met. If the unknown box was checked, the reason why the habitat criteria could not be observed must be noted. Record photo number of the habitat and UTM coordinates if different from the plot coordinates. If more space is needed, then use the notes space with a code to the extra note.
10	Use **notes and photo numbers** space to record important features not described elsewhere, or for explanatory notes keyed to other entries on the form. All photos taken for this form need the photo number recorded, and a brief description of what is shown in the photo. If more space is needed, use the back of the field sheet and indicate with a large and bold arrow that more information is recorded on the back of the form.
11	Not to be completed in the field. Data should be entered into a spreadsheet as soon as possible, ideally completed daily. Once data are entered into the spreadsheet, fill in the date entered and who entered it. Data entry should be checked by a second person and any discrepancies discussed with the original surveyor. Enter the name of the QA/QC checker once completed.

5.0 REFERENCES

BC MFR, and BC MOE. 2010. Field Manual for Describing Terrestrial Ecosystems. Land Management Handbook. 2nd edition. B.C. Ministry of Forests and Range and B.C. Ministry of Environment.

ELC Working Group. 2009. Ecosites of Ontario - Operational Draft. Ecological Land Classification Working Group, Ministry of Natural Resources.

Marshall, P. L., G. Davis, and V. M. LeMay. 2000. Using Line Intersect Sampling for Coarse Woody Debris. Forest Research Technical Report TR-003. Research Section, Vancouver Forest Region, BCMOF.

OMNR. 2015. Field Guide to the Substrates of Ontario. Ontario Ministry of Natural Resources.

RIC. 1998. Standard for Terrestrial Ecosystem Mapping in British Columbia. Prepared by Ecosystem Working Group, Terrestrial Ecosystems Task Force, Resources Inventory Committee.

APPENDIX A: FIELD FORMS FOR PRINTING

(see next page – formatted for printing)

Date (DD/MM/YY)	Plot Number	Crew ID	All Surveyors	Weather	Page	Of

Predetermined Site Information

UTM Zone	Easting/Lat.	Northing/Long.	GRTS Number	Polygon Number	Ecosite	Survey Type Full ☐ Gr. ☐ Vis ☐

Information Determined at Site

UTM Zone	Easting/Lat.	Northing/Long.	GPS Accur. (m)	Elevation (m)	Ecosite	Rational for Ecosite Change

Site Description

Slope %	Aspect °	Surface Shape CCV ☐ CVX ☐ STR ☐	Microtop Size	Meso Slope Pos. Crest ☐ Upper ☐ Mid ☐ Lower ☐ Toe ☐ Level ☐ Dep. ☐ Gully ☐	Floodplain? ☐	Drainage (1-7)

Site dist. ☐ n/a ☐ fire ☐ site prep ☐ terrain ☐ soil dist. _____ ☐ harvest ☐ planted ☐ biotic ☐ other _____	Humus/ Organic Form ☐ Mull ☐ Fibrimor ☐ Peatymor ☐ Moder ☐ Humimor ☐ Anmoor	Gleying or Mottling ☐n/a _____cm	Seepage ☐n/a _____cm

Humus Thickness_____cm	Ah? Ae? ☐ ☐ _____cm	Estimated Soil Depth _____cm	Estimated Rooting Depth _____cm	R.Z. Coarse Frag %	R.Z. Text.	Site Diagram

Substrate Series	Restricting Layer ☐n/a ____cm	Restricting Layer Type ☐ Cement ☐ Compact ☐ Water ☐ Pan ☐ Lithic ☐ Chem

Notes and Photo Numbers:

Date entered into spreadsheet: Entered by: QA/QC by:

Site and Soil Description Form

Date (DD/MM/YY)	Plot Number	Polygon Number	Surveyor(s)	Weather	Survey Type Full ☐ Grd. ☐	Page	Of

COL.	TREES	A1	A2	A3	A	B1	B2	B

COL.	SHRUBS	B1	B2	B

COL.	HERB LAYER (C)	%

COL.	MOSS/LICHEN/SEEDLING (D)	%

COL.	ADDITIONAL SPECIES	Layer	%

Notes and Photo Numbers:

% Cover by Layer	TREE (A)	SHRUB (B)	HERB (C)	MOSS/LICHEN (D)

Date entered into spreadsheet: Entered by: QA/QC by:

Vegetation Survey Form

Date (DD/MM/YY)	Plot Number	Polygon Number	Surveyor(s)	BAF (M^2/Ha)	Minimum DBH (cm)	Page	Of

Tree no.	Species	Stand/Fall	DBH (cm)	M or E	Rem. Bark (%)	Length				Estimated Length (m)	Cr. Class	Height to Live Crown	Wildlife Codes						Notes and Photo Numbers
						Top (%)	Bot. (%)	Bot. Pos. (m)	Slope Distance (m)				Appear	Crown	Bark	Wood	Lichen	Wildlife Use	
01																			
02																			
03																			
04																			
05																			
06																			
07																			
08																			
09																			
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18																			
19																			
20																			

Date entered into spreadsheet: Entered by: QA/QC by:

Tree Attributes for Wildlife Survey Form

Date (DD/MM/YY)	Plot Number	Polygon Number	Surveyor(s)	Page	Of

Coarse Woody Debris Survey Form

Tree no.	Transect #1							Transect #2							Degree and Type of Piling (in plot boundaries)						
	Azimuth		(0-359)	Sampled		of 24m		Azimuth		(0-359)	Sampled		of 24m								
	Species	Diameter (cm)	Class	Tilt Angle	Length (m)	Height of end (cm)	Angle grnd.	Species	Diameter (cm)	Class	Tilt Angle	Length (m)	Height of end (cm)	Angle grnd.	Pile #	Size of pile (m) Length	Width	Height	Diam (cm)	Inter. space	
01															1						
02															2						
03															3						
04															4						
05															5						
06															6						
07															7						
08															8						
09															Notes and Photo Numbers						
10																					
11																					
12																					
13																					
14																					
15																					
16																					
17																					
18																					

Date entered into spreadsheet: Entered by: QA/QC by:

Date (DD/MM/YY)	Plot Number	Polygon Number	Surveyor(s)	Survey Type F/G ☐ VIS. ☐	Non-Hab. Feature	Type				Page	Of
						Dist.					

Wildlife Habitat Assessment Form

ASSESSMENT	Hab Use/Sen		Plot Type				Plot in Context													
Species	Sp. L.R.	Ssn	FD	SH	TH	Note #	Habitat feature	Conf.	Distance (km)	F/C L.R.	Imp.	Habitat Feature	Conf.	Distance (km)	F/C L.R.	Imp.	FD	SH	TH	Suit.	Note #

EVIDENCE OF USE	Inside plot							Outside plot and inside ecosite														
Species	Sex	Life Stage	Activity		Des.	No.	Com.	Sex	Life Stage	Activity		Des.	No.	Sex	Life Stage	Activity		Des.	No.	Com.		

Notes and Photo Numbers	

Date entered into spreadsheet: Entered by: QA/QC by:

Significant Wildlife Habitat Survey Form					
Date (DD/MM/YY)	**Plot Number**	**Polygon Number**	**Surveyor(s)**	**Predetermined Ecosite**	**Final Ecosite**

Seasonal Concentration Areas for Wildlife Species			
Wildlife Habitat	**Ecosite Codes**	**Habitat Criteria**	**Habitat Details & Rationale, Photo Reference, UTM**
Waterfowl Stopover & Staging Area (Terrestrial)	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Waterfowl Stopover and Staging Area (Aquatic)	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Shorebird Migratory Stopover Area	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Colonially - Nesting Bird Breeding Habitat (Bank & Cliff)	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Colonially - Nesting Bird Breeding Habitat (Tree/Shrubs)	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Colonially - Nesting Bird Breeding Habitat (Ground)	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Eagle & Osprey Concentration Area	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Sharp-tailed Grouse Lek	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Bat Hibernaculum	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Bat Maternity Colony	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Amphibian Breeding Habitat	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Turtle Wintering Area	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Snake Hibernaculum	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Animal Movement Corridors			
Habitat	**Ecosite Codes**	**Habitat Criteria**	**Habitat Details & Rationale, Photo Reference, UTM**
Cervid Movement Corridor	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Amphibian Movement Corridor	☐Yes ☐No	☐Yes ☐No ☐ Unknown	

Significant Wildlife Habitat Survey Form			
Plot Number	**Polygon Number**	**Predetermined Ecosite**	**Final Ecosite**

Specialized Habitat for Wildlife			
Specialized Wildlife Habitat	**Ecosite Codes**	**Habitat Criteria**	**Habitat Details & Rationale, Photo Reference, UTM**
Waterfowl Nesting Area	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Wild Rice Stand	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Milkweed Patch	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Bald Eagle & Osprey Nesting Habitat	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Woodland Raptor Nesting Habitat	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Turtle Nesting Area	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Aquatic Feeding Habitat	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Seeps and Springs	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Mineral Lick	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Mammal Denning Site	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Marsh Bird Breeding Habitat	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Open Country Bird Breeding Habitat	☐Yes ☐No	☐Yes ☐No ☐ Unknown	
Notes and Photo Numbers:			
Date entered into spreadsheet:	Entered by:	QA/QC by:	

LIST OF SITES AND SITE INFORMATION TO BE SUPPLIED ONCE DESK-BASED MAPPING WORK IS COMPLETED

APPENDIX C: MAP OF FIELD SAMPLING LOCATIONS

MAP OF FIELD SAMPLING LOCATIONS TO BE SUPPLIED ONCE DESK-BASED MAPPING WORK IS COMPLETED

APPENDIX D: FIELD SUMMARY OF SIGNIFICANT WILDLIFE HABITAT CRITERIA SCHEDULES FOR ECOREGION 3W

(see next page – formatted for printing)

Seasonal Concentration Areas for Wildlife Species

Wildlife Habitat	Wildlife Species	Ecosite Codes	Habitat Criteria
Waterfowl Stopover and Staging Area (Terrestrial) **Rationale:** Habitat important to migrating waterfowl.	• Brant • Cackling Goose • Canada Goose • Snow Goose • Ross's Goose • Tundra Swan • Trumpeter Swan • Wood Duck • Gadwall • American Wigeon • American Black Duck • Mallard • Blue-winged Teal • Northern Shoveler • Northern Pintail • Green-winged Teal *Other:* • Sandhill Crane	Focus on sites that have appropriate vegetation and highest likelihood of seasonal water accumulation. These include: B060-062 B077-079 B093-095 B109-111 Plus evidence of annual spring flooding from melt water or run-off within identified ecosites.	Open areas with sheet water during Spring (mid-March to June or September to November). Areas with flooding during spring melt and run-off provide important invertebrate foraging habitat for migrating waterfowl. Cultivated fields with waste grains are commonly used by waterfowl, these are not considered SWH.
Waterfowl Stopover and Staging Area (Aquatic) **Rationale:** Important for local and migrant waterbird populations during the spring or fall migration or both periods combined. Sites identified are usually only one of a few in the ecodistrict.	• Brant • Cackling Goose • Canada Goose • Greater White-fronted Goose • Snow Goose • Ross's Goose • Tundra Swan • Trumpeter Swan • Wood Duck • Gadwall • American Black Duck • American Wigeon • Mallard • Blue-winged Teal • Northern Shoveler • Northern Pintail • Green-winged Teal • Canvasback • Redhead • Ring-necked Duck	B142-152	Ponds, marshes, lakes, bays, coastal inlets, and watercourses used during migration. Sewage treatment ponds and storm water ponds do not qualify as a SWH; however, a reservoir managed as a large wetland or pond/lake does qualify. These habitats may have an abundant food supply (mostly aquatic invertebrates and vegetation in shallow water). Sites with wild rice have a high likelihood of being a waterbird stopover and staging area.

Seasonal Concentration Areas for Wildlife Species

	• Lesser Scaup • Greater Scaup • Harlequin Duck • Surf Scoter • White-winged Scoter • Black Scoter • Long-tailed Duck • Bufflehead • Common Goldeneye • Common Merganser • Hooded Merganser • Red-breasted Merganser • Ruddy Duck Others: • Red-necked Grebe • American Coot • Sandhill Crane • Common Loon • Red-throated Loon Special Concern under Ontario's Endangered Species Act, 2007: • Horned Grebe		
Shorebird Migratory Stopover Area **Rationale:** High quality shorebird stopover habitat is extremely rare and typically has a long history of use.	• Black-bellied Plover • American Golden-Plover • Semipalmated Plover • Killdeer • Whimbrel • Hudsonian Godwit • Marbled Godwit • Ruddy Turnstone • Stilt Sandpiper • Sanderling • Dunlin • Baird's Sandpiper • Least Sandpiper • White-rumped Sandpiper	Potential ecosites include: B005-006 B154-156 B160-162 B170-172 B176-178 B186-188 Includes all other ecosites where criteria have been met	Shorelines of lakes, rivers and wetlands, including beach areas, bars and seasonally flooded, muddy and un-vegetated shoreline habitats or open areas. Great Lakes coastal shorelines, including groynes and other forms of armour rock lakeshores, are extremely important for migratory shorebirds in May to mid-June and late July to early October. Will likely require multiple field visits. Storm water retention ponds, cultivated fields, and sewage lagoons are not considered SWH.

	• Buff-breasted Sandpiper • Pectoral Sandpiper • Semipalmated Sandpiper • Short-billed Dowitcher • Wilson's Snipe • Spotted Sandpiper • Solitary Sandpiper • Greater Yellowlegs • Lesser Yellowlegs • Wilson's Phalarope		
Colonially - Nesting Bird Breeding Habitat (Bank and Cliff) **Rationale:** Historical use and number of nests in a colony make this habitat significant. An identified colony can be very important to local populations. All swallow populations are declining in Ontario.	• Northern Rough-winged Swallow (this species is not colonial but can be found in Cliff Swallow colonies) • Cliff Swallow **THREATENED** Bank Swallow are protected under Ontario's *Endangered Species Act, 2007*.	Cliff faces, banks, bridge abutments, silos, barns (Cliff Swallows). Habitat may be found in, but not limited to the following ecosites: B001-004 B157-159 B173-175	Any site or areas with exposed soil banks, undisturbed or naturally eroding that is not a licensed/permitted aggregate area. Does not include man-made structures (e.g. bridges or buildings) or recently (2 years) disturbed soil areas, such as berms, soil or aggregate stockpiles. Does not include a licensed/permitted Mineral Aggregate Operation.
Colonially - Nesting Bird Breeding Habitat (Tree/Shrubs) **Rationale:** Large colonies are important to local bird population, typically sites are only known colony in area and are used annually.	• Bonaparte's Gull • Black Tern • Double-crested Cormorant • Great Blue Heron	May include a wide variety of tall treed ecosites. Habitat selection based on close proximity to water body or on island: B011-019 B023-028 B033-043 B048-059 B064-076 B081-092 B097-108 B113-137 B161-162 B177-178 B222-224	Great Blue Herons nest in live or dead standing trees in wetlands, lakeshores, islands, and peninsulas. Shrubs and occasionally emergent vegetation may also be used. Most nests in trees are 11 to 15m from ground, near the top of the tree. Bonaparte's Gulls nest in coniferous trees (preferably spruce-fir) near fens, bogs, swamps, ponds or lakes. Double-crested Cormorants prefer to nest in trees but will nest on the ground as well where trees are limited or have died and fallen.

Seasonal Concentration Areas for Wildlife Species

Colonially - Nesting Bird Breeding Habitat (Ground) **Rationale:** Colonies are important to local bird population, typically sites are only known colony in area and are used annually.	• Ring-billed Gull • Herring Gull • Common Tern • Caspian Tern • Double-crested Cormorant • Brewer's Blackbird **THREATENED** American White Pelican are protected under Ontario's *Endangered Species Act, 2007*.	Any rocky island or peninsula (natural or artificial) within a lake or large river (two-lined on a 1;50,000 NTS map) may indicate candidate habitat. B160-165 B169-172 B176-181 B185-188 Close proximity to watercourses in open fields or pastures with scattered trees or shrubs (Brewer's Blackbird). B008-009 B020-021 B030-031 B045-046 B061-062 B078-079 B094-095 B110-111 B142-144	Nesting colonies of gulls and terns are on islands or peninsulas (natural or artificial) associated with open water or in marshy areas, lakes or large rivers (two-lined on a 1:50,000 NTS map). Brewers Blackbird colonies are found loosely on the ground or in low bushes in close proximity to streams and irrigation ditches within farmlands. Double-crested Cormorants prefer to nest in trees but will nest on the ground as well where trees are limited or have died and fallen.
Eagle and Osprey Concentration Area **Rationale:** Habitat of annual importance to migrating and/or wintering eagles and ospreys.	• Osprey Special Concern under Ontario's *Endangered Species Act, 2007*: • Bald Eagle **ENDANGERED** Golden Eagle are protected under Ontario's *Endangered Species Act, 2007*.	Important habitat includes forested ecosites within the vicinity of lakes and rivers that receive large runs of salmonids. Undisturbed mature trees or snags distributed evenly along shorelines are important. Most individual trees used for perching are "super canopy" trees.	Most sites have been used for several years. Mostly associated with large river systems and lake confluences. Can be important hunting locations in Spring, Fall or Winter. Storm water waste facilities, retention ponds and sewage lagoons are not considered SWH.
Sharp-tailed Grouse Lek **Rationale:** Leks are an important habitat feature required to maintain populations of sharp-tailed grouse.	• Sharp-tailed Grouse	B029-031 B044-046 B060-062 B077-079 B093-095 B109-111 B126	Leks are typically in a grassy field/meadow or peatlands such as fens and bogs separated by >15ha from adjacent shrublands and >30ha from adjacent treed areas. There is often a knoll or slightly elevated rise in topography associated with the site. This is a better drained site less likely to collect water.

		B136-141	Field/meadows are to be >15ha when adjacent to shrubland and >30ha when adjacent to deciduous stands. Leks will be used annually if not destroyed by cultivation or invasion by woody plants or tree planting.
Bat Hibernaculum **Rationale:** Bat hibernacula are rare habitats in all Ontario landscapes.	• Big Brown Bat **ENDANGERED** Little Brown Myotis, Northern Long-eared Myotis, Eastern Small-footed Myotis, and Tricolored Bat are protected under Ontario's *Endangered Species Act, 2007*.	Hibernacula may be found in abandoned caves, addits, mine shafts, underground foundations. Caves and mine shafts are the important features. Commonly associated as components of either Cliff or Rock Barren ecosites. Once feature is identified the substrate classification can be used to identify characteristics and potential/suitability of identified or suspected hibernacula.	The locations and site characteristics of bat hibernacula are relatively poorly known. Primary criteria is identification of known feature Buildings are not considered to be SWH)
Bat Maternity Colony **Rationale:** Identification of maternity roosts is difficult. Known locations of treed bat maternity colonies is extremely limited in all Ontario landscapes.	• Big Brown Bat • Silver-haired Bat **ENDANGERED** Little Brown Myotis, Northern Long-eared Myotis, Eastern Small-footed Myotis, Tricolored Bat are protected under Ontario's *Endangered Species Act, 2007*.	Maternity colonies considered SWH are found in treed ecosites. B011-019 B023-028 B033-043 B048-059 B064-076 B081-092 B097-108 B113-125 B126-133 B222-224 Aspen is an important feature in Ecoregion 3W, primarily the presence of larger diameter trees in older mixed-wood stands.	Maternity colonies can be found in tree cavities, vegetation and often in buildings (buildings are not considered to be SWH). Maternity roosts are generally not found in caves and mines in Ontario. Maternity colonies located in Mature (dominant trees >80yrs old) deciduous or mixed forest stands with >10/ha large diameter (>25cm dbh) wildlife trees. Female bats prefer wildlife trees (snags) of decay class 1 or 2 or class 2-4, can be living or with bark mostly intact. Silver-haired Bats prefer older mixed or deciduous forest and form maternity colonies in tree cavities and small hollows. Older forest areas with at least 21 snags/ha are preferred. Buildings are not considered to be SWH).
Amphibian Breeding Habitat **Rationale:** These habitats are extremely important to	• Eastern (Red-spotted) Newt • Spotted Salamander • Blue-spotted Salamander	May include swamps and thickets, vernal/seasonal pooling, riparian and variety of wetland interiors and margins B109-156	Wetlands and pools (including vernal pools) > 500m2 (about 25m diameter) supporting high species diversity are significant; small or ephemeral habitats may not be identified on MNRF mapping and could be important amphibian breeding habitats.

amphibian biodiversity within Northern Ontario landscapes.	• American Toad • Gray Treefrog • Boreal Chorus Frog • Wood Frog • Spring Peeper • Northern Leopard Frog • Green Frog • Mink Frog • Mudpuppy	B223-224	Spotted Salamanders are extremely rare in 3W; candidate wildlife habitat for this species could include artificial wetlands and/or ponds and ditches. Wetlands and pools need to persist until August. Presence of shrubs and logs increase significance of pond for some amphibian species because of available structure for calling, foraging, escape and concealment from predators.
Turtle Wintering Area Rationale: Turtle distribution in 3W is very limited and disjunct. Sites with the highest number of individuals are most significant.	• Painted Turtle Special Concern under Ontario's *Endangered Species Act, 2007*: • Snapping Turtle	Lakes, wetlands, ponds, and ecosites: B128-142 B145-152	For most turtles, wintering areas are in the same general area as their core habitat. Water has to be deep enough not to freeze and/or have soft mud substrates. Over-wintering sites can be permanent water bodies, large wetlands, and bogs or fens. Storm water waste facilities, retention ponds and sewage lagoons are not considered SWH. Both Midland and Western Painted Turtles are present in 3W.
Snake Hibernaculum Rationale: Generally hibernacula that provide adequate conditions for overwintering in 3W will be limited on the landscape. Sites with the highest number of individuals are most significant.	• Eastern Gartersnake • Northern Redbelly Snake	For all snakes, habitat may be found in any forested ecosite in northern Ontario. Talus, rock barren, crevice and caves are more typically related to these habitats. Many suitable conditions also observed in the very shallow ecosites particularly on fractured bedrock and lower veg cover Open and Sparse Tall/Low Treed or Shrub Systems.	For snakes, hibernation takes place in sites located below frost lines in burrows, rock crevices and other natural locations. Areas of broken and fissured rock are particularly valuable because they provide access to subterranean sites below the frost line. Observation of congregating snakes on sunny warm days in the spring or fall is a good indicator of a hibernaculum. The existence of rock piles or slopes, stone fences, and crumbling foundations. Large numbers of roadkill during period of emergence in spring can also be an indicator that a hibernaculum is nearby. The subspecies of Eastern Gartersnake, Red-sided Gartersnake, occurs in the northwest portion of 3W.

Animal Movement Corridors

Wildlife Habitat	Wildlife Species	Ecosite Codes	Habitat Criteria
Cervid Movement Corridor **Rationale:** Corridors are important for moose to be able to access seasonally important life-cycle habitats or to access new habitat for dispersing individuals by minimizing their vulnerability while travelling.	• Moose	Corridors may be found in all treed ecosites.	Movement corridor must be determined when **Aquatic Feeding Habitat and Mineral Lick** habitat are confirmed from Table 1.2.2 of this schedule. Corridors typically follow riparian areas, woodlots, areas of physical geography (ravines, or ridges). Corridors will be multi-functional i.e. these will function for any smaller mammal species as well.
Amphibian Movement Corridor **Rationale:** Movement corridors for amphibians that are moving from their terrestrial habitat to breeding habitat can be extremely important for local populations.	• Eastern Newt • Blue-spotted Salamander • Spotted Salamander • Northern Leopard Frog • Green Frog • Mink Frog	Corridors may be found in all ecosites associated with water. Corridors will be determined based on identifying the significant breeding habitat for these species in Table 1.2.2.	Movement corridors between breeding habitat and other habitat. Movement corridors must be determined when Amphibian breeding habitat is confirmed as SWH from Table 1.2.2 (**Amphibian Breeding Habitat – Wetland)** of this Schedule.

Specialized Habitat for Wildlife

Wildlife Habitat	Wildlife Species	Ecosite Codes	Habitat Criteria
Waterfowl Nesting Area **Rationale:** Important to local waterfowl populations, sites with greatest number of species and highest number of individuals are significant.	• Trumpeter Swan • Wood Duck • Gadwall • American Wigeon • American Black Duck • Blue-winged Teal • Northern Shoveler • Northern Pintail • Green-winged Teal • Ring-necked Duck • Bufflehead • Common Goldeneye • Hooded Merganser • Red-breasted Merganser • Common Merganser	All upland habitats located adjacent to ELC ecosites; B129-135 B140-152 B224 are Candidate SWH Note: includes adjacency to provincially Significant Wetlands	A waterfowl nesting area extends 120m from a wetland (>0.5ha) or a cluster of 3 or more small (<0.5ha) wetlands within 120m of each individual wetland where waterfowl nesting is known to occur. Upland areas should be at least 120m wide so that predators such as raccoons, skunks, and foxes have difficulty finding nests. Wood Ducks, Bufflehead and Hooded Mergansers utilize large diameter trees in forested habitat for cavity nest sites. Storm water waste facilities, retention ponds and sewage lagoons are not considered SWH.
Wild Rice Stand **Rationale:** Wetlands containing large stands of wild rice are important rearing and migratory stopover locations for waterfowl.	• *Zizania palustris* (Wild Rice)	B142-145 B148-152	Ponds, marshes, lakes, bays, coastal inlets, and watercourses with wild rice. These habitats have an abundant food supply for waterbirds. Sites with wild rice have a high likelihood of being a waterfowl stopover and staging area as well.
Milkweed Patch **Rationale:** Native milkweed patches are extremely rare in 3W and are specialised habitats for Monarch butterflies.	• *Asclepias incarnata* • *Asclepias syriaca*	*Asclepias incarnata* can be found within a variety of habitats. Most likely ecosites include: B126-156 B170-172 B222-224 *Asclepias syriaca* may be found within: B006 B008 B020-021 B029-030 B044-045	Extremely rare in 3W. Potential habitat includes fields and dry or wet meadows as well as shorelines of lakes and rivers. Horticultural or planted sites are not considered SWH.
Bald Eagle and Osprey Nesting Habitat	• Osprey	Typically nests in treed communities directly adjacent to riparian areas – rivers, lakes, ponds and wetlands.	Nests are associated with lakes, ponds, rivers or wetlands along treed shorelines, islands.

Specialized Habitat for Wildlife

Wildlife Habitat	Wildlife Species	Ecosite Codes	Habitat Criteria
Rationale: Nests are used annually by these species. Suitable nesting locations may be impacted due to shoreline development.	Special Concern under Ontario's *Endangered Species Act, 2007*: • Bald Eagle	However, species may nest further away from water.	Osprey nests are usually at the top of a tree whereas Bald Eagle nests are typically in super canopy trees in a notch within the tree's canopy. Nests located on man-made objects such as telephone or hydro poles will not normally be considered as SWH, however the MNRF District retains discretion regarding significance of constructed nesting platforms.
Woodland Raptor Nesting Habitat **Rationale:** These habitats may be used annually by some species. Nests sites for these species are rarely identified in advance of site investigations.	*Stick nesters/users:* • Sharp-shinned Hawk • Cooper's Hawk • Northern Goshawk • Broad-winged Hawk • Red-tailed Hawk • Great Horned Owl • Barred Owl • Great Gray Owl • Long-eared Owl • Merlin • Common Raven *Cavity nesters/users:* • Great Horned Owl • Northern Hawk Owl • Barred Owl • Boreal Owl • Northern Saw-whet Owl • American Kestrel (Note: Bufflehead, Common Goldeneye, Wood Duck and Common and Hooded Merganser also use tree cavities for nesting. Northern Flying Squirrel use cavities as roosting sites in winter)	May be found in all forested ELC Ecosites.	All natural or conifer plantation woodland/forest stands. Stick nests found in a variety of intermediate-aged to mature conifer, deciduous or mixed forests within tops or crotches of trees. Common Raven are included because nests may be used in subsequent years by raptors. Species such as Merlin or Coopers Hawk nest along forest edges sometimes on peninsulas or small off-shore islands. Some raptors rely on cavity trees for nesting. They do not excavate their own cavities, but rely on natural cavities of sufficient size and those excavated by Pileated Woodpeckers. Larger diameter trees are used most frequently, with nest cavities most often found in trembling aspen. Nests may be used again, or a new nest may be in close proximity to old nest.
Turtle Nesting Area **Rationale:** These habitats are rare and when identified will often be the only breeding	• Painted Turtle Special Concern under Ontario's *Endangered Species Act, 2007*: • Snapping Turtle	May include: B003 B006-007 B031 B171-172 B187-188	Best nesting habitat for turtles is close to water and away from roads and other sites less prone to loss of eggs by predation from skunks, raccoons or other animals. For an area to function as a turtle-nesting area, it must provide sand and gravel that turtles are able to dig in and be located in an open and sunny

Specialized Habitat for Wildlife

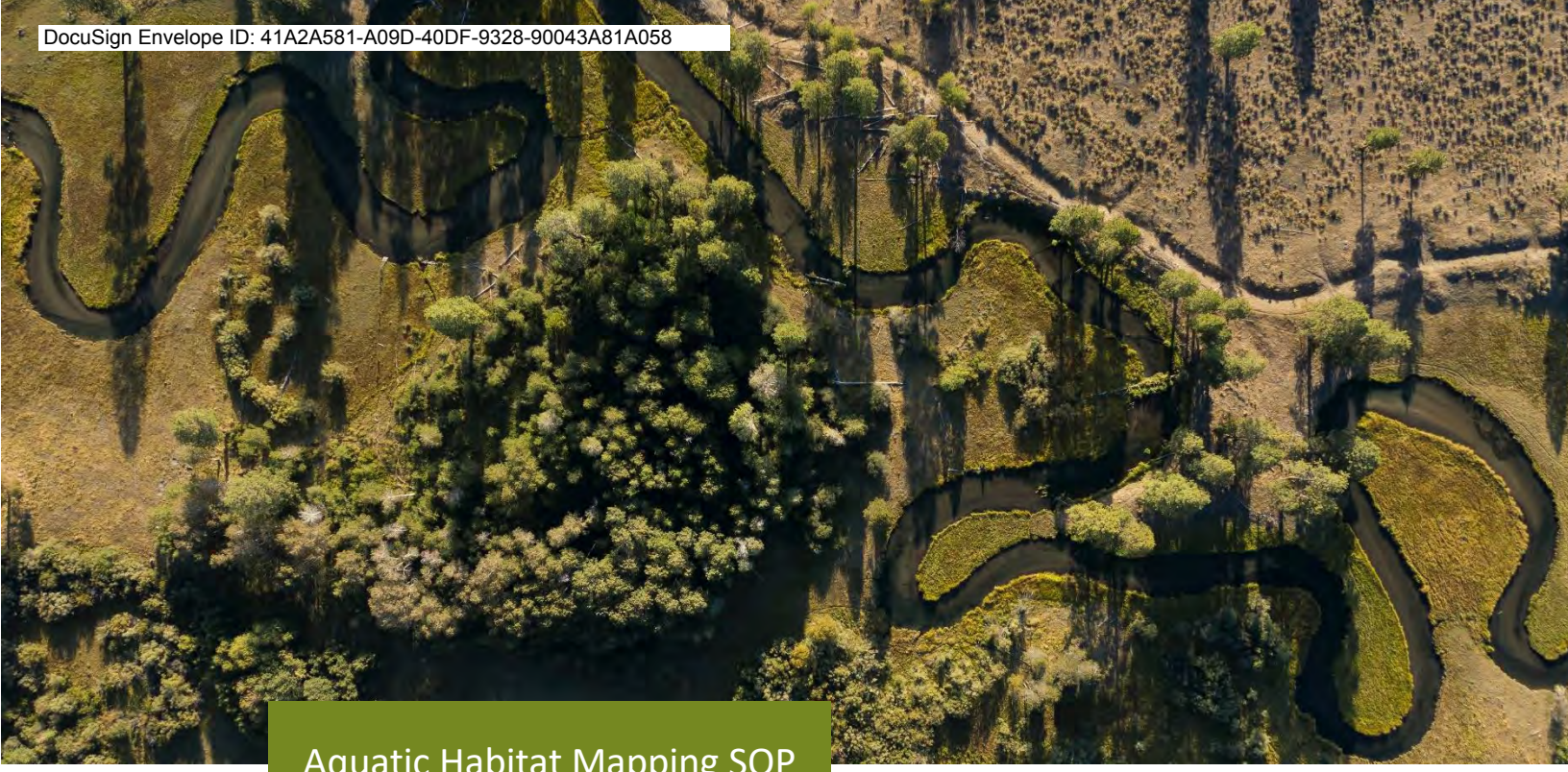
Wildlife Habitat	Wildlife Species	Ecosite Codes	Habitat Criteria
site for local populations of turtles.	**THREATENED** Blanding's Turtle are protected under Ontario's *Endangered Species Act, 2007*.		area. Nesting areas on the sides of municipal or provincial road embankments and shoulders are not SWH. Sand and gravel beaches adjacent to undisturbed shallow weedy areas of marshes, lakes, and rivers are most likely used.
Aquatic Feeding Habitat **Rationale:** Aquatic Feeding Habitats are an extremely important habitat component for moose and other wildlife as they supply important nutrients. Forest cover adjacent to these areas is important as well to provide for summer thermal cover, screening and escape cover.	• Moose	Habitat may be found in all forested ecosites adjacent to water.	MNRF maps these locations on Crown land and rates the site on a scale of 1 – 4, with 4 having the greatest potential. Feeding sites classed 3 or 4 are candidate significant areas. Identification of Aquatic Feeding Habitat for moose should follow the method outlined in MNRF's Selected Wildlife and Habitat Features: Inventory Manual. Wetlands and isolated embayments in rivers or lakes which provide an abundance of submerged aquatic vegetation such as pondweeds, water milfoil and yellow water lily are preferred sites. Adjacent stands of lowland conifer or mixed woods will provide cover and shade.
Seeps and Springs **Rationale:** Seeps/Springs are typical of headwater areas and are often at the source of coldwater streams.	Selected wildlife species that utilize this feature: • Spotted Salamander • Blue-spotted Salamander • Ruffed Grouse • Spruce Grouse • Moose • White-tailed Deer • Black Bear Other species: • Brook Trout **THREATENED** Forest-dwelling Woodland Caribou are protected under Ontario's *Endangered Species Act, 2007*.	Seeps are areas where groundwater emerges and can be identified as zones where surface saturation produces overland flow but there is no obvious source for the surface water. Often they are found within headwater areas within forested habitats. Any forested Ecosite within the headwater areas of a stream could have seeps/springs or ground water recharge areas.	Any forested area within the headwaters of a stream or river system. Seeps and springs are important feeding and drinking areas especially in the winter and will typically support a variety of plant and animal species.

Specialized Habitat for Wildlife

Wildlife Habitat	Wildlife Species	Ecosite Codes	Habitat Criteria
Mineral Lick **Rationale:** Mineral licks are a valuable habitat component but are also very rare on the landscape.	• Moose • White-tailed Deer Porcupine and other species may utilize these sites as well **THREATENED** Woodland Caribou are protected under Ontario's *Endangered Species Act, 2007*.	Habitat may be found in all treed ecosites. Most probable in clay ecosities: B110-B125	This habitat component is found in upwelling groundwater and the soil around these seepage areas. It typically occurs in areas of sedimentary and volcanic bedrock. In areas of granitic bedrock, the site is usually overlain with calcareous glacial till. Does not include anthropogenic sources such as roadsides.
Mammal Denning Site **Rationale:** Species are important fur-bearing mammals and den sites can be a limiting factor in sustaining populations.	• Mink • Otter • Gray Wolf • Coyote • Canada Lynx • Bobcat • Black Bear • Red Fox **Cavity Users** • Marten • Fisher **THREATENED** Algonquin Wolf, Cougar, Gray Fox and Wolverine are protected under Ontario's *Endangered Species Act, 2007*.	Habitat may be found in all treed ecosites.	Mink prefer shorelines dominated by coniferous or mixed forests with dens usually underground. Mink will often use old muskrat lodges. Mink may den in root masses along shorelines of water bodies. Otters prefer undisturbed shorelines along water bodies that support productive fish populations with abundant shrubby vegetation and downed woody debris for denning. They often use old beaver lodges or log jams and crevices in rock piles. Marten and fisher share the same general habitat, requiring large tracts of coniferous or mixed forests of mature or older age classes. Denning sites are often in cavities in large trees or under large downed woody debris. Wolves prefer a more interior forest condition for locating their den sites. Wolves require sandy ground, sloped for excavation (esker areas should be examined as potentially key sites). Lynx den sites are most often associated with the presence of downed woody debris. Black bears, particularly sub-adults, will often den in the base of hollow trees. In 3W such trees are rare and primarily consist of large diameter cedar or sometimes large white spruce.
Marsh Bird Breeding Habitat **Rationale:** Rich wetlands are productive breeding habitats for these bird species and rare in Northern Ontario landscapes.	• Trumpeter Swan • Ring-necked Duck • Green-winged Teal Northern Shoveler • Pied-billed Grebe • Red-necked Grebe • Virginia Rail • Sora	Ecosites: B134-B152	Nesting occurs in wetlands. All wetland habitats are to be considered as long as there is shallow water with emergent aquatic vegetation present. Storm water retention ponds and sewage lagoons are not considered SWH.

Specialized Habitat for Wildlife

Wildlife Habitat	Wildlife Species	Ecosite Codes	Habitat Criteria
	• American Coot • Sandhill Crane • Common Loon • American Bittern • Spotted Sandpiper • Sedge Wren • Marsh Wren Special Concern under Ontario's *Endangered Species Act, 2007*: • Yellow Rail • Black Tern		
Open Country Bird Breeding Habitat Rationale: This wildlife habitat is declining throughout Ontario and North America	• Upland Sandpiper • Black-billed Magpie • Western Meadowlark • Eastern Bluebird • Vesper Sparrow • Le Conte's Sparrow Special Concern under Ontario's *Endangered Species Act, 2007*: • Short-eared Owl **THREATENED** Bobolink and Eastern Meadowlark are protected under Ontario's *Endangered Species Act, 2007*.	All field, meadow, open bog or fen, and sparse shrub ecosites: B08-09 B20-21 B29-31 B44-46 B60-62 B77-79 B93-95 B109-111 B136-141	Large field/meadow areas (includes natural and cultural fields and meadows) >30ha. Field/meadow not Class 1 or 2 agricultural lands, and not being actively used for farming (i.e. no row cropping or intensive hay or livestock pasturing in the last 5 years). Field/meadow sites considered significant should have a history of longevity, either abandoned fields, mature hayfields and pasturelands that are at least 5 years or older. The indicator bird species are area sensitive requiring larger field/meadow areas than the common field/meadow species.



Aquatic Habitat Mapping SOP

THE NWMO BIODIVERSITY IMPACT STUDIES: STANDARD OPERATING PROCEDURE FOR AQUATIC HABITAT MAPPING

August 14, 2020

PREPARED BY Zoetica Environmental Consulting Services

SUBMITTED TO Melissa Mayhew
Nuclear Waste Management Organization
22 St. Clair Avenue East
Sixth Floor, Toronto, ON
M4T 2S3, Canada

ZOETICA
ENVIRONMENTAL CONSULTING SERVICES

OFFICE 102-22351 St Anne Ave, Maple Ridge, BC, V2X 2E7
PHONE 604 467 1111
WEBSITE www.zoeticaenvironmental.com

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A000	10-Jul-2020	First submission to NWMO	A. Buckman	D. MacKinnon	H. Bears
B000	14-Aug-2020	BIS Year 1 final submission to NWMO	A. Buckman	C. Chui	H. Bears

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GLOSSARY OF TERMS

BIS	Biodiversity Impact studies
CCME	Canadian Council of Ministers of the Environment
DO	Dissolved Oxygen
EC	Electrical Conductivity
EMBP	Environmental Media Baseline Program
GIS	Geographical Information System
GPS	Global Positioning System
GRTS	Generalized Random Tessellation Stratified
LSA_{AQU}	Aquatic Local Study Area
LUB	Left Upper Bank
MNRF	Ministry of Natural Resources and Forestry
MTO	Ministry of Transportation Ontario
NAD	North American Datum
NWMO	Nuclear Waste Management Organization
OS	OverSample
P1	PanelOne
PFD	Personal Floatation Device
QA	Quality Assurance
QC	Quality Control
RIC	Resource Inventory Standards
RISC	Resource Inventory Standards Committee
RSA_{AQU}	Aquatic Regional Study Area
RUB	Right Upper Bank
SAR	Species at Risk
SOP	Standard Operating Procedure
TDS	Total Dissolved Solids
UTM	Universal Transverse Mercator
PanelOne and OverSample points	PanelOne (P1) points are pre-selected points in a Generalized Random Tessellation stratified study design. If a P1 site is found to be inaccessible after reasonable effort, then the surveyor can visit/consider the next OverSample (OS) point as a replacement.

Waterbody	A waterbody is an accumulation of water in an area and includes lakes, ponds, and wetlands that do not have a defined channel that flows through it.
Lake	Open waterbody with a depth greater than 2 m and with less than 25% of its surface area covered with wetland vegetation.
Pond	A pond is a small body of still water formed naturally or by hollowing or embanking. Ponds differ from lakes in that they do not have an atrophic zone (an area deep enough that sunlight cannot reach the bottom).
Stream Reach	A relatively homogenous length of a stream, that flows on a perennial or seasonal basis, having a continuous channel bed and demonstrating fluvial processes (e.g., flowing water has scoured the channel bed or deposited any amount of mineral alluvium within the channel).
Watercourse	A watercourse is a natural or artificial channel through which water flows and includes streams, rivers and wetlands that include a defined channel that flows through them.
Wetland	An area where the water table is at, near, or above the surface, or where soils are saturated with water for sufficient time such that the principal determinants of vegetation and soil development are excess water and low oxygen. Waterbodies less than 2 m deep are considered wetlands. Wetlands are classified into four classes, marsh, swamp, bog, and fen (see Appendix I for definitions of the wetland types).

1.0 PURPOSE AND PRINCIPLE

The objective of this Standard Operating Procedure (SOP) is to provide instructions to the field contractor for conducting the field portion of the aquatic habitat mapping for the APM Project described in the Baseline Design Report. The SOP focusses on field sampling procedures based on available best practices and guidelines for fish habitat mapping; it follows protocols aimed at large inventory projects following the Reconnaissance (1:20 000) Fish and Fish Habitat Inventory: Standards and Procedures (RIC 2001), with modifications from the Ministry of Transportation Environmental Guide for Fish and Fish Habitat (MTO 2009) and the Ontario Wetland Evaluation System – Northern Manual (MNRF 2014), to align with Ontario standards.

The overall objectives of the baseline aquatic habitat mapping for the Biodiversity Impact Studies (BIS) are to:

1) Characterize the presence and distribution of fish habitat within the LSA_{AQU} and select control areas in the RSA_{AQU} under baseline conditions;
2) Detect any potentially important fish areas including spawning, rearing or overwintering habitat;
3) Assess the potential for SAR habitat within the study areas;
4) Evaluate the distribution of habitats within the LSA_{AQU} and select control sites in the RSA_{AQU} to select suitable sampling sites for fish community characterization studies (Tier 2); and,
5) Provide the necessary baseline data for the development of a potential monitoring program(s) to address the environmental, regulatory, and stakeholder/rights-holder concerns relevant to the APM Project.

2.0 GENERAL PRECAUTIONS

Fieldwork comes with inherent risk, especially when working around water. The field contractor should be trained in safety measures for working around water and in boats (for the waterbody surveys) and should be prepared to have all required safety equipment on hand. It is the responsibility of the contractor to ensure that all required personal gear and safety gear is carried in the field.

The location of the APM Project is remote, and access to many reaches may be difficult. Caution should be taken to determine safe access points, including from the air when ground access is not possible. In addition to access, wildlife is prevalent in remote areas of Northern Ontario.

3.0 EQUIPMENT AND MATERIALS

Equipment and materials required for bathymetry, limnology and water chemistry are described in the EMBP (CanNorth 2020). For fish habitat measurements the following equipment will be required:

Equipment for Field Measurements:

- Ruler (meter stick)
- Camera
- Tape measure (50 m)
- Range finder
- Clinometer or Survey equipment
- Hand level & measuring tape
- Water quality meter (e.g., YSI or HydroLab)
- Turbidity meter
- Bathymetry and limnology equipment (see EMBP, CanNorth, 2020)

Equipment for Cleaning:

- Spray bottles
- Rubber or disposable nitrile gloves
- Household bleach
- Tap water
- Distilled water
- Paper towels

General Field Equipment:

- Binoculars
- Waterproof notebook
- Pencils
- Clipboard
- Waterproof data forms
- Maps

Navigation Equipment:

- Access maps
- Air photos
- GPS unit
- Compass
- Georeferenced field maps on tablet or phone

General Safety Equipment:

- First aid kit
- Survival kit
- Emergency plan
- High visibility clothing
- Flagging tape
- Radio
- Satellite phone
- Bear spray

Boat Equipment (For Lakes/Ponds/Wetlands/ Large Rivers)

- Boat and motor equipped to meet Coast Guard safety standards (e.g., PFD, oars, bailing can)
- Fuel and oil (for motorboat)
- Tool-kit and spare parts (spark plugs, pull cord)
- Anchor with an adequate line for deep lakes
- The propeller, cotter pins, shear pins

Personal Gear

- Rain gear
- Waders and patch kit
- Lunch and water
- Polarized sunglasses
- Clear safety glasses
- Hat with brim
- Sunscreen
- Bug shirt and insect repellent

4.0 QUALITY CONTROL

Collection, acquisition, quality assurance (QA) and quality control (QC) of the immense volumes of data generated by large projects demand standardization and well-defined data management procedures. The field contractor is expected to develop their own QA/QC protocols to ensure that field-based activities (e.g., data collection and data entry) meet the expectations of the NWMO. QA/QC protocols must include a method to back up all field data collected and to prevent the loss of data stored in one location or manner (e.g., a lost field form).

Desk-based mapping via air photo analysis will include field checks by the field contractor to note any inconsistencies between field observations and mapped products. High-quality, field verified maps will permit extrapolation of field sampling information to areas that have not been sampled, and for which maps and air photos are the only information available.

Changes to methodology should be verified with Zoetica before implementation. The field contractor will be supplied with reach tables (**Appendix A** for watercourses and **Appendix F** for waterbodies) that identify reaches for sampling (based on a stratified and randomized sampling design). The list of reaches to be sampled will include contingency sampling reaches in order of selection, in the case where chosen reaches cannot be sampled (e.g., due to access or safety).

5.0 PROCEDURE

This document outlines the procedure for the aquatic habitat mapping of watercourses and waterbodies in the study area. The procedure section is subdivided into 1) Decontamination Procedures for equipment and personal gear before and between sampling in watercourses and waterbodies; 2) General Procedures for all watercourses and waterbodies; 3) Watercourse (Stream) Surveys that include rivers, streams, and tributaries, and wetland reaches that have a clear watercourse running through them; and 4) Waterbody Surveys that include lakes, ponds, and wetlands with no clear channel flowing through them. The field protocol follows the MTO *Environmental Guide for Fish and Fish Habitat* (MTO 2009) with adaptations from the *Reconnaissance (1:20 000) Fish and Fish Habitat Inventory: Standards and Procedures* (RIC 2001) for completing a large inventory project.

5.1 Decontamination Procedures

All equipment requires cleaning between sampling sites. The Canadian Council of Ministers of the Environment (CCME) *Protocols Manual for Water Quality Sampling in Canada* (CCME 2011) will be followed for the cleaning of sampling equipment and personal gear (e.g., waders, gloves) before surveying, and between sites. The effective cleaning of survey equipment prevents the transfer of aquatic invasive species from one waterbody to another. Section 5.1.1 briefly summarizes the cleaning protocol:

5.1.1 Decontamination of Equipment Before Fieldwork

1. **Drain** all water from watercraft, including the motor, live well, and bilge at the source or on land.
2. **Clean** and inspect all equipment and personal gear such as waders, watercraft, trailers, boots, gloves. **Remove** all plants, animals, and mud.
3. **Soak** all equipment in a diluted household bleach solution (>5% sodium hypochlorite) at a concentration of 100 ml (~3 ounces) of bleach to 20 L (~5 gallons) of water for at least 60 minutes. Triple-rinse all equipment with tap water. **Rinse** using high pressure, if possible. Extremely hot

tap water is preferable (50°C or 120°F). Alternatively, **freeze** all equipment and gear for two or more days.

4. **Dry** all watercraft and gear for at least 5 days in the hot sun.

5.1.2 Decontamination Between Sites

Decontamination must be performed before moving from site to site (i.e., different watercourse, waterbody, or wetland). Bleach will avoid the spread of invasive species as well as chytrid fungus, ranaviruses, and other amphibian diseases as per protocols found in the *Decontamination Protocol for Field Work with Amphibians and Reptiles in Canada* (Canadian Herpetofauna Health Working Group, 2017).

1. **Set up** a decontamination station by placing 3 tote bins on top of a tarp in a sunny location away from a watercourse or waterbody. Direct sunlight will help break down residual bleach. (Note: it is assumed that there will be no impermeable surfaces within the study area on which to dispose of rinse water.)
2. **Fill** one tote bin with soapy water (add liquid dish soap to tap water). Scrub the soles and sides of your rubber/hiking boots with a scrub brush to remove visible organic matter, dirt, and sediment. **Rinse** with clean tap water into the 3rd tote bin. Note: if it is not possible to carry large quantities of tap water into the field, the field crew can use water from the next surveying site to wash equipment, but rinsing should occur away from the waterbody at the rinse station to avoid contamination.
3. **Prepare** a fresh 1 in 10 dilution of household bleach in a spray bottle, i.e., 1 part bleach, 9 parts water. Fill a 2nd tote bin and a spray bottle with this bleach solution. Wear rubber gloves and safety glasses/goggles – bleach is corrosive!
4. **Immerse** all equipment and gear that may be contaminated (including personal gear such as boots, waders, and gloves) in the bleach solution. Spray larger items to soak thoroughly. Let sit for at least 1 min, then triple rinse with clean tap water into the 3rd tote bin.

Shelf life of decontamination solutions and disposal:

a. Bleach solution in the tote bin and spray bottle can be reused for up to 7 days.
b. Tote bin with soapy water can be reused until it becomes too dirty with visible particulate matter.
c. Tote bin with rinse water should be disposed of daily.
d. To dispose of old solutions, carry the bins back to camp and flush down the drain with plenty of water.

Notes: Even trace amounts of residual bleach can adversely affect amphibians on contact. If it is not feasible to carry large quantities of tap water, consider bringing multiple pairs of boots and change between sites. Decontaminate all boots back at the camp.

5. Watercraft, such as boats and motors, should also be cleaned between sites and thoroughly dried before using it at a new site. All visible mud, vegetation, and biotic material should be removed from watercraft at the site immediately after removal of the watercraft from the. Before moving watercraft to a new waterbody, the watercraft should be scrubbed down using a brush and bleach solution as described above, triple-rinsed and left to dry in the sun to break down any remaining bleach.

5.2 General Field Procedure

1. Before conducting fieldwork at each identified location for the survey, a field reconnaissance should be conducted using a helicopter to fly over the proposed survey reaches identified in the *Watercourse (Stream) Survey Reach Table and Map* (**Appendix A**) and the *Waterbody (Lake/Pond/Wetland) Survey Reach Table and Map* (**Appendix F**) to verify the reach breaks and major inlets and outlets to waterbodies mapped during the desk-based aquatic habitat mapping. Any changes that require being made to reach breaks should be conducted following the procedure outlined in Section 5.3.2.
2. Each day, a route should be determined using the list of pre-determined reaches and field maps provided in **Appendix A** for watercourses (streams) and **Appendix F** for waterbodies (lakes/ponds/wetlands). Route planning should ensure any sites not visited the previous day, due to time limitations, should be included in the next day's route. The field contractor should note any required use of helicopters to assist access or speed up field work.
3. When in the field, follow the route planned for the day to navigate to the next site. Sites have been determined using a GRTS design to create stratified and randomized points. As part of this design method, some points are pre-selected as PanelOne (P1) for sampling or OverSample (OS). If a P1 site is determined after a reasonable effort to be inaccessible, an OS site can be used in place of the P1 site. If the OS site is also deemed inaccessible after reasonable effort, the next OS site can be visited/considered. The field contractor should take care, however, not to skip points too quickly and without effort in favour of randomized points that happen to occur next to easily accessible areas (roads, cleared forest) as this may impose a site selection bias meant to be diminished via the use of pre-selected, random points. While points should be visited in order wherever possible, logistical constraints due to difficulty accessing areas may render it necessary to deviate from the ordering from P1 points and OS points from time to time, (e.g., in a situation where there is only one way into a large, difficult to access area and points can be selected along a hiking route). In such cases, the replacement of any P1 sites with an OS site should be noted in the comments section of the *Watercourse (Stream) Survey Form* (**Appendix B**) *or the Waterbody (Lake/Pond/Wetland) Survey Form* (**Appendix G**).
4. Using the list of pre-determined reaches, check what type of survey is meant to be conducted at each reach visited. Continuous boat surveys may be conducted along all reaches of the major watercourses (Mennin and Revell Rivers) to document aquatic habitat and fish habitat in these reaches. The survey type is pre-determined for the field contractor by Zoetica and will be provided in **Appendix A** for watercourses and **Appendix F** for waterbodies.
5. Assemble required gear for survey type according to the gear lists in Section 3.0.

5.3 Watercourse (Stream) Survey

This section describes the procedures for conducting watercourse (stream) surveys. The *Watercourse Reach Table and Survey Map* (**Appendix A**), the *Watercourse (Stream) Survey Form* (**Appendix B**), the *Watercourse Reference Guide* (**Appendix C**), and the *Aquatic Habitat Mapping Form* (**Appendix D**) accompany this SOP for the watercourse inventory. Both the *Watercourse (Stream) Survey Form* and the *Aquatic Habitat Mapping Form* are to be filled out at every site on each reach surveyed. Before fieldwork, reach tables (**Appendix A**) will be provided to the field contractor after desk-based mapping of watercourses has been conducted by Zoetica and will indicate which reaches will be surveyed in the field. A list of contingency reaches will also be supplied in the case where reaches are not able to be surveyed due to safety or access concerns.

The SOP procedure below is structured to provide instruction for conducting the field measurements to fill out each section of the *Watercourse (Stream) Survey Form* (**Appendix B**). Detailed instructions and field code definitions for filling out the form can be found in the *Watercourse Reference Guide* (**Appendix C**). Some sections of the form can be filled out by the field-contractor before conducting fieldwork by using the reach table to be provided by Zoetica (in **Appendix A**) after completion of desk-based mapping and site selection. The *Aquatic Habitat Mapping Form* (**Appendix D**) is used to map the habitat characteristics at each site visited. Instructions for Aquatic Habitat Mapping are described in Section 5.5.

Pre-field:

The *Watercourse (Stream) Survey Form* (**Appendix B**) is used to capture data collected in the field. As part of pre-field site referencing, transcribe the Project Number and Project Description from the reach table (**Appendix A**) (see General Information, Section 1) and reach referencing information (see Location Information, Section 5.3.2) onto the *Watercourse (Stream) Survey Form* before conducting field surveys.

In the field:

The field component of the watercourse (stream) aquatic habitat mapping survey includes the following:

1. description of survey and water conditions (General Information, Section 5.3.1);
2. survey site description including surrounding land use (Location Information, Section 5.3.2);
3. watercourse morphology characterization (Reach Section Type and Morphology, Section 5.3.3);
4. channel measurements (Reach Section Type and Morphology, Section 5.3.3);
5. cover and habitat inventory (Cover and Habitat, Section 5.3.4;
6. features identification (e.g. migratory obstructions) (Additional Information, Section 5.3.5);
7. wildlife observations (recorded in comments section) (Additional Information, Section 5.3.5);
8. photography (Photographing Watercourses, Section 5.3.1.1); and,
9. Mapping of reach information on the Aquatic Habitat Mapping Form (**Appendix D**) (see Section 5.5).

For wetland reaches, the minimum information required is location information (site referencing), cover estimates, water characteristics (temperature, pH, conductivity, turbidity), photos, and cover and habitat inventory.

5.3.1 General Information

This section details the procedure to fill out the General Information Section of the *Watercourse (Stream) Survey Form* (**Appendix B**) and provides directions for taking watercourse site photos.

1. Record Project information on survey form: **APM BIS Project Number: NWMO-BIS-AQH1**
2. Record Project Description: **Biodiversity Baseline Data Collection**
3. Record Survey Date, Collectors, Weather Conditions, Air Temperature, Time Started.
4. Remember to record Time Finished at the end of sampling.
5. Take water temperature, pH, conductivity, and turbidity measurements from mid-stream and mid-depth, if possible, using a portable conductivity meter (e.g., YSI, or HydroLab). If the meter does not automatically standardize to 25°C, record water temperature at the same time as conductivity and use a conductivity nomograph to convert the reading to 25°C. Record information on the survey form.

6. Take photographs of the site following photographic instructions below and record photo numbers and general descriptions on the *Watercourse (Stream) Survey Form* (**Appendix B**) and the *Aquatic Habitat Mapping Form* (**Appendix D**).

5.3.1.1 Photographing Watercourses

Photographs need to be carefully referenced on the *Watercourse (Stream) Survey Form* (**Appendix B**) and the *Aquatic Habitat Mapping Form* (**Appendix D**).

1. Take upstream-oriented and downstream-oriented photographs at each site to show general stream characteristics including:
    a. Channel morphology (e.g., riffles, pools, runs);
    b. Riparian vegetation;
    c. Obstructions to fish passage;
    d. Major disturbances;
    e. Culverts/water crossings (e.g., piers, abutments);
    f. Ditch lines (at crossing structure);
    g. Rare habitat/spawning/nesting sites;
    h. Specific habitat features that may be impacted by the project activities; and,
    i. Areas of potential compensation (e.g., where habitat has been degraded due to forestry roads).

At road crossings, take the photos of vantage points illustrated in **Figure 5-1**.

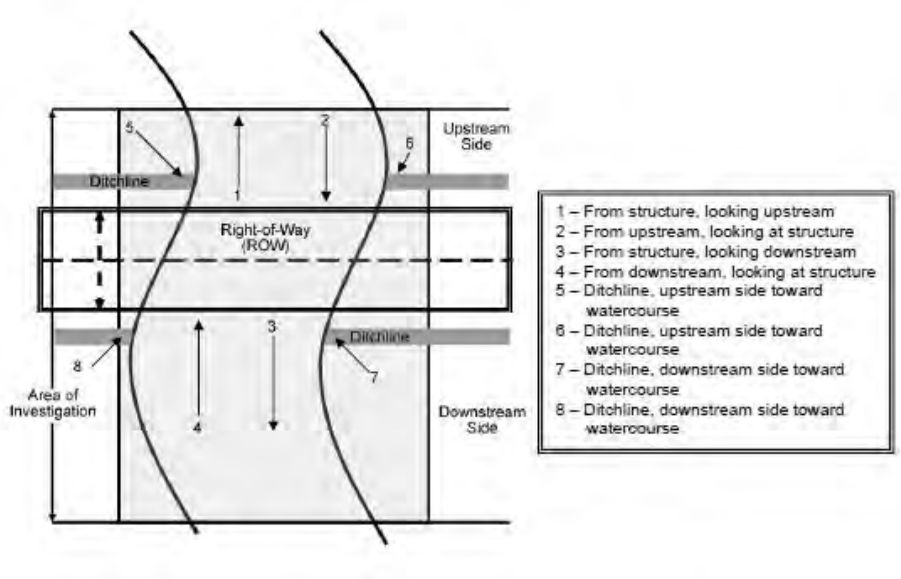

Figure 5-1. Photo vantage points to be taken at crossing locations. From MTO 2009.

2. For each photo record the photo ID (from the camera) and the general direction that each photograph was taken: U/S (upstream), D/S (downstream), X (across the stream), or Bd (towards stream bed) and any relevant descriptions of photos (see the list in bullet #1) on the *Watercourse (Stream) Survey Form*. On the *Aquatic Habitat Mapping Form,* note the location and photo number (from the camera) on the drawn map of all photos taken.

5.3.2 Location Information

This section details how to fill out the Location section of the *Watercourse (Stream) Survey Form* (**Appendix B**) and provides instruction on conducting the in-field verification of the desk-based reach mapping. Some information in this section can be filled into the survey form before heading to the field.

Pre-field:

1. Record the following information supplied in the reach tables (**Appendix A**) onto the *Watercourse (Stream) Survey Form* (**Appendix B**) before entering the field:
    a. Watercourse ID (desk-based unique ID)
    b. Watercourse Name (gazetted name and/or alias)
    c. Reach Number (derived by Zoetica using GIS and assigned in a sequential upstream order starting at the downstream end of the stream)
    d. GRTS Number (assigned by desk-based stratified and randomized reach selection)
    e. GPS Coordinates of the upstream end of reach (UTM Zone/Easting/Northing (NAD 83) based on GIS)

In the field:

1. In a helicopter with good visibility, conduct a reconnaissance flight of the reach before landing to survey, to determine if reach breaks are appropriate. If pre-determined reaches provided in the reach table (**Appendix A**) need to be split into separate reaches (e.g., due to non-homogenous characteristics identified in the field, or fish barriers), renumber the split reaches as sub-reaches using a decimal system (e.g., 1, 2.1, 2.2, 2.3, 3…) and record on the *Watercourse (Stream) Survey Form* (**Appendix B**). Take GPS coordinates of each new reach break (at downstream end) and record waypoint numbers in the comments section.
2. Record surrounding land use (e.g., forestry road, forestry clear cut) and any sources of pollution (e.g., runoff).

5.3.3 Reach Section Type and Morphology

This section details the procedures to fill out the information in the Reach Section Type and Morphology section of the *Watercourse (Stream) Survey Form* (**Appendix B**) and provides instruction for conducting physical field measurements at the site.

1. In the field, determine a representative section of the reach to sample.
2. Record Reach or Sub-reach Number (Note: if the Reach Number identified in Location Block is sub-divided further in the field, record Sub-Reach Number here).
3. Record Site Number. Site numbers are given in a sequential, upstream, ascending order and are used when more than one site is sampled in a reach. A new form is filled out for each new site in a reach.
4. Record Site Access: helicopter (H), two-wheel-drive (V2), four-wheel-drive (V4), foot (FT), boat (B), other (O).
5. Record Field UTM (the GPS Coordinates) of the downstream end of the site to be sampled using NAD 83. Record UTM Coordinates (Zone/Easting/Northing) to the meter level (uncertainty).
6. Type: Check the appropriate type (stream/river or channelized) **and** check either permanent, intermittent, or ephemeral.

7. Associated Wetland: Describe the wetland type that appears to be hydrologically connected to stream reach, if present, according to the Ontario Wetland Evaluation System (MNRF 2014). Wetland types include bog (Wb), fen (Wf), marsh (Wm), and swamp (Ws) (see **Appendix I** for definitions).
8. Site Length: Measure and record total site length using meter tape.
    a. Measure site length following the stream channel, including its bends and curves.
    b. If bankfull width is less than 10 m wide, sample a minimum of 100 m of the stream.
    c. If bankfull width is > 10 m wide, multiply the width generated from the first measurement by 10 to determine the minimum length of the survey site required.
    d. Increase the site length to capture habitat sequences (e.g. riffle-run sequences) adequately.
    e. Record the site length to the nearest 1.0 m or ± 5%
9. Gradient % and Code: Measure at a minimum of two sites along the survey site over as long a distance as possible. Measure in upstream and downstream directions to maximize the distance. Measurements are sighted from similar habitat units (e.g., riffle crest to riffle crest). Record the grade to the nearest 0.5% and the method code using the following:

Method Type	**Code**
Ground Estimates	GE
Clinometer	C
Abney type level	AL
Survey equipment	S

10. Stream Discharge: Record the amount of water passing through the channel at the time of the survey
    a. Observe the amount of water in the channel in relation to the bankfull depth.
    b. Look for low flow indicators:
        i. The distinct sequence of riffles and pools, or steps and pools
        ii. Wetted width significantly less than the channel width
        iii. Dry, unvegetated channel bars
    c. Look for high flow indicators:
        i. The distinction between riffles and pools or steps and pools is difficult
        ii. The water level at or over bank tops
        iii. Wetted width similar to or greater than the channel width
        iv. No visible bars or banks
    d. Check the appropriate code:

Definition	**Code**
Low (0 – 30% of Bankfull)	L
Moderate (31 – 90% of Bankfull)	M
High flow (> 90% of Bankfull)	H

11. Subsections: Check all that apply (Run, Pool, Riffle, Flats, Culvert, Other)
12. Percentage Area: for each type of subsection described in #11, record the percentage it accounts for of the site sampled.

13. Mean Depth Wetted (m): For each type of subsection described in #11, record the mean wetted depth:
    a. Measure the outlet crest depth and subtract it from the maximum upstream pool depth.
    b. Repeat this measurement at a minimum of 6 riffle-pool, cascade-pool, or step-pool sequences within the site.
    c. Classify glides as large morphology.
    d. Record the residual pool measurements to the nearest 0.1 m.
14. Mean Width Wetted (m): For each type of subsection described in #11, record the mean wetted width:
    a. Measure to ± 0.1 m the distance of the wetted surface from the right to the left side of the channel. Repeat a minimum of 6 times at equally spaced intervals in conjunction with the channel measurements.
    b. Include water under undercut banks, protruding rocks, logs, and stumps.
    c. Repeat measurement in conjunction with channel bankfull width measurements (see #15).
    d. Add widths of multiple channels for the total wetted width.
15. Mean Bankfull Width (m): For each type of subsection described in #11, record the mean wetted depth:
    a. Include all unvegetated gravel bars in the measurement.
    b. Where one or more vegetated islands separate multiple channels, measure the width as the sum of all the separate channel widths. The islands are excluded from the overall width measurement (see **Figure 5-2**).
    c. Measure the widths at a minimum of 6 sites, taken at equally spaced intervals. The 6 channel/wetted widths should be equally spaced over the site.
    d. Generally, do not take stream widths near stream crossings, unusually wide or narrow areas (e.g., impoundments), or disturbances.

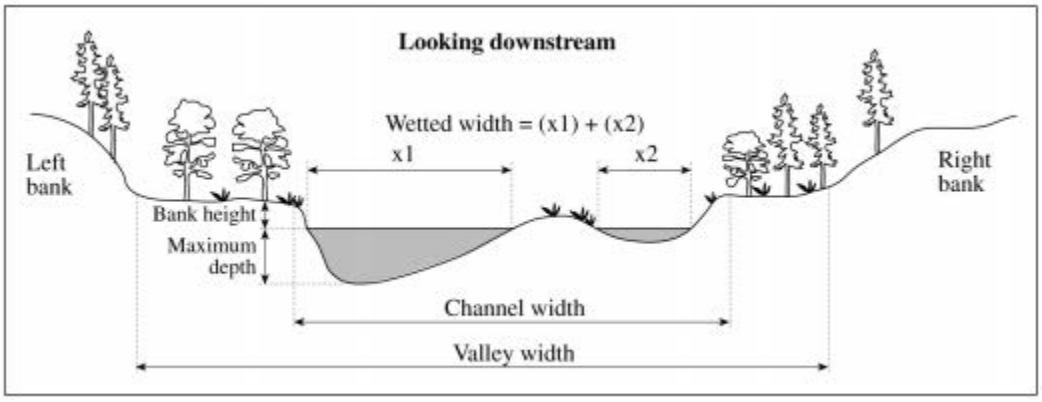

Figure 5-2. Measuring channel bankfull widths. From RIC, 1999.

16. Mean Bankfull Depth (m): For each type of subsection described in #11, record the mean wetted depth:
    a. Identify the top of both banks.

b. Extend the meter tape from the left banktop to the right banktop to simulate the bankfull water level.
c. Use a meter stick to measure the difference in elevation from the tape to the channel bed at a riffle-pool, cascade-pool, or step-pool break beneath the cross-section.
d. Place the meter stick at the deepest point in the channel along the cross-section at a riffle-pool, cascade-pool, or step-pool break. See **Figure 5-3** for a cross-sectional view of measuring bankfull depth.
e. If there is more than one channel (e.g., where vegetation islands separate flow), measure the bankfull depth in the main channel (usually the channel that is deepest and fastest flowing).
f. Collect a minimum of 3 measurements and record average and range to the nearest 0.1 m.

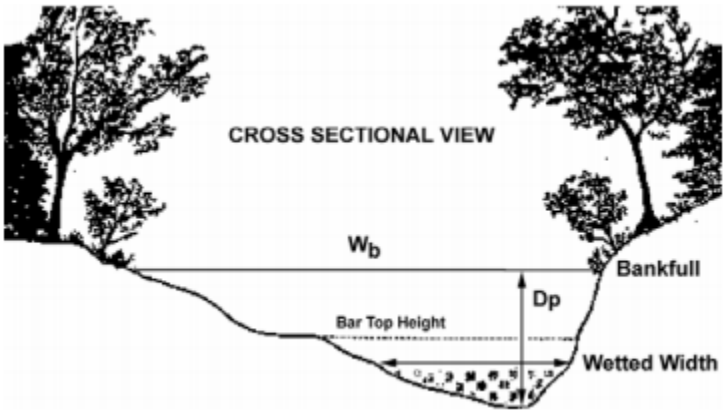

Figure 5-3. Cross-sectional view of measuring the bankfull channel width (Wb) and depth (Dp). From RISC (2008).

17. Substrate Percentage (%):
    a. Visually assess the percent cover of substrate type and record and classify percent type by bedrock, large boulder, small-medium boulder, cobble, gravel.
    b. For fine textures including sand, clay, muck, and detritus, place about ½ to 1 tablespoon of sediment in the palm, feel the mass by rubbing between fingers (see Appendix C) and record the percentage of each type.
    c. The total should add to 100%.

18. Bank Stability:
    a. Determine the category of bank stability for the left upstream bank and right upstream bank separately (see Watercourse Reference Guide: Stream Reaches in **Appendix C**).

5.3.4 Cover and Habitat

This section details the procedures to fill out the Cover and Habitat section of the *Watercourse (Stream) Survey Form* (**Appendix B**) and instructions for conducting cover and habitat measurements.

1. Instream Cover (% surface area):

   a. Locate the cover types (undercut banks, boulders, cobble, woody debris, vascular macrophytes, other) within the wetted channel or within 1 m above the water surface.
   b. Estimate the relative amount of cover that the individual cover type provides the entire site.
   c. Record the percent instream cover for each type of cover type.
2. Shore Cover (% stream shaded):
   a. Visually assess the amount of canopy closure provided by streamside/riparian vegetation, which projects over the stream channel to provide shade and is > 1 m above the water surface.
   b. Check off the appropriate percentage on the Watercourse (Stream) Survey Form.
3. Instream Vegetation Type (%) and Predominant Species:
   a. Visually estimate and record the percent of the stream bed covered by each vegetation type (submerged, floating, emergent).
   b. Record the predominant species of each vegetation type.
4. Riparian Vegetation Type and Stage:
   a. Visually assess riparian vegetation types and check the dominant type for stream section.
   b. Check the dominant riparian stage (initial, shrub/herb, pole-sapling, young forest, mature forest) for each bank (left upstream bank and right upstream bank).

5.3.5 Additional Information

1. Migratory Obstructions:
   a. Check off any permanent or seasonal natural (e.g., beaver dams, cascades, waterfalls, water depth and flow) and human-made (e.g., dams, weirs, perched culverts) obstructions that occur within the stream site surveyed.
   b. If barriers are noted outside of site location but within the full reach, note this in the comments section.
2. Potential Critical Habitat:
   a. Record locations of all evidence of spawning and potential groundwater discharge (e.g., springs, watercress, iron 'floc' or staining, bank seepage) or other types of habitat that may provide critical habitat functions such as nursery habitat, seasonal refugia, deep holding or staging pools.
3. Potential Enhancement Opportunities:
   a. Note any opportunities that exist for potential enhancement of stream section and reach. Provide as many details as possible.
4. Comments
   a. Note any additional information that may be useful in assessing fish habitat.
   b. Include incidental observations or signs of fish and other wildlife.

5.4 Waterbody (Lakes/Ponds/Wetlands) Survey

This section describes the procedures for conducting aquatic habitat surveys in lakes, ponds, and wetlands. A checklist for information that is required to be collected is supplied in the *Waterbody (Lake/Pond/Wetland) Checklist Form* (**Appendix E)**. The *Waterbody (Lake/Pond/Wetland) Survey Reach Table and Map* (**Appendix F**), the *Waterbody (Lake/Pond/Wetland) Survey Form* (**Appendix G**), and the

Aquatic Habitat Mapping Form (**Appendix D**) accompany this section of the SOP. Detailed instructions, including definitions for each data field on the *Waterbody (Lake/Pond/Wetland) Survey Form*, are included in a *Waterbody Reference Guide* (**Appendix H**). A description of wetland types is provided in the *Wetland Type Reference Guide* (**Appendix I**). Both the survey form and the mapping form are to be filled out at every site for each reach sampled. Before field work, the *Waterbody (Lake/Pond/Wetland) Survey Reach Table and Map* (**Appendix F**) will be provided to the field contractor after desk-based mapping of waterbodies has been conducted by Zoetica. The waterbody reach table will indicate which lake/pond/wetland reaches will be surveyed in the field. A list of contingency waterbody reaches will also be supplied in the case where reaches are not able to be surveyed due to safety or access concerns.

The SOP procedure below is structured to provide instruction for conducting the field measurements to fill out each section of the *Waterbody (Lake/Pond/Wetland) Survey Form* (**Appendix G**). Some sections of the form can be filled out by the field-contractor before conducting fieldwork by using the reach table to be provided by Zoetica after completion of desk-based mapping and site selection. The *Aquatic Habitat Mapping Form* (**Appendix D**) is used to map the habitat characteristics at each site visited. Instructions for Aquatic Habitat Mapping are described in Section 5.5.

Pre-field:

The *Waterbody (Lake/Pond/Wetland) Survey Form* (**Appendix G**) is used to capture data collected in the field. As part of pre-field site referencing, transcribe the Project Number and Project Description from the General Information section (see Section 5.4.1) and the reach referencing (Location) information (see Section 5.4.2) onto the *Waterbody (Lake/Pond/Wetland) Survey Form* before conducting field surveys.

In the field:

The field component of the waterbody (lake/pond/wetland) aquatic habitat mapping survey includes the following:

1. description of survey and water conditions (General Information, Section 5.4.1);
2. survey site description including the surrounding land use (Location Information, Section 5.4.2);
3. waterbody morphology characterization (Reach Type and Morphology, Section 5.4.3);
4. waterbody major inlet and outlet survey (to be conducted following the watercourse survey protocol on the Watercourse (Stream) Survey Form (**Appendix B**)
5. characterization of water quality and limnology (to be coordinated with the EMBP; CanNorth, 2020) and details to be recorded on the EMBP Forms (Limnology, Bathymetry, and Water Chemistry, Section 5.4.4);
6. bathymetric characterization (to be coordinated with the EMBP; CanNorth, 2020) and details to be recorded on the EMBP Forms (Limnology, Bathymetry, and Water Chemistry, Section 5.4.4);
7. bank and shoreline cover and habitat characterization (Cover and Habitat, Section 5.4.5);
8. In-water cover and habitat characterization (Cover and Habitat, Section 5.4.5);
9. wildlife observations (recorded in comments box) (Additional Information, Section 5.4.6);
10. photography (recorded in General Information, Section 5.4.1.1); and,
11. Mapping of waterbody on the Aquatic Habitat Mapping Form (**Appendix D**) (see Section 5.5).

For wetland reaches, the minimum information required is site referencing, cover (cover estimates), water characteristics (temperature, pH, conductivity, turbidity), photos, and habitat quality characterization.

5.4.1 General Information

This section details how to fill out the General Information section of the *Waterbody (Lake/Pond/Wetland) Survey Form* and provides directions for conducting measurements and taking waterbody site photos.

1. Record Project information on survey form: **APM BIS Project Number: NWMO-BIS-AQU1**
2. Record Project Description: **Biodiversity Baseline Data Collection**
3. Record Survey Date, Collectors, Weather Conditions, Air Temperature, Time started.
4. Remember to record Time Finished at the end of sampling.
5. Record surface conditions of the lake.
6. Take photographs of the site following photographic instructions below and record photo numbers and a general description on the survey form.

5.4.1.1 Photographing Lakes and Ponds

Photographs need to be carefully referenced on the *Waterbody (Lake/Pond/Wetland) Survey Form* (**Appendix F**) and the *Aquatic Habitat Mapping Form* (**Appendix D**).

1. Take the following photographs during lake inventories:
    a. Panoramic view of the surrounding area;
    b. Shoreline and riparian conditions;
    c. Inlet and outlet streams;
    d. Aquatic plant communities;
    e. Any other important and relevant features;
    f. Specific habitat features that may be impacted by the project activities; and,
    g. Areas of potential compensation (e.g., where habitat has been degraded).

2. For each photo, record the photo ID (from the camera) and the general direction that each photograph was taken: North/East/South/West (see the list in bullet #1) on the *Waterbody (Lake/Pond/Wetland) Survey Form*. On the *Aquatic Habitat Mapping Form,* note the location and photo number (from the camera) on the drawn map of all photos taken.

5.4.2 Location Information

This section details how to fill out the Location section of the *Waterbody (Lake/Pond/Wetland) Survey Form* (**Appendix F**) and provides instruction on conducting the in-field verification of the desk-based reach mapping. Some information can be filled into the survey form before heading to the field.

Pre-field

1. Record the following information supplied in the reach tables (**Appendix A**) onto the *Waterbody (Lake/Pond/Wetland) Survey Form* (**Appendix G**) before entering the field:
    a. Waterbody ID (desk-based unique ID)
    b. Waterbody Name (gazetted name and/or alias)
    c. Reach Number (assigned by desk-based GIS exercise)
    d. GRTS Number (assigned by desk-based stratified and randomized reach selection)
    e. GPS Coordinates (at Primary Inlet, derived by GIS)

In the field

1. In a helicopter with good visibility, conduct a reconnaissance flight of each waterbody (reach) before landing to survey, to determine appropriate access sites and verify information provided in the reach table. Record any changes in the comments section.
2. Record Site Access: helicopter (H), two-wheel-drive (V2), four-wheel-drive (V4), foot (FT), boat (B), other (O).
3. Record surrounding land use (e.g., forestry road, forestry clear cut) and any sources of pollution (e.g., runoff).

5.4.3 Reach Type and Morphology

This section details how to fill out the information in the Reach Section Type and Morphology section of the *Waterbody (Lake/Pond/Wetland) Survey Form* (**Appendix G**) and provides instruction for conducting physical field measurements at the site.

1. Record Field UTM (the GPS Coordinates) of the **downstream end of the site** to be sampled using NAD 83. Record UTM coordinates (Zone/Easting/Northing) to the meter level (uncertainty).
2. Check and record waterbody type (large lake, small lake, pond, wetland, reservoir, dug-out).
3. Check waterbody source (intermittent, runoff, spring-fed, not connected, by-pass, in-stream).
4. Record estimated waterbody dimensions (length and width in meters) using a range finder in meters.
5. Indicate if the main inlet and outlet streams are surveyed and indicate reach numbers for each. NOTE: Surveys of these stream reaches should be filled out on the Watercourse (Stream) Survey Form (**Appendix B**).

5.4.4 Limnology, Bathymetry and Water Chemistry

Limnology and Water Chemistry information is required for Aquatic Habitat Mapping. As these measurements are recorded as part of the EMBP, the Limnology, Water, and Plankton Datasheet - LSA form provided in the EMBP Final Design Report (CanNorth 2020). should be used to collect data in collaboration with the field contractor responsible for water quality measurements. This section outlines the specific requirements for limnological and water chemistry measurements for Aquatic Habitat Mapping as it pertains to fish habitat.

1. Collect dissolved oxygen concentrations and temperatures at 0.5 m intervals to bottom or a maximum depth of 10 m.
2. Take vertical dissolved oxygen (DO in mg/L) and temperature profiles (in °C) simultaneously using a meter (e.g., YSI or HydroLab).
3. Take two sets of readings at each depth interval: one during descent and the other during ascent to minimize bias due to adjustment of the meter to the water conditions at the subsequent depths.
4. Max Depth: Record max depth during dissolved oxygen measurements.
5. pH: Measure pH in the field using a hand-held pH meter. Ensure that the pH meter has been calibrated using a low ionic strength electrode and calibration standards. pH can be recorded at the same depth measurements as dissolved oxygen profile.
6. Conductivity: Make conductivity measurements from lake surface and bottom samples or to a max depth of 10 m.

   a. Measure electrical conductivity in the field using a portable conductivity meter (e.g., YSI, HydroLab).
   b. Note: Most conductivity meters automatically convert conductivity measurements to 25°C. If the meter does not automatically standardize to 25°C, record the water temperature at the same time as conductivity and use a conductivity nomograph to convert the reading to 25°C.
7. Water Temperature: Record water temperature in °C from the depth where conductivity measurements are made.
8. Determine Secchi depth on the shady side of the boat. Do not wear sunglasses. Take reading as close to mid-day as possible (10 am – 2 pm). Record depths in tenths of meters. Conduct Secchi depth determinations after the dissolved oxygen/temperature profiles have been conducted as not to disturb the water column.
   a. Lower the Secchi disk until it disappears.
   b. Note depth to the nearest tenth of a meter.
   c. Raise Secchi until it reappears.
   d. Note depth.
   e. The Secchi depth is the midpoint between these 2 depths.

5.4.5 Cover and Habitat

This section details how to fill out the Cover and Habitat section of the *Waterbody (Lake/Pond/Wetland) Survey Form* (**Appendix F**) and instructions for conducting cover and habitat measurements.

Bank Habitat:

1. In-Water Cover (% surface area): In-water cover provides an area for resting, shelter and predator avoidance for fish. Different types of cover are important in different habitat types, so it is essential to note in which part of the waterbody the features occur. A cover particle is any object that touches the water within the sample area, is **at least 100 mm wide** along the median axis and of sufficient density to block >75 % of sunlight from reaching the stream bottom. A cover particle can consist of a mat of materials such as twigs, macrophytes, or the bank. The mat must still meet the median diameter size and light penetration restrictions.
   a. Locate the cover types (undercut banks, boulders, cobble, woody debris, vascular macrophytes, other) within the wetted channel or within 1 m above the water surface.
   b. Estimate the relative amount of cover that the individual cover type provides the entire site.
   c. Record the percent In-water cover for each type of cover type.
2. Near Shore Slope (%):
   a. Use a handheld clinometer to measure nearshore slope.
   b. Tie a ribbon or mark up the shore at eye level.
   c. While standing on the shoreline, use the clinometer to determine the angle of slope.
   d. Record gradient in %.
3. Shoreline Substrate (%):
   a. Visually estimate percent cover of substrate type (bedrock, boulder, cobble, gravel).
   b. For fine substrate types (sand, silt, clay, muck, marl, detritus), place ½ to 1 tablespoon of sediment in the palm. Feel mass by rubbing between fingers and record the percentage of each type.

   c. The total should add to 100%.
4. Shoreline Type:
   a. Visually observe shoreline type and classify by percentage of presence of an appropriate type of shoreline (sand or gravel beach, low rocky shore, cliff or bluff shore, wetland shore, vegetated shore). The percentage should add up to 100%.
5. Shoreline Cover:
   a. Visually observe and record the percentage of shoreline coverage
      i. None 0%
      ii. Sparse < 5%
      iii. Moderate 5 – 20%
      iv. Abundant > 20%

In-Water Habitat

1. Vegetation Type (%):
   a. Visually observe and record the approximate percentage of each type of vegetation (submerged, floating, emergent).
   b. List predominant species of each type of vegetation observed.
2. Underwater Cover (% of surface area): Underwater cover provides an area for resting and predator avoidance for fish. Different types of cover are important in different habitat types, so it is essential to note in which part of the waterbody the features occur. Most types of cover should fit into the categories provided. Any additional features should be identified. The percentage should total 100% (of the surface area).
   a. Visually observe and record underwater cover types and percentage surface area of waterbody of each type (undercut banks, boulders, cobble, woody debris, organic debris, vascular macrophytes).
3. Bottom Substrate: Estimate the percent cover of each substrate type (bedrock, boulder, cobble, gravel, sand, silt, clay, muck, marl, detritus) visually (if possible).

5.4.6 Additional Information

1. Migratory Obstructions:
   a. Check off any permanent or seasonal natural (e.g., beaver dams, cascades, waterfalls, water depth and flow) and human-made (e.g., dams, weirs, perched culverts) obstructions that occur within the lake/pond and outlets sampled.
2. Potential Enhancement Opportunities:
   a. Note any opportunities that exist for potential enhancement of lake/pond/wetland reach. Provide as many details as possible.
3. Comments
   a. Note any additional information that may be useful in assessing fish habitat.
   b. Include incidental observations of fish and other wildlife.

5.5 Aquatic Habitat Mapping

For watercourses (streams reaches), and waterbodies (lakes/ponds/wetlands), this section describes the instructions for filling out the *Aquatic Habitat Mapping Form* (**Appendix D**). The map scale is expected to be approximately 1 cm = 5 m so that a 100 m section will fit on a letter-sized page. More than one map

page may be required for reach sections that are longer than 100 m in length and for larger lakes/ponds/wetlands. Small cross-sectional sketches of representative morphological features (e.g. through pools, runs) should be added, showing the bed and bank profiles. Where a defined valley configuration is present, a cross-sectional diagram should be included to show the entire river valley and floodplain. It may be necessary to estimate the dimensions of the flood plain and river valley for the cross-section diagram. If the vertical scale needs to be exaggerated to show features, then the scale must be recorded on the map.

5.5.1 Suggestions for Mapping Aquatic Habitat

1. A compass ring is provided on the *Aquatic Habitat Mapping Form* (**Appendix D**), and north should be marked on the page
2. The length of the section should be measured using a tape measure or hip chain, where possible and marked on the map.

Within the section, the stream banks or lake edge should be outlined on the *Aquatic Habitat Mapping Form* (**Appendix D**).

1. Begin by marking prominent landmarks that are evenly spaced along the section; large trees on the bank or sand bars are suitable landmarks. It is challenging to avoid distorting the map without using landmarks.
2. Outline the wetted width around these landmarks.
3. Any side channels or discharges into the water feature should be included.
4. Hydrologic features should be drawn in the diagram of the water feature using the symbols noted in the legend. Accurate positioning of each habitat feature on the map will allow distances or areas to be measured later (approximate distances should be noted for quick reference).
5. Make a note of the general substrate class and distribution, including locations of large boulders. If there is a definite change in the substrate, mark the transition of the change.
6. Map in-stream vegetation and cover with appropriate symbols (see legend provided on the *Aquatic Habitat Mapping Form* in **Appendix D**). The type, location and distribution of each cover type will allow calculation of distance or areas.
7. Include a cross-sectional diagram with the entire river valley and floodplain illustrated. Dimensions of the wetted channel are available from the Watercourse (Stream) Survey Form. An estimation of the entire flood plain and river valley may be required if it cannot be easily measured, for the cross-section diagram.
8. Note the conditions of the banks, particularly undercut banks, overhanging grasses or shrubs, eroding areas, and heavily stabilized areas.
9. Note any observations of fish or wildlife.
10. Mark all photo locations on the habitat map for reference.

6.0 MAINTENANCE

Maintenance should be conducted on all equipment required for fieldwork to keep equipment in good working order. The calibration of quantitative instruments should be conducted to ensure the accuracy and precision of data collected. If boats are required, regular maintenance of the boat and motor should be conducted to ensure that it is in working order and that it is cleaned between sampling locations (e.g.,

between lakes) not to spread possible invasive species between sampling locations. All measurement equipment should similarly be cleaned (see Section 5.1.2) and maintained between sampling sites.

The contractor should ensure that spare parts and batteries are at hand at all times and should have a contingency in place if the equipment requires repair. Safety and first aid equipment should be checked regularly and replaced when needed.

7.0 PROCEDURAL NOTE

The field contractor will be supplied with a watercourse (stream) reach table (**Appendix A**), and waterbody (lake/pond/wetland) reach table (**Appendix F**) with identified reaches for sampling (based on a stratified and randomized sampling design). The list of reaches to be sampled will include contingency sampling reaches in order of selection, in the case where chosen reaches cannot be sampled (e.g., due to access or safety).

8.0 REFERENCES

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MTO. 2009. Environmental Guide for Fish and Fish Habitat. Environmental Standards and Practices. Provincial and Environmental Planning Office, Ministry of Transportation.

RIC. 2001. Reconnaissance (1:20 000) Fish and Fish Habitat Inventory: Standards and Procedures. Prepared by BC Fisheries, Information Services Branch for the Resources Inventory Committee.

RISC. 2008. Reconnaissance (1:20,000) Fish and Fish Habitat Inventory: Site Card Field Guide. Prepared by Ministry of Environment Ecosystems Branch for the Resources Inventory Standards Committee.

9.0 APPENDICES

Appendix A: Watercourse (Stream) Survey Reach Table and Map

STREAM REACH TABLE AND MAP OF FIELD SURVEY LOCATIONS TO BE SUPPLIED ONCE DESK-BASED MAPPING WORK IS COMPLETED

Appendix B: Watercourse (Stream) Survey Form

(see next page)

Watercourse (Stream) Survey Form

GENERAL INFORMATION				
PROJECT #:	PROJECT DESCRIPTION:	SURVEY DATE Day: Month: Year:		
COLLECTORS:	WEATHER:	TIME STARTED:	TIME FINISHED:	
AIR TEMP: °C	WATER TEMP: °C	pH:	CONDUCTIVITY: µS/cm	TURBIDITY:
PHOTO NUMBERS & DESCRIPTIONS:				

LOCATION		
WATERCOURSE ID: - - - - - -	WATERCOURSE NAME:	
REACH #:	GRTS #:	REACH SUB-DIVIDERS: ◯ Yes ◯ No Sub-divider IDs:
GPS COORDINATES (upstream end of reach): Zone: Easting: Northing:		
SURROUNDING LAND USE:	SOURCES OF POLLUTION:	

REACH SECTION TYPE AND MORPHOLOGY			
REACH OR SUB-REACH #:	SITE #:	ACCESS:	Field Site UTM (downstream end of the site):
TYPE: Stream/River ◯ Channelized ◯ Permanent ◯ Intermittent ◯ Ephemeral ◯			ASSOCIATED WETLAND:
TOTAL SITE (SECTION) LENGTH (m):	GRADIENT %:	GRADIENT CODE:	STREAM DISCHARGE: L ◯ M ◯ H ◯

SUBSECTIONS:	Run ◯	Pool ◯	Riffle ◯	Flats ◯	Inside culvert ◯	Other ◯
Percentage of area	%	%	%	%	%	%
Mean depth wetted	m	m	m	m	m	m
Mean width wetted	m	m	m	m	m	m
Mean bankfull width	m	m	m	m	m	m
Mean bankfull depth	m	m	m	m	m	m

SUBSTRATE %:								
Bedrock %	Boulder %	Cobble %	Gravel %	Sand %	Silt %	Clay %	Muck %	Detritus %

Watercourse (Stream) Survey Form

BANK STABILITY:	Depositional	Protected	Vulnerable	Erosional
Left Upstream Bank:	◯	◯	◯	◯
Right Upstream Bank:	◯	◯	◯	◯

COVER & HABITAT

IN-STREAM COVER (% surface area):	Undercut banks %	Boulders %	Cobble %	Woody Debris Instream: % Overhanging: %	Vascular Macrophytes Instream: % Overhanging: %	Other %	None %

SHORE COVER (% stream shaded):	100 – 90% ◯	90 – 60% ◯	60 – 30% ◯	30 – 1% ◯

VEGETATION TYPE (%):	Submergent %	Floating %	Emergent %	None ◯
PREDOMINANT SPECIES:				

RIPARIAN VEGETATION TYPE:	Grass ◯	Shrub ◯	Coniferous Forest ◯	Deciduous Forest ◯	Mixed Forest ◯	Wetland ◯	None ◯

RIPARIAN STAGE:	Initial (< 5 % Total Cover) LUB ◯ RUB ◯	< 10 % Total Cover LUB ◯ RUB ◯	Pole Sapling LUB ◯ RUB ◯	Young Forest LUB ◯ RUB ◯	Mature Forest LUB ◯ RUB ◯

MIGRATORY OBSTRUCTIONS: None ◯ Seasonal ◯ Permanent ◯

OBSTRUCTION DETAILS:

POTENTIAL CRITICAL HABITAT LIMITING:	Spawning	Evidence of Groundwater	Other

POTENTIAL ENHANCEMENT OPPORTUNITIES:

COMMENTS:

Additional Notes Appended? ◯ No ◯ Yes Number of pages _____

Date entered into spreadsheet: Entered by: QA/QC by:

Appendix C: Watercourse Reference Guide: Stream Reaches

Information	Purpose/Description/Directions
General Information – some information can be obtained before entering the field	
Project Name	To identify the APM Project.
Project Description	A concise description of the project.
Survey Date	Date in Day/Month/Year format
Collectors	Identify all members of the field crew and identify the crew leader. Identify the person or people doing the fieldwork. Example: ABC Consultants: CG AM crew, AC leader
Weather Conditions	Record the general weather conditions at the time of surveying. This information should include any precipitation during the sampling, or if the sky is clear or overcast. Record any adverse weather conditions that may affect data (e.g., heavy rain, frost, freezing rain, snow).
Time Started/Finished	It is essential to record the time of day the sampling is done as well as the date. When the information is reviewed, it may be important to know if water temperatures were taken early in the morning or early afternoon (typically the warmest time of the day). Local or regional weather conditions could be reviewed and related to the water levels.
Air Temp (°C)	Record in °C
Water Temp (°C)	Record in °C from YSI or HydroLab
pH	Record water pH from YSI or HydroLab
Conductivity (µS/cm)	Make conductivity measurements from mid-stream and mid-depth if possible. Electrical conductivity (EC) is dependent on the total dissolved salt concentration (TDS) in the water; the higher the conductivity, the higher the salt (e.g., sodium, calcium, sulphate) concentration. Measure EC in the field using a portable conductivity meter (e.g., YSI, HydroLab). Most conductivity meters automatically convert conductivity measurements to 25°C. If your meter does not automatically standardize to 25°C, record the water temperature at the same time as conductivity and use a conductivity nomograph to convert the reading to 25°C.
Turbidity	Using a turbidity meter, record turbidity at the same sample locations for conductivity measurements.
Photo Numbers and Descriptions	Record photograph numbers (from the camera), direction (Upstream (US)/Downstream (DS)/Cross Stream (X)/ Streambed (Sb)/ Left Upstream bank (LUB)/ Right Upstream Bank (RUB)and a description of what each photo shows (e.g., Photo 004 – US Beaver Dam).
Location – some information can be obtained before entering the field	
Watercourse ID	Desk-based unique ID provided by Zoetica in the Reach Table (Appendix A) and used to describe the characteristics of each watercourse.
Watercourse Name	Many watercourses have had an official name gazetted, and this will be published in the Ontario edition of the "Gazetteer of Canada." If the name does not appear in the Gazetteer, then the name used on a published map should be used. If there is a commonly used local name, it should be included with the official name and flagged as a local name.
Reach Number	Derived by Zoetica using desk-based GIS mapping and provided in the Watercourse Reach Table (**Appendix A**)
GRTS Number	PanelOne (P1) points are pre-selected points in a Generalized Random Tessellation Stratified study design and can be found in the Watercourse Reach Table (**Appendix A**). If a P1 site is found to be inaccessible after reasonable effort, then the surveyor can visit/consider the next OverSample (OS) point as a replacement.

GPS Coordinates	Record the GPS coordinates (UTM) and GPS accuracy (e.g., +/-5 m) of the station. Try to get within 3m accuracy or less.
Surrounding Land Use	A concise description of the surrounding land uses to assist in determining potential impacts to fish or fish habitat, such as resorts, tree harvest areas, and agriculture.
Sources of Pollution	A concise description of any visible sources of: 1. Point or non-point source pollution including outfalls, bank seepage, oil slicks. 2. Nutrient loading evident by the presence of algal blooms or dense growth of aquatic macrophytes. 3. Sediment loading evident by the presence of mid-channel bars, extended point bars around bends; pools filled with fines; sand dunes in shallow areas.
REACH SECTION TYPE AND MORPHOLOGY	
Reach or Sub-reach #	Unique Identifier name for the reach. Note: if the Reach Number identified in the Location Block is sub-divided further in the field, record Sub-Reach Number here
Site (Section) #	If more than one site is surveyed on a given reach, the site number is used as an identifier and should be assigned in a sequential upstream order starting at the downstream end of the reach.
Access	Record how the site was accessed for the survey: helicopter (H), two-wheel-drive (V2), four-wheel-drive (V4), foot (FT), boat (B), other (O)
Field Site UTM	Record the UTM at the downstream end of the site to be sampled using NAD 83. Record UTM Coordinates (Zone/Easting/Northing) to the meter level (uncertainty).
Type	*Check the appropriate type (natural/altered and flow regime)*
Stream / River	'Natural' watercourse containing flowing water at least part of the year.
Channelized	Constructed or altered/straightened channel, drain, ditch, canal or aqueduct that is straight and uniform in structure.
AND check either	
Permanent	A stream that flows for 9 or more consecutive months per year under average annual precipitation conditions. It has a channel with a defined bed and banks of a permanent nature.
Intermittent	A stream that flows for less than 9 consecutive months per year under average annual precipitation conditions and usually is dry in May/June. It has a poorly defined channel.
Ephemeral	A stream that flows for short periods in the spring or in response to runoff events, and usually or insufficient duration to create a defined channel (e.g., field swale, or gully).
Associated Wetland	Name and describe any wetland that appears to be hydrologically connected to the stream reach. Write N/A if no wetland present. Wetland types include Wetland Marsh (Wm), Wetland Swamp (Ws), Wetland bog (Wb) and Wetland fen (Wf). Please see **Appendix I** for descriptions or the Ontario Wetland Evaluation System Manual for full details.
Site/Section Length (m)	Record length of the reach/site surveyed in m. Minimum length of 100 m.
Gradient % and Code	Measure at a minimum of two sites along the sample site over as long a distance as possible. Measure in upstream and downstream directions to maximize the distance. Measurements are sighted from similar habitat units (e.g., riffle crest to riffle crest). Record method used to assess gradient as follows: Ground Estimate (GE); Clinometer (C); Abney type level (AL); Survey equipment (S)
Stream Discharge	Record the amount of water passing through the channel at the time of the survey in relation to the bankfull depth as follows: Low (L) = 0 – 30% of bankfull; Moderate (M) = 31 – 90% of bankfull; High (H) = >90% bankfull
Subsections	*Check the appropriate types present and channel dimensions*

Riffle	Areas of relatively shallow fast, turbulent flow where the water's surface is typically broken. Riffles have a hydraulic head of 8 mm or greater and fast velocities ranging from 0.25 – 0.40 m/s.
Run	Areas typically found at the head of a pool with rapidly flowing water and a similar hydraulic head (≥8 mm) and velocity (0.25-0 – 40 m/s) as a riffle but greater depth. The water's surface is typically not agitated by bed material but may be turbulent.
Pool	Areas of a stream that are deep with a relatively low velocity and a smooth unagitated surface. Pools have a hydraulic head of 0 to 3 mm and a velocity less than 0.05 – 0.15 m/s.
Flats	Low flowing water with a smooth unagitated surface (not as deep as a pool). Flats have a hydraulic head of 4-7 mm and velocity between 0.15 – 0.30+ m/s.
Channel Dimensions	
Mean depth wetted (m)	Maximum within the specific morphological feature (e.g., pool). Provide average and range if several features (e.g., pools) within a section. For larger crossings, measure at least five locations spread equally across a transect. It should be the same transect used to measure the wetted width of the waterbody. Measurements can be made easily using a meter stick, ensuring it is not sinking into the substrate. The mean should be calculated using the formula: Mean depth = (D1 + D2 + ... Dn)/n Where D = depth measurement, and n = total number of measurements
Mean width wetted (m)	The distance from high water mark on one stream bank to the opposite stream bank. It is a transect taken perpendicular to the direction of flow. High water marks are either visibly stained on the stream bank or taken at the beginning of rooted vegetation on the stream bank/lakeshore or top of the bank. When measuring the wetted width of the stream, subtract the width of islands and include undercuts to the nearest tenth of a metre.
Mean bankfull width (m)	The width at the elevation point of incipient flooding, indicated by deposits of sand or silt at the active scour mark, break in stream bank slope, perennial vegetation limit, rock discoloration, and root hair exposure.
Mean bankfull depth (m)	The average depth measured at the elevation point of incipient flooding and should be measured at the same transect used to measure the bankfull width and calculated as per *mean wetted depth*.
Substrate (%)	An estimate of the percent cover of the substrate type can be made visually. The substrate should be classified according to the categories described below:
Bedrock	Exposed rock with no overburden.
Large Boulder	Particles with an intermediate width (median axis) of >1024 mm.
Small-Medium Boulder	Particles with an intermediate width (median axis) of 256-1024 mm.
Cobble	Particles with an intermediate width (median axis) between 64 and 256 mm.
Gravel	Particles with an intermediate width (median axis) between 2 to 64 mm.
Finer textures	Particles with an intermediate width (median axis) of less than 2 mm. For the fine-textured material, place about ½ to 1 tablespoon of sediment in the palm. Feel the mass by rubbing between fingers.
Sand	Loose and single grained. The individual grains can readily be seen or felt. If squeezed in the hand, it will form a cast but will crumble when touched.
Clay	The finest of the substrate types. It is quite plastic and usually sticky when wet. When the moist soil is pinched out between the thumb and fingers, it will form a long flexible ribbon.
Muck	A mixture of decomposing organic matter, silt and/or clay. It tends to be dark in colour and greasy to the touch.
Detritus	Organic material with large pieces of sticks and leaves accounting for at least 85% of the mass.

Bank Stability	Stable banks are characterized by the presence of boulders, rocks, or rooted vegetation that reduces the bank's susceptibility to erosion. Unstable banks are characterized by the presence of exposed raw dirt, lack of rooted vegetation, steep-sloped banks, undercuts, and often slumping banks. Determine the category of bank stability for the left upstream bank and right upstream bank separately.
Deposition Zone	Gentle, <45-degree slope. Generally, materials that have been deposited by the river during its flood condition.
Protected Bank	Steep, >45-degree non-erodible materials (e.g., rock, boulders or hardened clay). Vegetation may or may not be present and includes banks armoured by humans.
Vulnerable Bank	Steep, >45-degree erodible materials which show no recent signs of erosion (i.e., undercuts or slumping) and protected by a mat of live vegetation.
Eroding Bank	Steep, >45-degree erodible materials undercut (by at least 5 cm) or shows signs of recent slumping (i.e., no or little vegetation present).
Cover and Habitat	
In-Stream Cover (% Surface Area)	The in-stream cover provides an area for resting, shelter and predator avoidance for fish. Different types of cover are important in different habitat types, so it is essential to note in which part of the waterbody the features occur. A cover particle is any object that touches the water within the sample area, is **at least 100 mm wide** along the median axis and of sufficient density to block >75% of sunlight from reaching the stream bottom.
Undercut banks	A bank that has had its base eroded away and now overhangs the water. These often occur under tree root systems. The undercut area should be probed with a meter stick to determine its size and depth.
Boulders	In-stream rocks larger than 25 cm in diameter are considered suitable cover for many larger fish. They create back eddies for fish to rest out of the current and are also large enough to hide fish from predators.
Cobble	In-stream rocks 8 to 25 cm in diameter provide interstitial spaces (gaps between rocks) that can be used as cover by small or juvenile fish. These interstitial spaces are important overwintering and/or spawning areas for many fish.
Woody Debris	Living or dead woody materials (includes fallen trees, stumps, logs, mats of twigs, shrubs).
Vascular Macrophytes	Living aquatic and terrestrial non-woody plants in-stream or overhanging (< 1 m above the water surface) vegetation can provide shelter, protection and an attachment surface for food items.
None	
Shore Cover (% Stream Shaded)	A visual estimate of the percent of the watercourse that is shaded by overhanging trees or shrubs that are more than 1 m above the water surface.
Vegetation Type (%)	In-stream vegetation is an important component of fish habitat. It provides cover for fish to seek shelter from predators and provides shade from the sun. Food production is usually excellent in areas with a variety of vegetation. Visual estimation of the percent of the stream bed covered by each vegetation type should be made, and plant species (at least predominant species) identified.
Submergent	Group of plants that remains under the water surface at most times. Both vascular and non-vascular plants are included. Examples of these plants include pondweeds and milfoil. Algae often form an extensive mat covering the stream bed. However, the value in terms of fish cover is limited, so it should not be included in that category.
Floating	Vascular plants, usually with broad leaves floating on the water surface. Common in calm, still waters (e.g., water lilies).
Emergent	Vascular plants which root to the stream or lake bottom while their stems extend above the water surface (e.g., cattails, bulrushes, and sedges). Insects with aquatic

	life stages are often abundant in areas with emergent vegetation. These insects are an essential food source for many fish.
Riparian Vegetation Type	Check the dominant types of vegetation present at the stream section assessed (grass, shrub, coniferous forest, deciduous forest, mixed forest, wetland, none)
Riparian Stage	Check the dominant riparian stage (initial, shrub/herb, pole-sapling, young forest, mature forest) for each bank (Left upstream bank (LUB) and right upstream bank (RUB)).
Migratory Obstructions	Natural or constructed obstructions (e.g., dams, weirs, perched culverts) that block the movement of fish. Barriers may also result from insufficient water depth, for example, through a culvert with no low flow channel, or due to high slope and/or velocity. A description of the obstruction should be provided.
None	
Seasonal	The barrier is only present during lower water levels (i.e., is low enough that fish can move over it during higher flow periods/water levels; insufficient depth during low flow period may develop in a culvert with no low flow channel) or high flow periods (i.e., extreme velocities).
Permanent	The barrier is present under all flow conditions and during all times of the year.
Potential Critical Habitat	
Spawning	All evidence of spawning fish should be recorded in relation to the specific habitat. Potential spawning habitats should be identified in relation to the fish community. Known spawning areas (from background data) should be inspected.
Groundwater	The locations of all evidence of potential groundwater discharge such as springs, watercress, iron 'floc' or staining, and bank seepage should be recorded.
Other	Other types of habitat that may provide critical habitat functions should be identified, such as nursery habitat, seasonal refugia, deep holding or staging pools, should be recorded. Concentrations of fish may evidence these areas.
Potential Enhancement Opportunities	
	Opportunities to improve existing habitat conditions, such as removal of barriers to movement, rehabilitation of degraded conditions (e.g., bank failure, debris clean-up), diversification of homogeneous habitats or addition of features that may be limiting productivity (e.g., pools, gravels).
Comments	Additional comments. Include observations of wildlife, and any additional pertinent information.

Appendix D: Aquatic Habitat Mapping Form

(see next page)

Section Identifier:	Section location:	Section length (m);	Scale (cm / m)

Project #	
Mapper:	
Name of waterbody:	
Reach #:	
Site # (if more than one per reach):	
Survey Date: DD – MM – YY	

Legend

10d	Depth (cm)
6w	Width
➡	Riffle
⇨	Run/Glide
⬭	Pool
▓	Island/Bar
⁘	Fine Substrate
###	Gravel Substrate
oOooO	Cobble/Boulder
* * *	Debris
CT	Cattail
SV/FV	Submerg/Float Veg
EV	Emergent Vegetation
W	Watercress
Fe	Iron Staining
///////	Eroded Bank
XXX	Riprap / Other Stabilization
▭	Instream Log/Tree
^^^	Dam/Weir/Obstruction
®	Riparian Tree
⊢➤	Seep/Spring
- - - -	Undercut Bank
▬	Barrier to Fish Movement
-S-	Seasonal Barrier
-x--x-	Fence Line
⌴	Culvert

Profile:	Horz. Scale	Vert. Scale

Appendix E: Waterbody (Lake/Pond/Wetland) Checklist Form

Field Requirements Checklist

Field Lake Survey	**Lake Inlets and Outlets**
☐ Waterbody location and referencing ☐ Survey information ☐ Access ☐ Terrain characteristics ☐ Shoreline characteristics	☐ Identify tributaries, outlet locations ☐ Survey first reach of each tributary/ outlet (on Watercourse (Stream) Survey Form)
Bathymetry	**Photographic Documentation**
☐ Conduct a full bathymetric survey ☐ Benchmark ☐ Maximum depth (from bathymetry) ☐ High water level ☐ % littoral area (from bathymetry)	☐ Panoramic view of the surrounding area ☐ Shoreline and riparian conditions ☐ Inlet and outlet streams ☐ Aquatic Plant Communities ☐ Benchmark ☐ Any important/relevant features ☐ Record location/direction on outline map
Limnological Sampling	**Wildlife and Aquatic Flora**
☐ Establish limnological station ☐ Location on map and UTM ☐ Station Number ☐ Date and Time ☐ EMS number ***Record field measurements:*** ☐ Dissolved oxygen profile ☐ Temperature profile ☐ Secchi depth ☐ Water colour ☐ pH (surface and bottom) ☐ Conductivity (surface and bottom) ☐ Hydrogen sulphide detection	In the comments section record observations specific to: ☐ Rare and endangered species (non-fish) ☐ Aquatic species (non-fish) ☐ Comment on wildlife activities ☐ Identify and record dominant aquatic flora to species level

Appendix F: Waterbody (Lake/Pond/Wetland) Reach Table and Map

WATERBODY REACH TABLE AND MAP OF FIELD SURVEY LOCATIONS TO BE SUPPLIED ONCE DESK-BASED MAPPING WORK IS COMPLETED

Appendix G: Waterbody (Lake/Pond/Wetland) Survey Form

(see next page)

Waterbody (Lake/Pond/Wetland) Survey Form

GENERAL INFORMATION										
PROJECT #:	PROJECT DESCRIPTION:	SURVEY DATE Day: Month: Year:								
COLLECTORS:	TIME STARTED:	TIME FINISHED:								
WEATHER:	AIR TEMP: °C	SURFACE CONDITIONS: Calm ◯ Rippled ◯ Wavy ◯ Rough ◯								
PHOTO NUMBERS AND DESCRIPTIONS:										
LOCATION										
WATERBODY ID: - - - - - -	REACH #:									
GPS COORDINATES (upstream end of reach): Zone: Easting: Northing:	NAME OF WATERBODY:	GRTS #:								
ACCESS:	SURROUNDING LAND USE:	SOURCES OF POLLUTION:								
REACH TYPE AND MORPHOLOGY										
TYPE: Large Lake ◯ Small Lake ◯ Pond ◯ Wetland ◯ Reservoir ◯ Dug-out ◯										
SOURCE: Intermittent ◯ Runoff ◯ Spring Fed ◯ Not Connected ◯ By-pass ◯ In-stream ◯										
WATERBODY DIMENSIONS: Length: m Average Width: m										
MAIN INLET STREAM SURVEYED Yes ◯ No ◯	MAIN OUTLET STREAM SURVEYED Yes ◯ No ◯									
MAIN INLET REACH #	MAIN OUTLET REACH #									
LIMNOLOGY, BATHYMETRY, AND WATER CHEMISTRY										
LIMNOLOGY CONDUCTED? Full ◯ Partial ◯ No ◯	BATHYMETRY CONDUCTED? Full ◯ Partial ◯ No ◯	WATER CHEMISTRY CONDUCTED? Full ◯ Partial ◯ No ◯								
Max Depth: m										
COVER AND HABITAT										
BANK HABITAT										
IN-WATER COVER (% Surface area):	Undercut Banks %	Boulders %	Cobble %	Woody Debris In-water: % Overhanging: %	Vascular Macrophytes In-water: % Overhanging: %	Other %				
SHORELINE SUBSTRATE (add to 100%):	Bedrock %	Boulder %	Cobble %	Gravel %	Sand %	Silt %	Clay %	Muck %	Marl %	Detritus %
SHORELINE TYPE:	Sand/Gravel Beach %	Low, Rocky Shore %	Cliff or Bluff Shore %	Wetland Shore %	Vegetated Shore %	Other %				

Waterbody (Lake/Pond/Wetland) Survey Form

SHORELINE VEGETATION COVER (% of shoreline coverage):	Abundant (>20%) ◯	Moderate (5 - 20%) ◯	Sparse (< 5%) ◯	None ◯						
Predominant Species:										
IN-WATER HABITAT										
VEGETATION TYPE:	Submergent %	Floating %	Emergent %	None ◯						
PREDOMINANT SPECIES:										
UNDERWATER COVER (% surface area):	Undercut Banks %	Boulders %	Cobble %	Woody Debris %	Organic Debris %	Macrophytes %				
BOTTOM SUBSTRATE:	Bedrock %	Boulder %	Cobble %	Gravel %	Sand %	Silt %	Clay %	Muck %	Detritus %	Marl %
MIGRATORY OBSTRUCTIONS:	None ◯	Seasonal ◯	Permanent ◯							
OBSTRUCTION DETAILS:										
POTENTIAL ENHANCEMENT OPPORTUNITIES:										
COMMENTS:										
Additional Notes Appended? ◯ No ◯ Yes Number of pages _____										
Date entered into spreadsheet:	Entered by:	QA/QC by:								

Appendix H: Waterbody Reference Guide: Lakes, Ponds, and Wetlands

Information	Purpose/Description/Directions
General Information – some information is obtained before entering the field	
Project	To identify the APM Project.
Project Description	A concise description of the project.
Survey Date	Date in Day/Month/Year format
Collectors	Identify all members of the field crew and identify the crew leader. Identify the person or people doing the fieldwork, e.g., ABC Consultants: CG AM crew, AC leader
Time Started/Finished	It is essential to record the time of day the sampling is done as well as the date. When the information is reviewed, it may be important to know if water temperatures were taken early in the morning or early afternoon (typically the warmest time of the day). Local or regional weather conditions could be reviewed and related to the water levels.
Weather Conditions	Record the general weather conditions at the time of sampling. This information should include any precipitation during the sampling, or if the sky is clear or overcast. Record any adverse weather conditions that may affect data (e.g., heavy rain, frost, freezing rain, snow).
Air Temp (°C)	Record in °C
Surface Conditions:	Waterbody surface conditions
Calm	Nearly or completely motionless
Rippled	Small waves
Wavy	Large waves
Rough	
Photo Numbers and Descriptions	Record photograph numbers (from the camera), direction (North/East/South/West) and a description of what each photo shows (e.g., Photo 004 – N bank Beaver Dam).
Location – some information is filled in before entering the field	
Watercourse ID	Desk-based unique ID provided by Zoetica in the Reach Table (Appendix A) and used to describe the characteristics of each watercourse.
Waterbody Name	Many waterbodies have had an official name gazetted, and this will be published in the Ontario edition of the "Gazetteer of Canada." If the name does not appear in the Gazetteer, then the name used on a published map should be used. If there is a commonly used local name, it should be included with the official name and flagged as a local name.
Location of Station	A concise description of the geographic location of the station site should be made. Attempt to relate it to a feature easily identified on a map such as a pier, dock, and local roads.
Reach #	Derived by Zoetica using desk-based GIS mapping and provided in the Waterbody Reach Table (**Appendix F**)
GRTS #	PanelOne (P1) points are pre-selected points in a Generalized Random Tessellation Stratified study design and can be found in the Watercourse Reach Table (**Appendix F**). If a P1 site is found to be inaccessible after reasonable effort, then the surveyor can visit/consider the next OverSample (OS) point as a replacement.
GPS Coordinates	Record the GPS coordinates (UTM) and GPS accuracy (e.g., +/-5 m) of the station. Try to get within 3m accuracy or less.
Access	Record how the site was accessed for the survey: helicopter (H), two-wheel-drive (V2), four-wheel-drive (V4), foot (FT), boat (B), other (O).
Surrounding Land Use	A concise description of the surrounding land uses to assist in determining potential impacts to fish or fish habitat, such as resorts, tree harvest areas, and agriculture.
Sources of Pollution	A concise description of any visible sources of: 1. Point or non-point source pollution including outfalls, bank seepage, oil slicks. 2. Nutrient loading evident by the presence of algal blooms or dense growth of aquatic macrophytes.

	3. Sedimentation evident by the presence of turbid waters, sediment plumes, infilling, accumulation of fines on submergent macrophytes and along the shoreline.
Reach Type and Morphology	
Field Site UTM	Record the UTM at the approximate centre of the waterbody (where limnology measurements are to be taken) using NAD 83. Record UTM Coordinates (Zone/Easting/Northing) to the meter level (uncertainty).
Type	*Check the waterbody type.*
Large Lake	A natural body of deep standing freshwater larger than a small pond >5 ha.
Small Lake	A natural body of deep standing freshwater larger than a pond.
Pond	A shallow body of standing water. It may dry seasonally, typically smaller than 0.5 ha.
Reservoir	A human-made lake with a dam controlling discharge flows.
Dug-out	A excavated "pond" usually for water collection for agricultural purposes.
Source	*Check waterbody source type*
Intermittent	A waterbody that is seasonally dry.
Runoff	The waterbody is supplied primarily by surface runoff (e.g., a shallow pond).
Spring-fed	The waterbody is supplied primarily by groundwater. The ground surface is permeable (e.g., kettle lake).
Not Connected	The waterbody is not connected to a watercourse.
By-pass	Water by-pass.
In-stream	The waterbody is behind an impoundment in a watercourse.
Lake/Pond Dimensions:	
Length (m)	Estimated length.
Average Width (m)	Estimated width.
Inlet and Outlet Surveys	Check if the main inlet and outlets to the waterbody have been surveyed. Record the reach numbers for the main inlet and outlet reaches.
Limnology, Bathymetry, and Water Chemistry **NOTE: Information in this section to be recorded on the Water Chemistry LSA Form provided in the EMPB Design Report. Check Y/N for each Question on the Waterbody (Lake/Pond/Wetland) Survey Form (Appendix G)**	
Waterbody Dimensions	Record the estimated length, width and max depth in m (from Bathymetry profile).
Dissolved O2 Profile	Collect dissolved oxygen concentrations and temperature at 0.5 m intervals to bottom or a maximum of 10m. Take vertical dissolved oxygen (DO) and temperature profiles simultaneously, as your meter (e.g., YSI, HydroLab) will provide a temperature reading for every depth at which the DO is measured. Take two sets of readings at each depth interval: one during descent and the other during ascent, to minimize bias due to adjustment of the meter to the water conditions at the subsequent depths. Dissolved oxygen is a measure of the concentration of oxygen dissolved in water expressed in mg/L, or in parts per million (1 mg/L is equivalent to 1 ppm). Record information on the Limnology, Water, and Plankton Datasheet – LSA form provided in the EMBP Final Design Report (CanNorth 2020).
Max depth (m)	As measured during dissolved oxygen/temperature profile.
pH	pH is a measure of the hydrogen-ion concentration in water. It operates on a scale of 0 (highly acidic) to 14 (highly basic), with a pH of 7 being neutral. Measure pH in the field using a hand-held pH meter (a low ionic strength electrode and calibration standards may be required). These measurements are to be conducted along with dissolved O2 measures at each depth and recorded on the Limnology, Water, and Plankton Datasheet – LSA form provided in the EMBP Final Design Report (CanNorth 2020).
Conductivity (µS/cm)	Make conductivity measurements from the lake surface and bottom samples. Electrical conductivity (EC) is dependent on the total dissolved salt concentration (TDS) in the water; the higher the conductivity, the higher the salt (e.g., sodium, calcium, sulphate) concentration. Measure EC in the field using a portable conductivity meter (e.g., YSI,

	HydroLab). Most conductivity meters automatically convert conductivity measurements to 25°C. If your meter does not automatically standardize to 25°C, record the water temperature at the same time as conductivity and use a conductivity nomograph to convert the reading to 25°C. These measurements are to be conducted along with dissolved O2 measures at each depth and recorded on the Limnology, Water, and Plankton Datasheet – LSA form provided in the EMBP Final Design Report (CanNorth 2020).
Water Temperature	Record water temperature in °C from the depth where conductivity measurements are made. These measurements are to be conducted along with dissolved O2 measures at each depth and recorded on the Limnology, Water, and Plankton Datasheet – LSA form provided in the EMBP Final Design Report (CanNorth 2020).
Secchi Depth (m)	Determining the Secchi Depth 1. Lower the Secchi disk until it disappears. 2. Note the depth to the nearest tenth of a metre. 3. Raise the Secchi until it reappears. 4. Note the depth. 5. The Secchi depth is the midpoint between these 2 depths. NOTE: Take the reading on the shady side of the boat. Do not wear sunglasses. Take the reading as close to mid-day as possible (10 am – 2 pm). Record depths in tenths of meters. Also, conduct Secchi depth determinations after the dissolved oxygen/temperature profiles have been conducted so that the water column is not disturbed. These measurements are to be conducted along with dissolved O2 measures at each depth and recorded on the Limnology, Water, and Plankton Datasheet – LSA form provided in the EMBP Final Design Report (CanNorth 2020).
Cover and Habitat	
Bank Habitat	
In-Water Cover (% Surface Area)	In-water cover provides an area for resting, shelter and predator avoidance for fish. Different types of cover are important in different habitat types, so it is essential to note in which part of the waterbody the features occur. A cover particle is any object that touches the water within the sample area, is **at least 100 mm wide** along the median axis and of sufficient density to block >75 % of sunlight from reaching the stream bottom. A cover particle can consist of a mat of materials such as twigs, macrophytes, or the bank. The mat must still meet the median diameter size and light penetration restrictions.
Undercut banks	A bank that has had its base eroded away and now overhangs the water. These often occur under tree root systems. The undercut area should be probed with a meter stick to determine its size and depth.
Boulders	In-water rocks larger than 25 cm in diameter are considered suitable cover for many larger fish. They create back eddies for fish to rest out of the current and are also large enough to hide fish from predators.
Cobble	In-water rocks 8 to 25 cm in diameter provide interstitial spaces (gaps between rocks) that can be used as cover by small or juvenile fish. These interstitial spaces are important overwintering and/or spawning areas for many fish.
Woody Debris	Living or dead woody materials (includes fallen trees, stumps, logs, mats of twigs, shrubs).
Organic Debris	Branches, leaves and other material will often collect in piles in streams and lakes, creating areas of good cover.
Vascular Macrophytes	Living aquatic and terrestrial non-woody plants in-water or overhanging vegetation (<1 m above the water surface) can provide shelter, protection and an attachment surface for food items.
None	

Nearshore Slope (%)	A reasonable measurement of the slope can be obtained using a handheld clinometer. Tie a ribbon or mark up the shore at eye level. While standing on the shoreline, use the clinometer to determine the angle of slope. Record gradient in %.
Shoreline Substrate (%)	An estimate of the percent cover of the substrate type can be made visually (total 100%). The substrate should be classified according to the categories described below:
Bedrock	Exposed rock with no overburden.
Large Boulder	Particles with an intermediate width (median axis) of >1024 mm.
Small-Medium Boulder	Particles with an intermediate width (median axis) of 256-1024 mm.
Cobble	Particles with an intermediate width (median axis) between 64 and 256 mm.
Gravel	Particles with an intermediate width (median axis) between 2 to 64 mm.
Finer Textures	Particles with an intermediate width (median axis) of less than 2 mm. For the finer textured material, place about ½ to 1 tablespoon of sediment in the palm. Feel the mass by rubbing between fingers.
Sand	Loose and single grained. The individual grains can readily be seen or felt. If squeezed in the hand, it will form a cast but will crumble when touched.
Fines	Sand, silt and clay < 2 mm.
Clay	The finest of the substrate types. It is quite plastic and usually sticky when wet. When the moist soil is pinched out between the thumb and fingers, it will form a long flexible ribbon.
Muck	A mixture of decomposing organic matter, silt and/or clay. It tends to be dark in colour and greasy to the touch.
Marl	White to gray accumulation on lake bottoms caused by precipitation of calcium carbonate ($CaCO_3$) in hard water lakes. Marl may contain many snail and clamshells, which are also calcium carbonate. Marl also precipitates phosphorus, resulting in low algae populations and good water clarity.
Detritus	Organic material with large pieces of sticks and leaves accounting for at least 85% of the mass.
Shoreline Type	The type of immediate shoreline as defined by the 5 categories below. Includes all area affected by the lake margin from the low water mark to the average annual high water mark, and the riparian zone around the lake
Sand or gravel beach	Often associated with low rocky shoreline areas or adjacent to inlets
Low, rocky shore	Cobble, boulder or bedrock substrate, prevalent along the base of steeper shorelines
Cliff or bluff shore	Areas adjacent to steeper slopes. Usually indicates a steep-sided lake basin or sudden drop-off.
Wetland Shore	Characteristic of lakes in lowland areas. Often associated with abundant emergent vegetation such as sedges, reeds, cattails.
Vegetated shore	Characteristic of lakes in lowland areas. Vegetation is commonly shrubs and small trees.
Shore Cover (% Shaded)	Shoreline cover refers to the debris and overhanging vegetation present at the shoreline and one metre above the water**.**
In-Water Habitat	
Vegetation Type	Vegetation type refers to the vegetation emerging and present at the shoreline and in the water. Estimate and record to the nearest 10% of the lakeshore perimeter and include the most predominant species. Record the dominant aquatic plant species, to the genus level, or the species level if possible.
Submergent	Group of plants that remains under the water surface at most times. Both vascular and non-vascular plants are included. Examples of these plants include pondweeds and milfoil. Algae often form an extensive mat covering the stream bed and should be included in this group. However, the value in terms of fish cover is limited, so it should not be included as fish cover.
Floating	Vascular plants, usually with broad leaves floating on the water surface. Common in calm, still waters (e.g., water lilies).

Emergent	Vascular plants which root to the stream or lake bottom while their stems extend above the water surface (e.g., cattails, bulrushes, and sedges). Insects with aquatic life stages are often abundant in areas with emergent vegetation. These insects are an essential food source for many fish. An estimate of the amount of water interspersion and water depths in the emergent vegetation will be important in determining the value of this vegetation type as fish cover.
None	
Underwater Cover (% Surface Area)	Underwater cover provides an area for resting and predator avoidance for fish. Different types of cover are important in different habitat types, so it is essential to note in which part of the waterbody the features occur. Most types of cover should fit into the categories provided. Any additional features should be identified. The percentage should total 100% (of the surface area).
Undercut banks	A bank that has had its base eroded away and now overhangs the water. These often occur under tree root systems. The undercut area should be probed with a meter stick to determine its size and depth.
Boulders	In-water rocks larger than 25 cm in diameter are considered suitable cover for many larger fish. They create back eddies for fish to rest out of the current and are also large enough to hide fish from predators.
Cobble	In-water rocks 8 to 25 cm in diameter provide interstitial spaces (gaps between rocks) that can be used as cover by small or juvenile fish. These interstitial spaces are important overwintering and/or spawning areas for many fish.
Woody Debris	Living or dead woody materials (includes fallen trees, stumps, logs, mats of twigs, shrubs).
Organic Debris	Branches, leaves and other material will often collect in piles in streams and lakes, creating areas of good cover.
Vascular Macrophytes	Living aquatic and terrestrial non-woody plants in-water or overhanging vegetation (<1 m above the water surface) can provide shelter, protection and an attachment surface for food items.
None	
Bottom Substrate	An estimate of the percent cover of the substrate type can be made visually (if possible). The substrate should be classified according to the categories described above (see Shoreline Substrate).
Migratory Obstructions	
None	
Seasonal	Include type in the details section of the form
Permanent	Include type in the details section of the form
Potential Enhancement Opportunities	
	Opportunities to improve existing habitat conditions, such as rehabilitation of degraded conditions (e.g., shore/bank failure, debris clean-up), diversification of homogeneous habitats or addition of features that may be limiting productivity.
Comments	
	Include observations of wildlife, and any additional pertinent information

Appendix I: Wetland Type Reference Guide

According to the Ontario Wetland Evaluation System Manual (from MNRF, 2014), wetlands are classified into the following four classes/types:

- ***Marsh (Wb)***: Marshes are wet areas periodically inundated with standing or slowly moving water, and/or permanently inundated areas characterized by robust emergent, and to a lesser extent, anchored floating plants and submergents. Marsh wetlands have mineral and sometimes well-decomposed peat soils. When peat soils are present, they are often enriched with mineral materials. Waters are nutrient-rich with near neutral to basic pH. Surface water levels typically fluctuate seasonally with declining levels exposing matted vegetation or mudflats. Emergent vegetation includes grasses, cattails, sedges, rushes, and reeds, which cover more than 25% of the wetland surface.

- ***Swamp (Ws)***: Swamp wetlands are wooded wetlands with 25% cover or more trees or tall shrubs. In swamps standing or gently flowing water occurs seasonally or may persist for long periods and have an abundance of pools and channels. Many are typically flooded in spring and dry with relict pools later in the season. Swamps include forest swamps (having mature trees) and thicket swamps (having shrub-carrs characterized by thick growths of tall shrubs such as willow, red-osier dogwood, buttonbush and speckled alder.). There is pronounced internal water movement from adjacent mineral areas, making the water nutrient-rich. If peat is present, it is mainly well-decomposed wood and occasional sedges. The vegetation is typically dominated by coniferous or deciduous trees or dense shrubs and herbaceous species.

- ***Fen (Wf)***: Fens are peatlands characterized by surface layers of poorly to moderately decomposed peat, often with well-decomposed peat near the base and less than 25% live tree cover. Fen peats usually consist of mosses and sedges. Two main types of fens exist nutrient-rich fens, fed by groundwater and have a high pH and can be dominated by sedges and grasses; and nutrient-poor fens, with less groundwater and lower pH and may consist of low shrubs or ericaceous species. Fens have a higher diversity of plants compared to bogs. Fen wetlands have organic soils and a water table at or above the surface. Soils are primarily moderate to well-decomposed sedge and non-sphagnum moss peats. Waters are mainly nutrient-rich with a near neutral to slightly acidic pH. The vegetation consists primarily of sedges, grasses, reeds, mosses, and some shrubs. Scattered trees may be present.

- ***Bog (Wb)***: Bogs are peat covered areas or peat-filled depressions with a high water table and a surface carpet of mosses, primarily Sphagnum. Raised peat hummocks are present in bogs, and the wetland is ombrotrophic (i.e. dependent on atmospheric moisture for its nutrients). Bogs have low plant diversity with less than 14 species and few to no fen indicator plant species and few or no tamaracks or eastern white cedar. Bog wetlands have organic soils with a water table at or near the surface. Soils are predominantly composed of poorly to moderately decomposed sphagnum moss peats. The bog surface is usually unaffected by groundwaters, and thus waters are generally acidic and low in nutrients. Bogs are usually covered with sphagnum mosses and ericaceous (belonging to or similar to heath family, genus Erica) shrubs, and may be treed or treeless but with less than 25% tree cover.

eDNA Field SOP

THE NWMO BIODIVERSITY IMPACT STUDIES: STANDARD OPERATING PROCEDURE FOR AQUATIC ENVIRONMENTAL DNA FIELD SAMPLING

August 14, 2020

PREPARED BY Zoetica Environmental Consulting Services

SUBMITTED TO Melissa Mayhew
Nuclear Waste Management Organization
22 St. Clair Avenue East
Sixth Floor, Toronto, ON
M4T 2S3, Canada

ZOETICA
ENVIRONMENTAL CONSULTING SERVICES

OFFICE 102-22351 St Anne Ave, Maple Ridge, BC, V2X 2E7
PHONE 604 467 1111
WEBSITE www.zoeticaenvironmental.com

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Rev. Number	Issue Date	Description	Prepared By	Checked By	Approved By
A000	10-Jul-2020	First submission to NWMO	C. Chui	R. Hanner	H. Bears
B000	14-Aug-2020	BIS Year 1 final submission to NWMO	C. Chui	D. MacKinnon	H. Bears

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GLOSSARY OF TERMS

APM	Adaptive Phased Management
eDNA	Environmental DNA
NWMO	Nuclear Waste Management Organization
PQP	Project Quality Plan
QA/QC	Quality Assurance / Quality Control
SOP	Standard Operating Procedure
Contamination	Unwanted DNA in the sample. Contamination can occur by improper cleaning of equipment that touches the sample (i.e., filter housing, tweezers) or the habitat from which a sample is to be collected (e.g., boots), careless gear or sample handling/storage (e.g., reusing gloves between sites, damp filter membranes stored together), or various laboratory processes not described in this SOP.
Duplicate	A second ***sample*** collected at the same ***location***. The collection of duplicates acts as a QA/QC check for reproducibility (i.e., how reliable the results are) and also increases the probability of eDNA detection by increasing the volume of water sampled. Also known as a 'biological replicate.'
Field negative	Sample collection at a location where target species is/are known to be absent. For the APM Project, there will be no true field negatives as we cannot be sure that a species is not present at the sampling site. Instead, field negative samples will consist of "collection" of distilled water to ensure that gear has been properly decontaminated.
Field positive	Sample collection at a location where the target species is/are known to be present. If positive controls show up negative in the results, then there are likely error(s) in the field and/or laboratory methods. Positive controls also help increase confidence in the use of eDNA methods for detecting species and biodiversity monitoring, compared to traditional surveys.
GRTS	Generalized Random Tessellation Stratified. Conducted using the 'spsurvey' package in R, GRTS is a stratified random approach to study design and site selection that is also spatially balanced. The resulting PanelOne (P1) sites should be preferentially sampled in the order specified. OverSample (OS) sites are plotted in case replacement sites are needed due to logistical difficulties in the field.
Habitat grouping	The type of watercourse, waterbody, or wetland to be sampled. There are seven habitat groupings proposed for initial eDNA studies: - Watercourses: 1) rivers/streams in wetlands, 2) rivers/streams not in wetlands - Waterbodies: 3) lakes/ponds <= 1 ha, 4) lakes/ponds > 1 ha - Wetlands: 5) marshes, 6) swamps, and 7) peatlands (bogs and fens) Habitat groupings were determined through desk-based analysis of Ontario ecosite data; however, these classifications should be verified by the field contractor on the ground.
Lentic / Lotic habitat	A lentic ecosystem or habitat refers to standing or relatively still water, such as lakes, ponds, and wetlands. A lotic ecosystem or habitat refers to flowing water, such as rivers and streams.

Microhabitat	A consideration for selecting appropriate ***sampling locations*** on the ground. Microhabitats that are generally more suitable for wildlife and fish species include areas with emergent or submergent vegetation, large woody debris or rocks for cover, undercut banks, shade, gravel or cobble substrates for spawning fish, muddy substrates for turtles, etc.
Replicate	Typically, one of three ***samples*** to be collected at each ***site***. Replicates are collected at different ***sampling locations*** at a site by moving along the shoreline. The collection of replicates is meant to cover more area, which may increase the probability of species detection by sampling different microhabitats within the site.
Sample	Collection and filtration of water (ideally, 2 L) at a single ***sampling location***. One sample = one filter membrane that is labelled, stored, and analyzed separately.
(Sampling) Location	The exact location where sampling takes place, to be determined by the field contractor on the ground (geographic coordinates to be recorded on the data form = sampling location). ***Sampling site*** and sampling location may differ depending on access. GRTS site selection does not consider practicality and may select a point in the middle of a lake; however, for Year 1 eDNA studies, sampling will be conducted along the shore.
(Sampling) Site	The specific watercourse, waterbody, or wetland (i.e., ***habitat grouping***) selected for sampling through a GRTS study design. Sites are indicated as PanelOne (P1) or OverSample (OS) on the field map(s) provided by Zoetica. A site corresponds to the watercourse segment, waterbody polygon, or wetland polygon, as determined through GIS analysis.
Stream reach	A section of a stream or river that is relatively homogenous, with similar hydrologic conditions such as discharge, depth, area, and slope. The ***sampling site*** for watercourses should consist of a single stream reach at least 200 m long.
Study areas (AOI, LSA, RSA)	There are three types of study areas defined for the Biodiversity Impact Studies: Area of Interest (AOI), Local Study Area (LSA), and Regional Study Area (RSA). Year 1 of eDNA studies will focus on ***sampling sites*** within the AOI and the LSA; however, ***field positives*** or other biodiversity study design control sites may be selected within the RSA.
Wetland	Several wetland classification systems can be applied in Ontario, including the *Canadian Wetland Classification System*, Ontario's Ecological Land Classification (ELC) system, and the Ontario Wetland Evaluation System (OWES). For the APM Project, we will identify four wetland types (marsh, swamp, bog, fen) according to the OWES, where shallow open water wetlands are grouped with marshes.

1.0 PURPOSE AND PRINCIPLE

The objective of this SOP is to instruct the field contractor in conducting eDNA studies for the APM Project's Biodiversity Impact Studies and Environmental Media Baseline Program according to the study design developed by Zoetica and to the standards expected by the NWMO. This SOP focuses on field sampling procedures based on available best practices and guidelines for eDNA sampling. This SOP is intended to be aligned with the standard protocols of Dr. Robert Hanner's laboratory at the University of Guelph for eDNA sample collection, filtration, storage, and transport. The Hanner laboratory will also conduct eDNA laboratory analyses according to their internal SOPs and QA/QC checks. (As of July 2020, these university SOPs have not been permitted for distribution.)

2.0 GENERAL PRECAUTIONS

Suggestions and precautions noted herein should not be interpreted as prescriptive or exhaustive. The field contractor is responsible for their own field safety protocols, which includes the planning of field safety gear.

There are very few hazards inherent to eDNA sampling, except muscle strain from carrying the OSMOS eDNA sampling kit. Since work will be conducted in watercourses, waterbodies, and wetlands, there is a very slight possibility of drowning. Additional caution should be applied around water, and the field contractor should plan for safety when working around water as they see fit.

Field hazard identification and controls, safety equipment, and site emergency protocols should be outlined on the field contractor's safety forms. In general, Zoetica predicts that slips, trips, and falls are likely the biggest safety risk due to uneven terrain, followed by eye-poking by sharp objects/branches in the field. However, the field contractor will be responsible for conducting their own risk assessment associated with the perceived risks of this work. Zoetica recommends that the field contractor, within their field safety plan, consider appropriate footwear, safety glasses, sunscreen, and appropriate clothing (e.g., bug shirt) to protect against weather conditions and biting insects. Zoetica also recommends that redundant, functional navigational gear be carried, and that protocols for helicopter safety and communications be followed.

3.0 EQUIPMENT AND MATERIALS

3.1 Sample Collection, Filtration, and Storage

Halltech OSMOS eDNA sampling kit, including:

- Battery-powered backpack
- 4-12' telescopic pole with tripod and pivot clamp
- 2 x 29.4 V Lithium-Ion batteries (keep extra battery as a spare)
- Smart battery charger
- Rugged field case with wheels

OSMOS reusable filter housings*

Cellulose nitrate filters** (0.45-5 µm)

Water quality instrument

Densiometer (to measure canopy cover)

Disposable nitrile gloves

Plastic tweezers

- Small paper coin envelopes***
- Ziploc bags (S, M, L)
- Silica gel desiccant packs
- Sharpie markers and pencils
- Waterproof data forms in clipboard
- Distilled water
- Cooler and ice packs
- Refrigerator (optional)

* Reusable filter housings must be decontaminated with a 50% bleach solution between uses (see Section 6.1.2). It may be feasible to carry enough filter housings such that decontamination can be done at the end of the day at camp. E.g., if 8 sites can be visited per day, each team would need to bring at least 30 filter housings (24 replicates + 4 duplicates + 2 field negatives). Alternatively, if proper decontamination can be done in the field, each team can carry fewer filter housings.

** eDNA has been successfully captured using a variety of filter materials. Cellulose nitrate filters are considered ideal; however, if there are delays or difficulties in procuring these filters, glass fibre is the next preferred filter type of the Hanner laboratory. Glass fibre filters also have the benefit of being more cost-effective.

*** In humid environments, paper envelopes may not be ideal for storing dried filters (see Section 6.4.2). To protect samples from rain and humidity, and to prevent cross-contamination between samples and controls, an alternative approach is to store each filter separately in its own small plastic Ziploc bag with silica gel desiccant packs.

3.2 Decontamination

- Household bleach (5-6% sodium hypochlorite)
- Liquid dish soap
- Tap water
- Distilled or deionized water
- Tote bins (3), ideally with leak-proof or leak-resistant lids
- Pressure sprayer (with 1-2 gal tank)
- Small plastic (HDPE) containers (e.g., Nalgene bottles)
- Spray bottle
- Scrub brush
- Rubber gloves
- Safety glasses/goggles
- Paper towels

3.3 Standard Field Gear

- High rubber boots and hiking boots
- Binoculars, waterproof notebook, camera
- Navigational equipment: GPS, compass, hard copy field maps and written coordinates, tablet or smartphone with georeferenced digital maps
- General safety equipment: hi-vis clothing, safety glasses, flagging tape, long-range radio, first aid/survival kit, bear spray, field knife and/or hatchet
- Optional or specialized safety equipment (i.e., not everyone will carry these): SPOT beacon, satellite phone, bear rifle

4.0 QUALITY CONTROL

Quality control measures for eDNA studies include:

1. Strict decontamination procedures to avoid contamination (see Section 6.0)
2. Collection of duplicate, field negative, and field positive samples (see Section 6.4.3)
3. Data entry verification and backup management (see Section 6.6)
4. Calibration of quantitative instruments (see Section 8.0)
5. Protocols developed by the field contractor as per their PQP (not described in this SOP)
6. Laboratory QA/QC protocols; optimized and validated methods for DNA extraction, PCR amplification, library preparation, MiSeq sequencing, and bioinformatics analyses for detecting the biodiversity of the Revell Batholith Area (not described in this SOP)

5.0 GENERAL GUIDELINES FOR SAMPLING

1. **Timing** – appropriate biological windows when eDNA concentration should be higher, e.g., the breeding season of target species. For this project, Zoetica recommends seasonal sampling:
    - Spring (early-mid April)
    - Summer (mid-late June)
    - Fall (mid-late September)
    - Winter (December to February) – *will only be conducted if species of interest are present in the fall, if the habitat could be used as an overwintering site, and if that potential overwintering site could potentially be affected by the APM Project.*

2. **Conditions** – it is not recommended to sample during or immediately after heavy rain, or during high-flow events.

3. **Locations** – suitable microhabitats where the target species is most likely to occur:
    - **Lentic systems** (waterbodies and wetlands) – can be chosen for ease of access and should represent a variety of microhabitats and as much of the spatial extent of the shoreline as possible.
    - **Lotic systems** (watercourses) – headwater streams and tributaries (rather than the main stem); stream margins, and thalweg are recommended. Always collect samples sequentially from downstream to upstream.

4. **Sampling effort** will depend on budget and time. The goal is to collect 3 replicate samples (and sometimes 1 extra duplicate sample; see Section 6.4.3) x 2 L water per sample at each site, wherever possible. (Note that each 2 L is considered a separate sample for filtering and lab analysis.) If these volumes cannot be attained (e.g., high algae/sediment levels clog the filter quickly), try to keep total sample volumes consistent between sites to allow the calculation of detection probabilities. Installing filters with a larger pore size may also help increase the volume of water that can be collected.

5. Sampling recommendations when using the **OSMOS eDNA sampler** or other backpack sampling and filtration systems:

- **Lentic systems** (waterbodies and wetlands) – mobile sampling is preferred to increase the area surveyed, wherein the surveyor will walk along the shoreline while running water through the system. Replicates can be collected by continuing mobile sampling along the shoreline (i.e., rather than doubling back to sample along the same reach).
- **Lotic systems** (watercourses) – stationary point sampling can be used, wherein the surveyor will stand in one spot while running water through the system. Replicates should be collected downstream to upstream and spaced 100 m apart (Bedwell and Goldberg 2020) within a single stream reach.

6. **Avoid standing in the water** while collecting samples to avoid contamination from your boots. For streams, do not enter the water upstream of the collection site.

   Notes: We prefer not to enter the water at all. The OSMOS eDNA sampler has an extendable pole that should help reduce the need to enter the water. However, northwestern Ontario is largely made up of wetlands, and avoidance may be difficult. Any gear that has entered the water (including boots) should be sprayed with bleach solution and rinsed between sites (see Section 6.1.1) to avoid the small chance of contamination of one site with DNA from another.

7. **Avoid** collecting samples in areas with **visibly disturbed sediment** in the water column, including sediment stirred up by standing in the water. Sediments will clog up your filter membrane. If the water feature has high natural turbidity, installing filters with a larger pore size (up to 5 µm) can help increase the flow rate – *record filter pore size on the data form for each sample collected*.

8. **Sampling from boats** is not recommended in lentic habitats unless the boat surface can be decontaminated with 1 in 10 bleach solution between sites. Depending on a species' habitat preference, boat sampling may increase detection. Lotic sampling is acceptable if collection occurs upstream from the boat (also decontaminated, ideally).

9. **Depth of sampling** – for Year 1 of this project, sample collection will occur along the shoreline at or just under the water surface to avoid stirring up sediment. The seasonal timing of surveys is planned to coincide with the periods when fish and amphibian species are closer to shore. However, sampling at depth is also possible, and the use of a boat to reach deeper areas of a watercourse or waterbody may improve the detection of deep-water species.

6.0 PROCEDURE

6.1 Decontamination Procedures

6.1.1 Decontamination Between Sites

1. Decontamination is not needed between replicates at the same site but must be performed before moving to the next site (i.e., different watercourse, waterbody, or wetland).

2. Bleach will break down DNA and will avoid the spread of chytrid fungus, ranaviruses, and other amphibian diseases when following the *Decontamination Protocol for Field Work with Amphibians and Reptiles in Canada* (CHHWG 2017).

3. Set up a decontamination station by placing 3 tote bins on top of a tarp in a sunny location ***away from a watercourse or waterbody***. Direct sunlight will help break down residual bleach. (Note: it is assumed that there will be no impermeable surfaces within the study area on which to dispose of rinse water.)

4. Fill one tote bin with soapy water (add liquid dish soap to tap water). Scrub the soles and sides of your rubber/hiking boots with a scrub brush to remove visible organic matter, dirt, pebbles, etc. Rinse with clean tap water into the 3rd tote bin.

5. Prepare a fresh 1 in 10 dilution of household bleach, i.e., 1 part bleach, 9 parts water. Fill a 2nd tote bin and a spray bottle with this bleach solution. *Wear rubber gloves and safety glasses/goggles – bleach is corrosive!*

6. Immerse all equipment and gear that may be contaminated (including boots, extendable pole, scrub brush, etc.) in the bleach solution. Spray larger items to soak thoroughly. Let sit for at least 1 min, then using the pressure sprayer, triple rinse/spray with clean water into the 3rd tote bin.

   Notes: Even trace amounts of residual bleach can adversely affect amphibians on contact. If it is not feasible to carry large quantities of tap water, there are two options: 1) use water from the ***next*** site for rinsing, or 2) consider bringing multiple pairs of boots and change between sites. Decontaminate all boots back at the camp.

7. Shelf life of decontamination solutions and disposal:
   a. Bleach solution in the tote bin and spray bottle can be reused for up to 7 days.
   b. Tote bin with soapy water can be reused until it becomes too dirty with visible particulate matter.
   c. Tote bin with rinse water should be disposed of daily.
   d. To dispose of old solutions, carry the bins back to camp and flush down the drain with plenty of water.

6.1.2 Daily Decontamination of eDNA Equipment

1. Any equipment or materials that directly touch eDNA samples or filters should be decontaminated with a 50% bleach solution (Goldberg and Strickler 2017, Hobbs et al. 2017).

2. Disposable nitrile gloves are single use only. Dispose of nitrile gloves after handling each sample. If contamination is suspected or if you accidentally touch something before handling the sample, change gloves before proceeding.

3. By using the OSMOS backpack sampler and filtration system, only reusable filter housings and plastic tweezers should require this stronger bleach decontamination procedure (which can be performed at camp at the end of each field day).

4. To prevent contamination and for ease of cleaning, reusable filter housings should be stored in Ziploc bags, and used tweezers will be kept in a small, labelled Nalgene (HDPE) bottle (see Section 6.4.2).

5. Prepare a fresh 1 in 2 dilution of household bleach, i.e., 1 part bleach, 1 part water. Fill a small HDPE container with this bleach solution (HDPE is more resistant to bleach than other plastics). *Wear rubber gloves and safety glasses/goggles – bleach is corrosive!*

6. Dismantle the reusable filter housings so that all parts can be properly decontaminated. Let tweezers and filter housings soak in the bleach solution for at least 1 min, then rinse thoroughly with distilled or deionized water.

7. Set tweezers onto clean paper towels to dry completely. Reusable filter housings can be dried if time allows; however, drying is not necessary before reloading with a clean filter membrane.

8. Be very cautious in handling the cleaned equipment to avoid possible contamination. Clean plastic tweezers should be placed in a clearly labelled, small Ziploc bag or small Nalgene bottle (with handles facing out). Do not touch the pincer tips.

9. Reassemble reusable filter housings, taking care not to touch the inside that will hold the filter membrane. Place clean filter housings into a clean Ziploc bag.

10. The bleach solution can be stored for up to 7 days. Old bleach solution can be flushed down the sink with plenty of water.

Notes: Decontamination of OSMOS reusable filter housings may need to be completed in the field if not enough units are available for a full day of field work by two teams. In this case, ensure that the bleach solution and rinse water do not come into contact with the environment. Even trace amounts of residual bleach can adversely affect amphibians on contact.

6.2 Daily Field Planning and Reporting

1. Zoetica has developed a GRTS design for eDNA sampling. Certain watercourses, waterbodies, and wetlands have been pre-selected as PanelOne (P1) sites for sampling, shown on the field map a as orange points (see **Appendix A – eDNA Field Map**). OverSample (OS) sites are also shown on the map as white points in case it is impossible to access a P1 site. However, reasonable efforts should be made to reach the P1 site.

2. Before each field day, the field contractor lead should prepare a daily route plan for each survey team, considering the potential need for helicopter “hopscotching” to pick up/drop off each team as well as refueling location(s) and timing.

3. It is expected that each survey team can sample 6-10 sites per day. Plan for more in case sampling proceeds more efficiently than expected. Conversely, if fewer sites can be sampled than expected,

ensure that the remaining sites are on the next day's planned routes. In the field, maintain communication with the pilot and the other survey team to coordinate site visits, if needed.

4. Travel to the sampling sites will occur via helicopter or ATV, if possible. Given the prevalence of wetlands in the area, however, it is likely that the site will need to be reached on foot. Record the waypoint of the helicopter drop-off point or ATV "parking spot" on the GPS and mark with flagging tape.

5. To balance the time available with the number of samples that need to be collected, Zoetica recommends that the survey team walk no farther than 1 km from the landing spot or vehicle restriction point. If the distance to the sample site is greater than 1 km, consider sampling at the nearest OS site as a replacement if this location is more accessible.

6. If OS sites are used, record the habitat grouping of the replaced P1 site and the replacement OS site, so that the planned sampling proportions in each habitat grouping within each study area are still maintained, as much as possible, by the end of the field campaign.

7. At the end of each day, program all actual sampling site coordinates onto the tablet with the georeferenced digital map (e.g., Avenza Maps). Review the area covered and the number of sites per habitat grouping completed. Use this information to plan the next day's surveys.

8. At the end of each day, hold a team debrief meeting to discuss any questions or concerns, including hazards encountered, access and/or sampling difficulties, etc. Discuss lessons learned and any improvements that can be made.

9. Prepare a daily report (email) to Zoetica and NWMO regarding progress made to date (e.g., information gathered from steps 7 and 8). Study design changes, if needed, must be approved by Zoetica and the NWMO.

6.3 Wetland Verification and Microhabitat Selection

1. The GRTS study design includes stratification of wetland types based on ELC data; however, wetland classification by air photo interpretation has inherent uncertainty. After arriving at a wetland site, the experienced biologist will verify the wetland type as either marsh, swamp, bog, or fen (MNRF 2014).

2. Upon arrival at any pre-selected site, the experienced biologist(s) will also assess the general shoreline area to select suitable microhabitats for sampling (as these features are not discernible from remote sensing data used by Zoetica to develop the study design). Suitable microhabitats will include areas with submergent or emergent vegetation, logs or rocks for cover, or other habitat features preferred by wildlife or fish species.

3. For watercourses, the sampling site should consist of a single stream reach (i.e., a relatively homogenous length of stream). The field contractor should verify that the stream reach is at least

200 m long before initiating sample collection at a watercourse, i.e., Replicate 1 will be collected at 0 m, replicate 2 will be collected at 100 m, and replicate 3 will be collected at 200 m.

6.4 Collecting and Filtering eDNA Samples

6.4.1 Lentic vs. Lotic Systems

1. As discussed in Section 5.0, mobile sampling will be used for lentic systems (waterbodies and wetlands), while stationary point sampling will be used for lotic systems (watercourses).

2. Mobile sampling – walk along the shoreline while running 2 L water through the system, sampling at or just under the water surface to avoid stirring up sediment. If it is necessary to enter the waterbody or wetland, extend the retractable pole as far as possible and keep the sampler in front of you (to avoid contamination from boots as well as collecting stirred up sediment). Replicates for lentic systems can be collected by continuing mobile sampling along the shoreline (i.e., rather than doubling back to sample along the same reach).

3. Stationary point sampling – stand in one spot on the shore (can set up OSMOS tripod) while running 2 L water through the system, sampling at or just under the water surface to avoid stirring up sediment. Always collect replicates from downstream to upstream to avoid contamination. Space replicates 100 m apart (Bedwell and Goldberg 2020) within a single stream reach.

6.4.2 Collecting "Unknown" Samples

Review the Halltech OSMOS eDNA Sampler video on YouTube and follow the training provided by the Hanner Lab technician.

https://www.youtube.com/watch?v=7yivEu909yE

1. Each survey team may consist of one Hanner Lab technician, one contracted field staff, and one local field assistant. At each site, there can be 3 roles:
    a. eDNA sample collector – to handle the eDNA equipment and samples
    b. Environmental data collector – to take environmental measurements (e.g., water quality)
    c. Record keeper – to record all information reported by the other two personnel onto the field data form. (If there are only two personnel on the survey team, the environmental data collector should also act as the record keeper.)

2. Use a GPS to record site coordinates (UTM NAD 83) of the starting location for all 3 replicates. Mark as waypoints on the GPS unit (e.g., unique site code R1, R2, R3) and record on the field data form. *Optional*: mark the physical location with flagging tape to help find the sampling site during the next seasonal campaign and subsequent years of study.

3. Take geotagged photos of each collection location to document habitat characteristics, including a wide-angle photo for overall context and the microhabitat(s) where sampling will take place. These photos will be linked to the unique site code and replicate number when a photo of the completed data form is taken (step 16).

4. Record site and sample info on a waterproof field data form (see **Appendix B – eDNA Field Data Form**):

General Information

- Unique site code
- Replicate number
- Survey crew (initials of eDNA sample collector, environmental data collector, record keeper)
- Date and time
- Location (geographic coordinates in UTM NAD 83)

Environmental Conditions

- Weather (cloud cover, air temperature, precipitation)
- Canopy cover (measured using a densiometer)
- Water quality data (water temperature, pH, dissolved oxygen, conductivity) – *note: record these parameters **after** sampling to avoid contamination*
- Geotagged photo numbers
- Site/habitat characteristics (presence of emergent vegetation, substrate type, riparian and upland vegetation, beaver modifications, human disturbance, etc.)
- Incidental wildlife and fish observations

Sampling Conditions

- Method (OSMOS eDNA sampler)
- Filter type and pore size – abbreviate cellulose nitrate as CN, glass fibre as GF
- Input parameters: sample volume and pump pressure
- Output parameters: total volume pumped, average flow rate, runtime (to be read from OSMOS final results screen)

5. Prepare the OSMOS eDNA sampler. Find a suitable place to fix the tripod. Place the pivoting boom clamp on the tripod and lock it in place.

6. Attach the inlet tube to the bottom of the unit using the quick connect.

7. Turn ON the unit (inside) and close the door.

8. Hang the unit on the tripod for added stability. Ensure the tripod feet are firmly in the ground.

9. Attach the aluminum filter housing to the end of the pole. The filter housing should be stored in a Ziploc bag until use. Avoid touching it with your hands while assembling the equipment.

10. Extend the pole to the required length. Adjust the pole to the required height (to sample at or just under the water surface to avoid stirring up sediment) and lock it in place.

11. Adjust the running parameters using the keypad:
    a. Volume Limit = 2.0 L

b. Pre Filters = 1
c. Pressure = -60 kPa
d. Hose Length = 15 ft
e. Enable Heater? OFF
f. Enable Remote Switch? NO
g. Shutoff Automatic? Auto

12. Press ENT to start. The unit will display the key parameter values in real-time: pressure, temperature, flow, and quantity.

    Notes: If the flow rate is very low due to high levels of particulates in the water, the Hanner Lab technician may choose to install filters with increased pore size. This method is preferable to turning up the pump pressure, as high filtration pressures may reduce eDNA retention (Thomas et al. 2018) and/or could rupture the membrane. Any modifications made to the initial (standard) parameters must be documented on the field data form.

13. Once the target volume has been reached, the unit will beep.

14. Invert the pole and follow the onscreen prompt to continue. Let the unit run until all the water has been pumped out.

15. The final results screen will show the total volume pumped, average flow rate, and runtime. (Note: it only takes a few minutes to pump 1-2 L of water.)

16. Record final results information on the field data form (i.e., output parameters). Take a photo of the completed data form.

17. Retract the pole to retrieve the filter. Invert the filter housing and lock in place.

(Steps 18-20 can be performed by the environmental data collector or record keeper while waiting for sampling to complete.)

18. Dried filter membranes will be placed in small paper coin envelopes.* Using a Sharpie marker, label envelopes with the following sample identifiers (must match the info recorded on the field data form):
    - Unique site code
    - Initials of eDNA sample collector
    - Date and time
    - Sample/replicate number (1, 2, 3)

19. Prepare a medium Ziploc bag to hold the sample envelopes once completed. Designate one bag per site for all “unknown” and control samples.*

* *If the environment is wet or humid, paper envelopes (step 18) may not be ideal for storing dried filters. To protect from rain and humidity and to prevent cross-contamination between samples*

and controls (step 19), store each filter separately in its own small plastic Ziploc bag with silica gel desiccant packs.

20. Wear sterile nitrile gloves before handling desiccant packs. Place a handful (~20) of desiccant packs at the bottom of the bag, enough to line the bottom.

    Note: Zoetica recommends pre-packing the miniature coin envelopes into the Ziploc bags with desiccants. This step will minimize the chances of a large-scale envelope contamination event (e.g., accidentally dripping sample water across all envelopes, which could lead to contamination of the filter that will go inside the envelopes).

21. Use sterilized gloves while removing the membrane from the filter housing. Gloves that will be used to handle samples should be stored in a large Ziploc bag for easy access to the wrist ends and to avoid contamination. Shake the gloves around in the bag and tip one toward the bag opening so you are able to grab the wrist part of a glove without touching any other gloves or the inside of the bag, which may transfer contamination to the remaining gloves.

22. Gently wiggle the filter housing back and forth to disconnect the top and bottom parts to expose the membrane.

23. Grab a pair of clean tweezers by the handle (from a Ziploc bag or bottle) using the dominant hand. Do not touch the pincers. You can place the tweezers on the palm of a gloved hand if you need to adjust positioning.

24. Use the tweezers to pick up the filter ring. Hold the edge of the ring with your free (non-dominant) hand. Use the tweezers to gently fold one side of the filter membrane toward the other. Use the untouched side of the filter ring to hold down the fold, then use the tweezers to gentle crease the membrane in half. Pick up the folded filter membrane with the tweezers. *By using the filter ring and tweezers, your fingers should not need to touch the membrane at all.*

25. Put the filter ring aside and pick up a labelled sample envelope using your non-dominant hand. Use a clean gloved finger to lift the flap, then gently squeeze the sides to open the envelope. Without touching the inside of the envelope, place the filter membrane deep into the envelope.

26. Seal the envelope and place it in the prepared Ziploc bag with desiccants. Store in a cooler with ice packs while completing the rest of the day's sampling.

27. Replace the filter ring into the aluminum filter housing and place it back into the labelled Ziploc bag. The unit must be decontaminated before reuse (see Section 6.1.2).

28. Remove gloves and keep in a labelled bag designated for used gloves; dispose of used gloves in the garbage back at camp. Place contaminated tweezers into the labelled Nalgene bottle designated for used tweezers. Put on a new pair of gloves and use clean tweezers for each sampling location (including replicates and duplicates) and when collecting field positive and negative samples.

29. Disconnect the pole from the unit (the quick connect at the bottom). Roll up tubing and pack up the tripod so that it can be stored safely and carried comfortably to the next sampling location.

6.4.3 Collecting Duplicate, Field Negative, and Field Positive Samples

1. A **duplicate sample** will be collected at every other site as a QA/QC check to assess the reproducibility of the results. Duplicates allow for the measurement of variability between samples and will potentially reveal lab analytical errors. Collecting a duplicate also doubles the volume of water sampled at a location and may increase the chances of eDNA detection.

2. The collection of a duplicate sample should mimic the collection of the first "unknown" sample at that location (as above, Section 6.4.2). In the 'Replicate no.' field of the data form, record the replicate number and the duplicate as "dup."

3. A **field negative sample** of distilled (DNA-free) water is "collected" to ensure that no contamination is occurring throughout the decontamination and collection procedures. If there is no contamination, a field negative should not display any DNA markers. This QA/QC check helps confirm that samples collected are representative of the site and do not contain any extra DNA.

4. One field negative should be collected every three sites. Run 2 L of distilled water through the OSMOS system and handle the filter like an actual sample (as above, Section 6.4.2). In the 'Replicate no.' field of the data form, record as "field neg."

5. Whenever possible, collect a **field positive sample** at a site where the species of interest is/are known to occur (which can be within any study area: AOI, LSA, or RSA). This QA/QC check helps to confirm that your protocols are working, establish rates of false negatives (which should be low), and also helps to confirm traditional surveying techniques.

6. The collection of field positives should mimic the collection of actual samples (as above, Section 6.4.2). In the 'Replicate no.' field of the data form, record as "field pos."

6.5 Storage and Transport of Samples to Laboratory

1. At the end of the day, back at camp, store all samples within a larger bag, securely and in the dark at room temperature or cooler (but do not freeze). The longevity of desiccant storage is thought to be 6-12 months (Hobbs et al. 2017); however, it is best to send them to the lab for DNA extraction as soon as possible.

2. Document the sample filter storage conditions (e.g., temperature, duration) until the point when samples are shipped to the Hanner laboratory for analysis. Submission of eDNA samples should be accompanied by both hard copy and electronic data forms, and site photos.

3. Ziploc bags of samples should be packaged securely (e.g., in a bubble envelope or box) and shipped via courier with tracking enabled. No special conditions (e.g., ice packs) or permits are required.

4. Send samples to:

   Hanner Laboratory
   Room 2409, Summerlee Science Complex
   University of Guelph
   50 Stone Road East
   Guelph, Ontario, N1G 2W1

6.6 Data Entry and Management

1. Transcribe information recorded on field data forms into an electronic spreadsheet as soon as possible, ideally on a daily basis. This person must input their initials into the "Entered by" field of both the hard copy and the spreadsheet.

2. A second person should verify (double-check) data entry against the original data form. This person must input their initials into the "QA/QC by" field of both the hard copy and the spreadsheet. Any discrepancies or ambiguities should be verified with survey team members.

3. All photographs taken for the site (including habitat and data form backup) should be organized into a folder labelled by the unique site code. All sites completed each day should be organized into a folder labelled by the survey date.

4. Photographs and spreadsheets should be uploaded to ZoeticaNet (or another server/database approved by the NWMO) daily or as soon as practicable. If online upload is not always possible, data must be stored on a secure hard drive until the opportunity to upload files arises.

5. At the end of the field campaign, GPS waypoints and tracks, modified or annotated Avenza Maps, and any other documentation should also be uploaded to ZoeticaNet (or another server/database approved by the NWMO).

7.0 EXPECTED RESULTS

The Hanner laboratory will present the eDNA metabarcoding methods and results in a summary report focused on species detected (and/or higher taxa as appropriate to the taxonomic resolution of the marker gene and reference database used to infer analysis). In addition, because existing morphologically-based taxonomy can overlook cryptic species, a summary of "molecular operational taxonomic units" (MOTUs) will also be included to further facilitate site comparisons. Raw data will be appended (e.g., as an Excel spreadsheet) and archived.

8.0 MAINTENANCE

Follow all maintenance procedures specified by Halltech Aquatic Research Inc. for the OSMOS eDNA sampling kit. Calibration of quantitative instruments (e.g., water quality meter, GPS unit) should be conducted before use to ensure the accuracy and precision of data collected. Ensure that all electronic devices have power, recharge daily (if possible), and carry extra batteries. Tie flagging tape to all equipment to prevent losing means of communication and navigation.

9.0 REFERENCES

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Hobbs, J., C. S. Goldberg, C. C. Helbing, and N. Veldhoen. 2017. Environmental DNA Protocol for Freshwater Aquatic Ecosystems, Version 2.2. Prepared for BC Ministry of Environment, Ecosystems Branch.

MNRF. 2014. Ontario Wetland Evaluation System: Northern Manual. 1st edition. Ministry of Natural Resources and Forestry.

Thomas, A. C., J. Howard, P. L. Nguyen, T. A. Seimon, and C. S. Goldberg. 2018. ANDe™: A fully integrated environmental DNA sampling system. Methods in Ecology and Evolution 9:1379–1385.

APPENDIX A – eDNA FIELD MAP

MAP OF FIELD SAMPLING LOCATIONS TO BE SUPPLIED ONCE DESK-BASED MAPPING WORK IS COMPLETED

APPENDIX B – eDNA FIELD DATA FORM

(see next page – formatted for printing)

NWMO APM PROJECT – eDNA Field Data Form

Unique site code & Replicate no.: _____ Date: _____ Time: _____

Survey crew initials: _____ eDNA sample collector _____ environ. data collector _____ record keeper

Location (NAD 83): Easting _____ Northing _____ UTM zone _____

ENVIRONMENTAL CONDITIONS

Weather: _____ cloud cover (%) _____ air temperature (°C) _____ precipitation

Water quality: _____ water temperature (°C) _____ dissolved oxygen (mg/L) _____ pH

_____ conductivity (µs/cm)

Canopy cover: _____ % Geotagged photo no.: _____

Site characteristics (substrate, aquatic/riparian/upland vegetation, beaver activity, human use, etc.)

Incidental wildlife/fish observations:

SAMPLING CONDITIONS

Method: _____ Filter type and pore size: _____

Input parameters: _____ sample volume (L) _____ pump pressure (kPa)

Output parameters: _____ total volume pumped (L) _____ average flow rate (L/s) _____ runtime (s)

Comments/modifications:

Unique site code & Replicate no.: _____ Date: _____ Time: _____

Survey crew initials: _____ eDNA sample collector _____ environ. data collector _____ record keeper

Location (NAD 83): Easting _____ Northing _____ UTM zone _____

ENVIRONMENTAL CONDITIONS

Weather: _____ cloud cover (%) _____ air temperature (°C) _____ precipitation

Water quality: _____ water temperature (°C) _____ dissolved oxygen (mg/L) _____ pH

_____ conductivity (µs/cm)

Canopy cover: _____ % Geotagged photo no.: _____

Site characteristics (substrate, aquatic/riparian/upland vegetation, beaver activity, human use, etc.)

Incidental wildlife/fish observations:

SAMPLING CONDITIONS

Method: _____ Filter type and pore size: _____

Input parameters: _____ sample volume (L) _____ pump pressure (kPa)

Output parameters: _____ total volume pumped (L) _____ average flow rate (L/s) _____ runtime (s)

Comments/modifications:

Date entered into spreadsheet: _____ Entered by: _____ QA/QC by: _____