freshwater
Ecosystem Services

Summary Report: R-20230309

Otter Lake – eDNA Biodiversity Assessment

Submitted to Otter Lake Ratepayers' Association (OLRA)

Attention: Brian Hindley & Kerry Mueller

Table of Contents

Freshwater Ecosystem Services is please to submit this summary report to the Otter Lake Ratepayers' Association (OLRA) for the initial biodiversity assessment of Otter Lake and Little Otter Lake (Seguin, Ontario), conducted during the summer of 2022.

Introduction

Genetic material or deoxyribonucleic acid (DNA) is present in environmental samples (eDNA) such as sediment, water, and air, including cells, extracellular DNA (e.g., saliva, mucus, hair, urine, faeces, skin, sperm, eggs and other organic tissue and fluids), which are expelled or shed, as well as whole micro and macroscopic organisms (Ruppert, *et., al.* 2019). Methods in eDNA research have recently expanded to be able to assess whole communities from a single sample. eDNA metabarcoding is a novel method of assessing biodiversity wherein samples are taken from the environment via water, sediment, or air, from which the DNA of many species is extracted, and then amplified using general or universal primers in Polymerase Chain Reaction (PCR) and sequenced using next-generation sequencing, to generate thousands to millions of reads (Ruppert, *et., al.* 2019). From this data, species presence can be determined, and overall biodiversity assessed.

Despite being a relatively new method, eDNA metabarcoding has already proven to have enormous potential in biodiversity monitoring. Conventional methods for surveying richness and abundance are limited by taxonomic identification, may cause disturbance or impact habitats or sensitive species, and may rely on methods in which it is difficult to detect small or elusive species, thus making estimates for entire communities impossible (Ruppert, *et., al.* 2019). eDNA can complement these methods by targeting different species, sampling greater diversity, and increasing taxonomic resolution. Additionally, eDNA can detect rare species, so it is ideal for supplementing traditional studies. It has useful applications in detecting the first occurrences of invasive species, the continued presence of native species thought to be extinct, or otherwise threatened, and other elusive species occurring in low densities that would be difficult to detect by traditional means (Ruppert, *et., al.* 2019). Ecosystem-wide applications of eDNA metabarcoding have the potential to not only describe communities and biodiversity, but also to detect interactions and functional ecology over large spatial scales (Ruppert, *et., al.* 2019). Additionally, eDNA metabarcoding increases speed, accuracy, and identification over traditional methods, and decreases cost (Ruppert, *et., al.* 2019).

Diverse freshwater ecosystem habitats at Sandy Bay, Otter Lake. 6 August 2022

eDNA can remain viable for variable periods of time from weeks to hundreds of thousands of years, allowing for applications in molecular biology, ecology, palaeontology, and environmental sciences, particularly when coupled with next-generation sequencing (Ruppert, *et., al.* 2019). eDNA metabarcoding has superior species detectability and is more efficient and more likely to detect species presence than traditional sampling, and that species identified with metabarcoding were comparable to those identified by traditional sampling. This method also requires lower effort, causes no ecosystem disturbance, allows detection without a priori knowledge of species, and can be implemented in areas where traditional surveys are impossible, making it extremely promising for the future of biodiversity monitoring (Ruppert, *et., al.* 2019). This methodology also provides new opportunities for large-scale biodiversity studies as well as being an efficient and economical approach to studying ecosystem structure.

eDNA samples contain DNA of all the species which were present or interacted with that environment within a ~24 to 72 hour period, in the case of water; longer (~>72 hrs) - as in the case of soils (varies by depth, exposure to oxygen, surface temperature, water percolation, etc.); and shorter (~<24 hrs) – as in the case of air, can be collected and sequenced. Species DNA may be present in the water or soil, from species which may have been active a couple of days prior to the sampling event, the night, or the day before the sampling event, early in the morning prior to the sampling event, and from species which evade or escape during the sampling event. Species detections can range from bats which are eating emerging or flying insects at dawn, dusk or during the night above creeks, wetlands and lakes and defecating in the water, to species like otters, voles, kingfishers, bears, deer, herons, and others which come down to the water to drink or feed or might be crossing or moving through creeks and wetlands.

The use of eDNA metabarcoding in freshwater ecosystem health and biodiversity assessments is relatively novel within Canada, primarily because freshwater biodiversity has not been part of the regulatory policies and frameworks of Federal and Provincial legislation, nor part of any standard or approved baseline or monitoring designs. Baseline and biomonitoring programs within freshwater ecosystems have historically been focused on commercial and recreational fisheries (i.e., large-bodied fish species, bait fish, benthic invertebrates, and habitat requirements for these selected species). Applications and use of eDNA within Canada over the last 10 years have primarily been focused on single-target species, particularly Species-at-Risk and aquatic invasive species, using single-species quantitative (qPCR) primers - developed for each target species.

NatureMetrics' development of eDNA metabarcoding services allow for many species to be detected from a single sample, whereby all the DNA from a multitude of species is sequenced and compared to genetic reference libraries and databases to match up with the DNA of species found in a water sample. By applying primers for all macroinvertebrates, fish, amphibians, reptiles, birds and mammals, a more holistic freshwater biodiversity measurement of species present within a water sample or freshwater location can be made. Using this eDNA approach allows for a full range of freshwater species and those that interact with a particular freshwater ecosystem to be detected, thereby incorporating more of the biodiversity at a location, into baselines and monitoring programs.

The OLRA Board conducted a freshwater biodiversity assessment, using environmental deoxyribonucleic acid (eDNA) to detect various species in the water within Otter Lake and Little Otter Lake, on 6 August 2022. The field sampling program was undertaken by Brian Hindley (OLRA) and Cameron von Bratt (Freshwater Ecosystem Services).

This report summarizes field and laboratory methods undertaken, as well as a summary of the analysed eDNA results and data provided by NatureMetrics North America Ltd.

Methods

The field program was conducted in two parts on 6 August 2022, an early morning subsampling event in six tributaries which flow into Otter Lake and Little Otter Lake at various confluence locations around the lakes (**Map 1**), followed by a short demonstration of the use of the eDNA kits and sampling procedures to a few of the Otter Lake community members, as well as a mid-afternoon lake subsampling event within Otter Lake and Little Otter Lake. At each confluence, subsamples from within the lake were collected and added to the corresponding subsamples from the tributaries, thereby compositing the water samples for each confluence location, resulting in a total of six composite water samples. Composite samples were roughly split 50/50 between the tributary subsamples and the lake subsamples.

The six tributaries were accessed via road, while the six corresponding lake confluence locations in Otter Lake and Little Otter Lake were accessed via boat. Site photos were taken (**Appendix A**). Samples were collected using the standard sampling methodology recommended by NatureMetrics (**Appendix B**). Within the tributaries, subsamples were taken from a water depth of ~0.3 m, while in lake locations, water depths ranged from 2.5 to 6 m with subsamples collected from the surface 0.3 m of depth (**Table 1**). Water temperatures were a constant 26℃ within the lake sampling locations, while in the tributaries, water temperatures ranged from 15℃ to 23℃ (**Table 1**). Additional field data, including GPS coordinates, observed species and site locations notes were recorded (**Table 1**). Sample water from each composited location was filtered through a NatureMetrics 0.8 µm eDNA filter (**Appendix B**). To the extent possible, the entire collected sample was processed through the filter. Between 600 mL and 2.9 L of water was filtered for each composite sample (**Table 1**). Each filter dried and preserved.

Table 1: Field data recorded during the Otter Lake eDNA Biodiversity Assessment, 6 August 2022

Station Name	GPS Information			Temp. (C)	Water Depth (m)	Observed species	Field Notes	Volume (mL)
	Waypoint Number	Latitude	Longitude					
Broken Link Tributary	159	45.269809	-79.907262	15	0.3	Northern Leopard Frog	Sample taken d/s of culvert. Large wetland u/s	600
Sunny Point Bay	166	45.267358	-79.907887	26	6	None	Wetland shoreline @ inlet	
Rankin Lake Tributary	160	45.298555	-79.930963	22	0.3	Green Frog	Sample taken d/s of culvert	2,900
Little Otter Bay	170	45.297647	-79.936826	26	4.6	None	Wetland shoreline @ inlet	
Link Property Tributary	161	45.272201	-79.931471	20	0.3	Blue Jay, Pileated Woodpecker, Green Frog	Sample taken u/s of culvert	2,800
Link Shore	167	45.266683	-79.929358	26	6.4	People	Wetland shoreline @ inlet	
Blue Lake Road Beaver Pond	164	45.292108	-79.980526	23	0.3	Beaver, Green Frog	Sample taken d/s of culvert. Large wetland u/s & d/s	900
Sand Bay	171	45.289983	-79.978565	26	2.5	None	Wetland shoreline @ inlet	
Salmon Lake Tributary	163	45.260713	-79.966970	22	0.3	Northern Leopard Frog	Sample taken u/s of culvert	2,900
Salmon Lake Bay	169	45.261357	-79.968331	26	4.1	None	Wetland shoreline @ inlet	
Sovereign Lake Tributary	162	45.260211	-79.946364	23	0.3	Green Frog	Sample taken u/s of culvert. Salmon Lake outlet	2,900
Mud Bay	168	45.261627	-79.946980	26	4.3	None	Wetland shoreline @ inlet	

Note: m – metre; mL - millilitre

Preserved filters from each composited location were shipped to the NatureMetrics laboratory in Guelph, Ontario. The laboratory conducted the eDNA extractions and sent extracted DNA to their main laboratory in Guildford, United Kingdom (UK) for sequencing, bioinformatics, data analyses, and reporting (**Appendix C & D**).

Freshwater Ecosystem Services conducted further analysis on the NatureMetrics results (**Appendix C & D**). Additional GIS maps, simplified tables, and data interpretations were provided.

Map 1: Boyne River Watershed - Otter Lake sampling locations. 6 August 2022. ● In-flowing tributary locations; ● Corresponding Lake locations. ©Google Earth Imagery (8/11/2022)

Results Summary and Discussion

As indicated by the reports in **Appendix C**, the quality of the DNA collected at each location was good and PCR reactions were successful for all six samples. Both negative and positive controls performed as expected for each of the assays conducted, with very few sequences discarded and minimal PCR and sequencing errors (**Appendix C**). Taxonomic assignments were made for each taxon or species using sequence similarity searches against DNA reference databases, the National Centre for Biotechnology Information's nucleotide genetic reference database (NCBI 2023), and the Barcode of Life Database (BOLD, 2023; **Appendix C**). Thresholds of 98%, 95% and 92% were used for species-, genus- and higher-level assignments respectively. Assignments were made to the lowest possible taxonomic level where there was consistency in the DNA matches. Assigned species were checked against GBIF occurrence records for presence in Canada and elevated to higher taxonomic levels if there were no occurrences and unconfirmed species identifications were only reported as genus/family/order as appropriate (**Appendix C**).

The results indicate the presence of various species at each location based on the detection of their unique DNA sequences, but does not provide an indication of species abundance, nor does it confirm the absence of any species (**Appendix C**). Unidentified or misidentified species can result from incomplete or incorrect reference databases. While some species may have unknown barcode references at the present time, their unique genetic DNA sequence suggests variation in the genetic diversity of different species populations, either at a sub-species or geographic level (**Appendix E**). Genetic diversity plays a major role in the make-up of populations, sub-populations, and sub-species within most organisms. At the scale of small-bodied fish, amphibians, and macroinvertebrates, genetic diversity at a species level can be assumed through scalable geographic isolation, where distances between the six sampling locations across Otter Lake and Little Otter Lake would be comparable to scalable distances equal to interprovincial or across Canada populations in mammal, fish, and human population terms. Our unique cities, towns, cultures and ethnicities across Canada, our varied First Nation communities, cultures, beliefs and traditions even within each province and territory - are easily recognisable scalable comparisons of the importance of population diversity. Another example is our unique fish sub-populations across each of the provinces, particularly Atlantic Salmon, where populations across various parts of our east coast, Quebec and Ontario are at risk and have federal protection under the *Species at Risk Act* (GOC, 2023).

The diversity of insects, reptiles, birds, mammals, from neighbouring ponds, wetlands, creeks and lakes, the continued flow and mixing of genes required to keep populations genetically healthy and ensure genetic resilience, allow for the survival of species, is equally important. An example of this is shown by the results of the Northern Spotted Owl (*Strix occidentalis caurina*) in the western parts of North America, where populations have declined and loss of genetic variation is a threat to the persistence subspecies' (Funk, *et.al*. 2010). If the same scale of species-level diversity importance is applied to the macroinvertebrate species detected within the six sampling locations at Otter Lake and Little Otter Lake, the variation of species, sub-species, and population genetic diversity within these taxonomic groupings, is of major importance for the sustained health of biodiversity and overall ecosystem health of the lake. Under most legislation globally, including here in Canada, a "species" includes entities that reflect various infra-specific levels that are all part of a broader species distribution and reflect to varying degrees the population-species continuum (Cotes, *et.al.* 2018). The more genetically diverse a species or population is, the greater it will be adapted to changes in the environment (i.e., genetic resilience), and a lack of genetic diversity increases vulnerability to disease, parasites, and other threats (OAG, 2022).

The abundance of each species cannot be directly inferred from the proportion of total sequence reads, even though the proportion of sequence reads is a consequence of abundance, as it is impacted by biomass, activity, surface area, condition, distance from the physical sample, primer bias and species-specific variation in the genome (**Appendix C**). Relative abundance, in terms of the amount of genetic material present from many sources and amounts of DNA (e.g., spawning events, hatching events, insect emergences, temperature, school of fish vs. single individual, herd of deer vs. single deer, etc.), can be inferred over time in long-term datasets, as seasonal regimes, flow fluctuations & climate patterns, produce enough data to interpret natural and impacts trends and cycles in biodiversity change.

Based on the results of the biodiversity assessment using eDNA, a total of 417 species were detected in the six eDNA samples collected at the various locations around Otter Lake and Little Otter Lake, using three metabarcoding assays for macroinvertebrates, fish, and vertebrates (**Appendix C, D & E**).

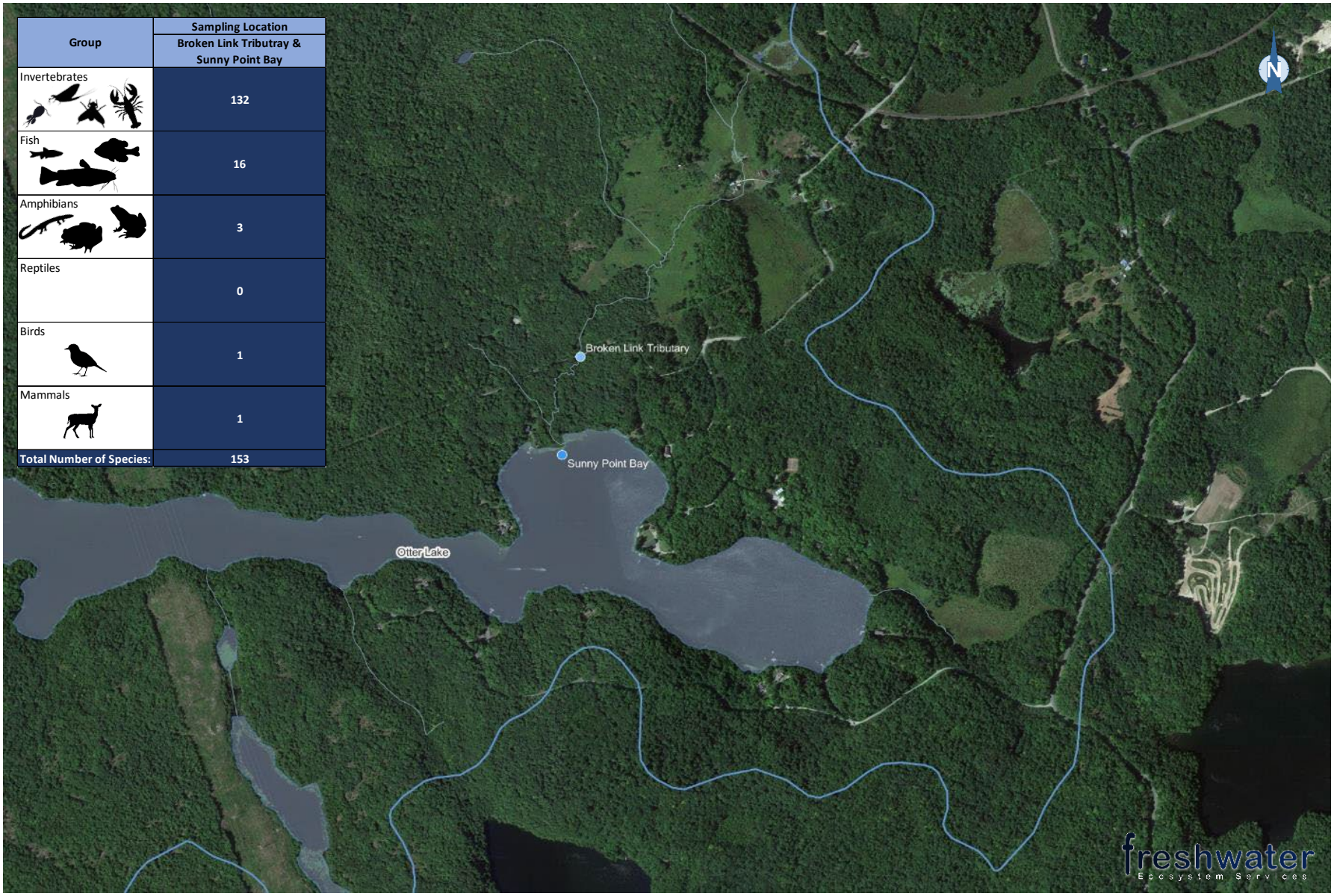
Within each composited sampling location around Otter Lake the number of species detected within each grouping varied (**Table 2 & Appendix E**). Species DNA detections ranged from 61 species detected at the Link Property Tributary & the Link Shore location (**Map 1; Table 2**), to 153 species detected at the Broken Link Tributary and Sunny Point Bay location (**Map 1; Table 2**).

Table 2: Biodiversity (i.e., diversity of species) detected using eDNA, in various sampling locations around Otter Lake, 6 August 2022

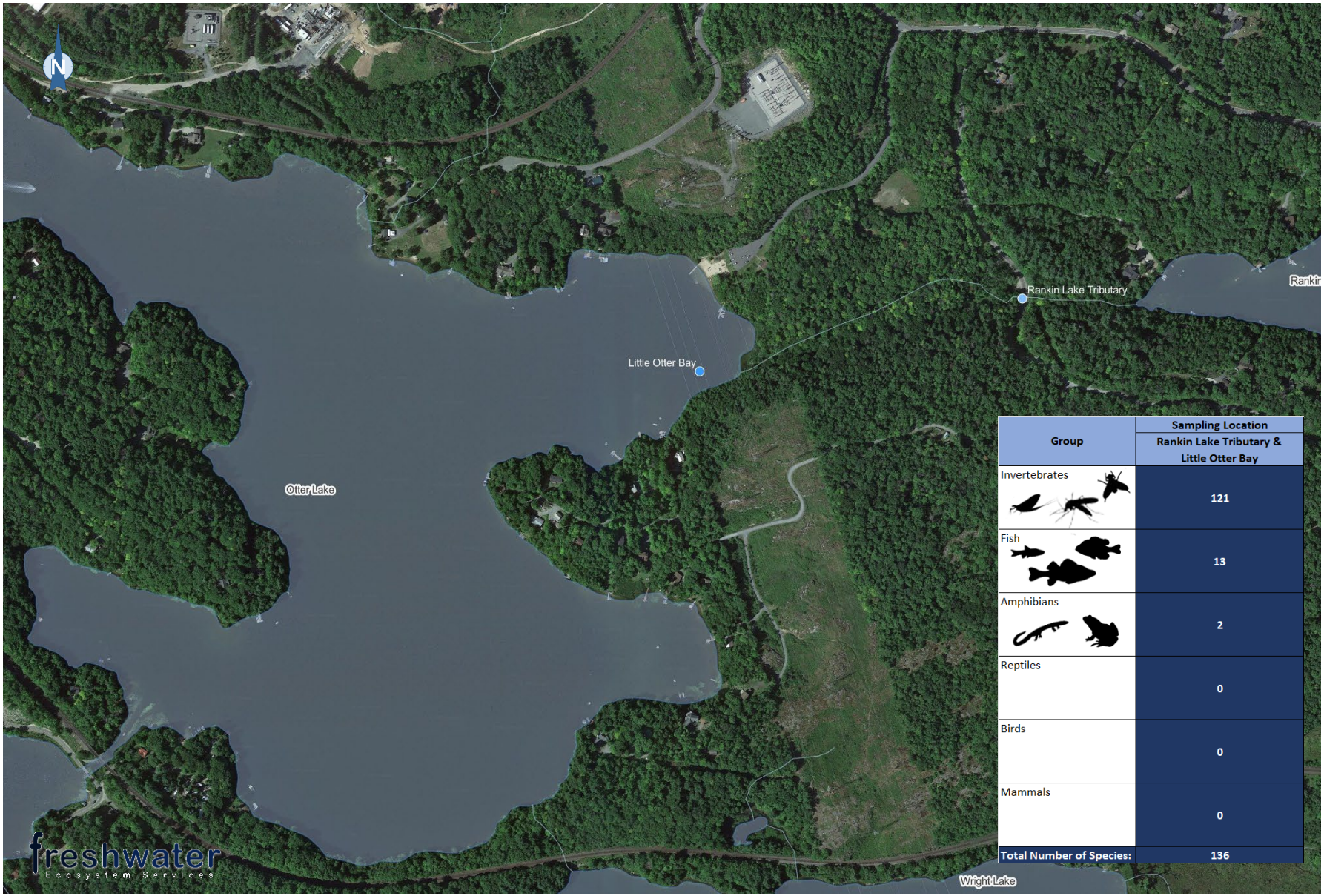
Group	Sampling Locations					
	Broken Link Tributray & Sunny Point Bay	Rankin Lake Tributary & Little Otter Bay	Link Property Tributray & Link Shore	Blue Lake Road Beaver Pond & Sand Bay	Salmon Lake Tributray & Salmon Lake Bay	Sovereign Lake Tributary & Mud Bay
Macroinvertebrates	132	121	42	76	123	115
Fish	16	13	18	15	11	19
Amphibians	3	2	1	6	3	2
Reptiles	0	0	0	2	0	0
Birds	1	0	0	1	0	1
Mammals	1	0	0	4	2	2
Total Number of Species:	**153**	**136**	**61**	**104**	**139**	**139**

Individual maps of each of the locations with a summary of the species detected are shown below (**Maps 2 to 7**). The Broken Link Tributary and Sunny Point Bay sampling locations are within a thickly forested part of Otter Lake (**Map 2, Appendix A**). Habitat quality was good with few human impacts in the immediate sampling location. Upstream of the creek sampling location land use changes, including a cottage, and a railway which crosses two upstream headwater source zones, are present (**Map 2**). The Rankin Lake Tributary & Little Otter Bay sampling locations are within proximity to busy bays of Rankin Lake and Little Otter Lake (**Map 3, Appendix A**). Numerous human activities, including the boat launch to Little Otter Lake are present in this area (**Map 3**).

The Link Property Tributary & Link Shore sampling locations are within a thickly forested part of Otter Lake (**Map 4, Appendix A**). Habitat quality was good with few human impacts in the immediate sampling location of the Link Property Tributary sampling location. A cottage is present near the Link Shore sampling location (**Map 4**). At the Blue Lake Road Beaver Pond & Sandy Bay sampling locations, the habitat is comprised of a wetland complex. Several cottages are present near the two locations (**Map 5, Appendix A**). The Salmon Lake Tributary & Salmon Lake Bay sampling locations are close together and separated by a thin strip of land between Salmon Lake and Otter Lake (**Map 6, Appendix A**). Several cottages are located here. The Sovereign Lake Tributary & Mud Bay sampling locations are within a thickly forested part of Otter Lake, with a few cottages present near these locations (**Map 7, Appendix A**).

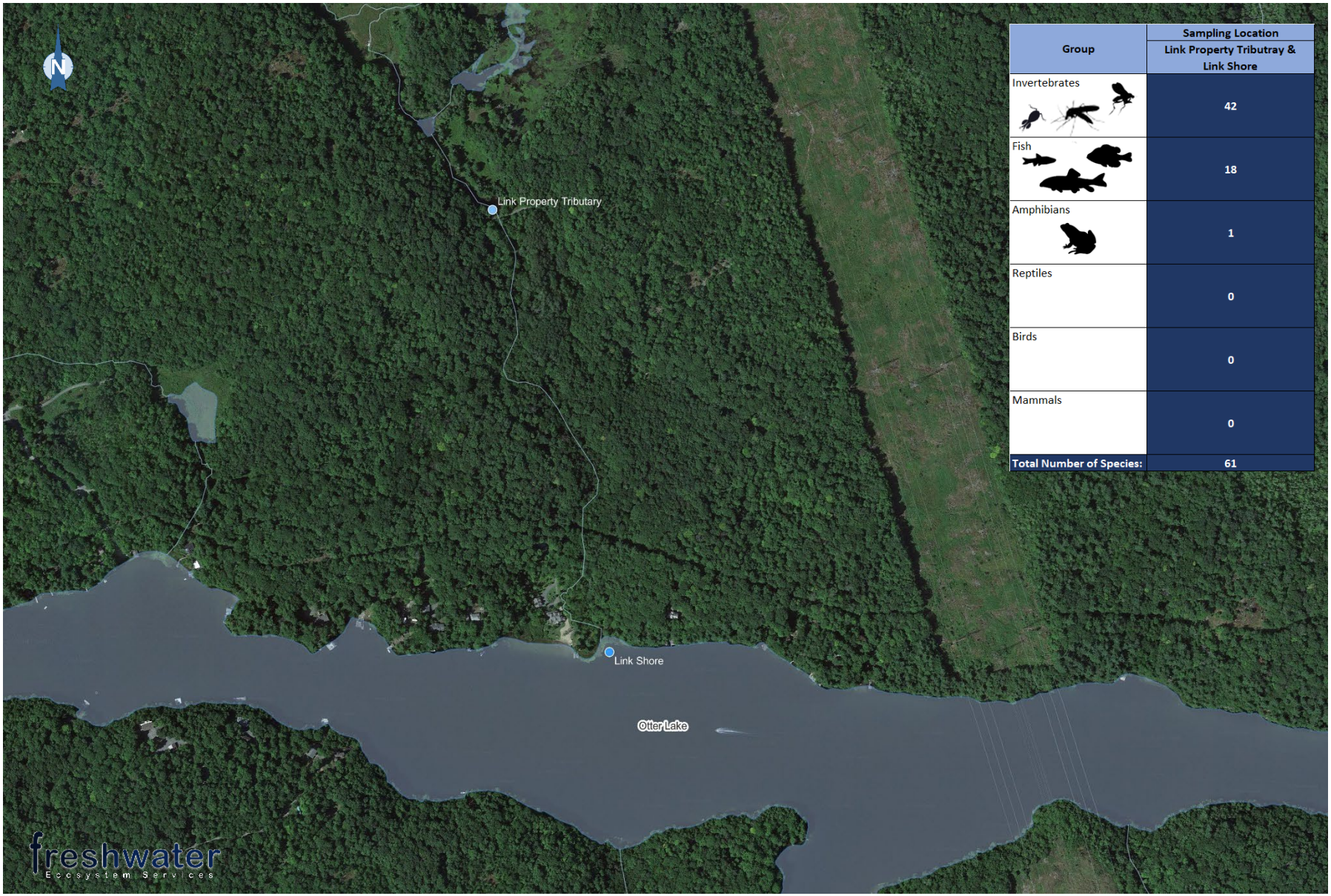


Map 2: Otter Lake – Broken Link Tributary & Sunny Point Bay sampling locations. 6 August 2022. ● In-flowing tributary location; ● Corresponding Lake location. Total number of species by group - detected by eDNA. ©Google Earth Imagery (8/11/2022)



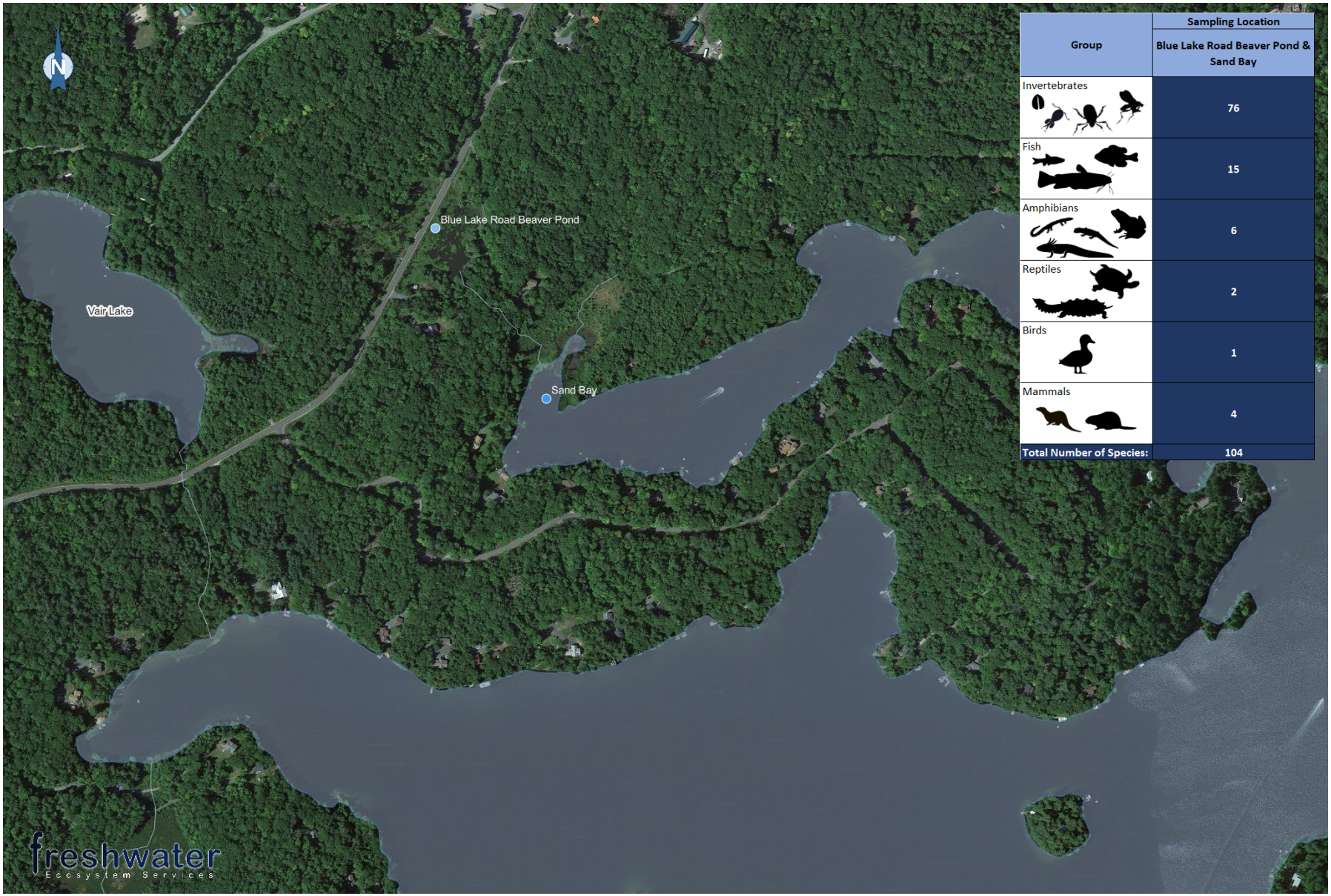
Group	Sampling Location: Rankin Lake Tributary & Little Otter Bay
Invertebrates	121
Fish	13
Amphibians	2
Reptiles	0
Birds	0
Mammals	0
Total Number of Species:	**136**

Map 3: Otter Lake – Rankin Lake Tributary & Little Otter Bay sampling locations. 6 August 2022. ● In-flowing tributary location; ● Corresponding Lake location. Total number of species by group - detected by eDNA. ©Google Earth Imagery (8/11/2022)



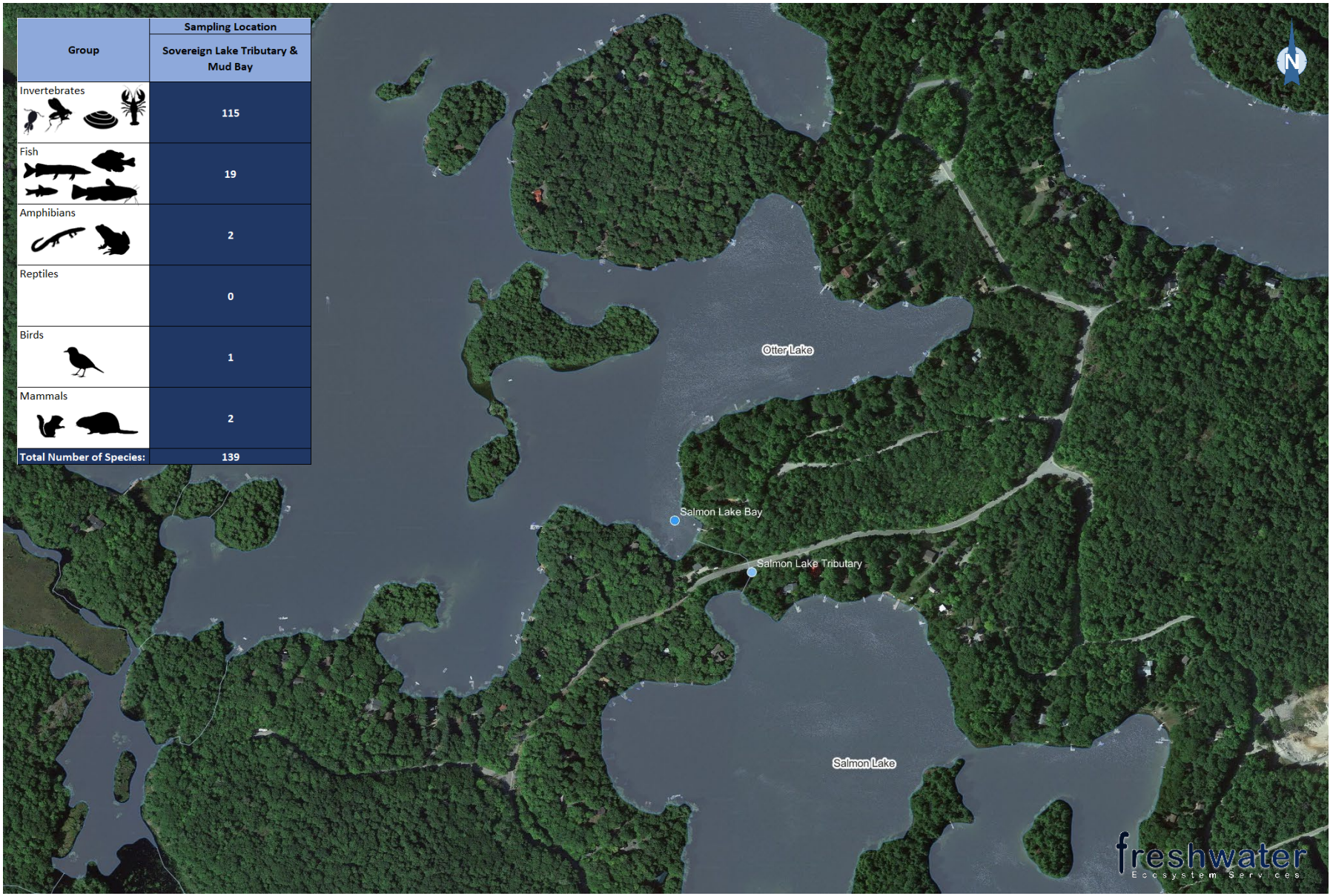
Group	Sampling Location: Link Property Tributray & Link Shore
Invertebrates	42
Fish	18
Amphibians	1
Reptiles	0
Birds	0
Mammals	0
Total Number of Species:	**61**

Map 4: Otter Lake – Link Property Tributary & Link Shore sampling locations. 6 August 2022. ● In-flowing tributary location; ● Corresponding Lake location. Total number of species by group - detected by eDNA. ©Google Earth Imagery (8/11/2022)

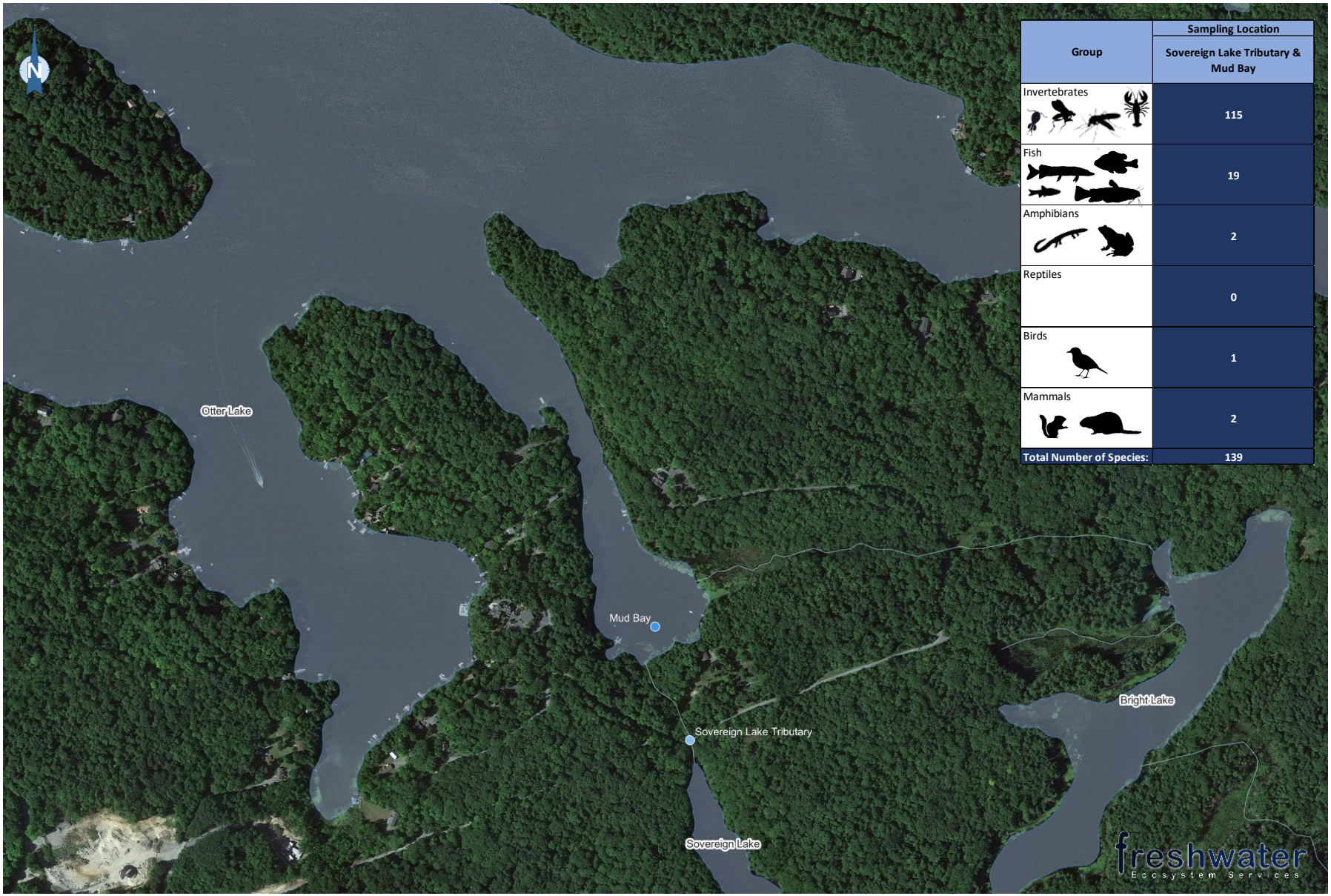


Group	Sampling Location: Blue Lake Road Beaver Pond & Sand Bay
Invertebrates	76
Fish	15
Amphibians	6
Reptiles	2
Birds	1
Mammals	4
Total Number of Species:	**104**

Map 5: Otter Lake – Blue Lake Road Beaver Pond & Sandy Bay sampling locations. 6 August 2022. ● In-flowing tributary location; ● Corresponding Lake location. Total number of species by group - detected by eDNA. ©Google Earth Imagery (8/11/2022)



Map 6: Otter Lake – Salmon Lake Tributary & Salmon Lake Bay sampling locations. 6 August 2022. ● In-flowing tributary location; ● Corresponding Lake location. Total number of species by group - detected by eDNA. ©Google Earth Imagery (8/11/2022)



Group	Sampling Location: Sovereign Lake Tributary & Mud Bay
Invertebrates	115
Fish	19
Amphibians	2
Reptiles	0
Birds	1
Mammals	2
Total Number of Species:	**139**

Map 7: Otter Lake – Sovereign Lake Tributary & Mud Bay sampling locations. 6 August 2022. ● In-flowing tributary location; ● Corresponding Lake location. Total number of species by group - detected by eDNA. ©Google Earth Imagery (8/11/2022)

Macroinvertebrates

Of the 364 macroinvertebrate species detected, 89 were identified to species level, while many macroinvertebrate species were only identifiable to family or genus level (**Appendix E, Table E-1**). The most common detected species were two Phantom midges (*Chaoborus punctipennis* and *C. flavicans*) and the Non-biting midge (*Ablabesmyia monilis*).

Macroinvertebrate species which were only detected at one of the various sampling locations, included the following species (**Appendix E, Table E-1**):

- Early brown spinner Mayfly (*Leptophlebia cupida*) and various Caddisfly species within the Trichoptera Order at the Broken Link Tributary and Sunny Point Bay location. The deep pool with followed by mixed gravels, pebbles, and sandy habitats in Broken Link Tributary suites these two groups of predator species, which favour these habitats (**Appendix A**).
- Spiny crawler Mayfly (*Eurylophella verisimilis*), Water scorpion (*Ranatra nigra*) and various Stoneflies within the Plecoptera order at the Rankin Lake Tributary & Little Otter Bay location (**Appendix A**). The flow in Rankin Lake Tributary, with shallow and mixed moderate and slow flows suites these three predator species, which favour these habitats (**Appendix A**).
- An unknown Copepod species in the Cyclopidae family, two midge species (*Atrichopogon* sp. and Brezzia sp.) and six species of Non-biting midge in the Chironomidae family, at the Link Property Tributary & Link Shore location. The open water, weedy and shallow depths of these two locations is well suited for these species (**Appendix A**). Habitat availability or quality limitations along the shoreline at the Link Shore location may be limiting the diversity of macroinvertebrate species. Species which are sensitive to water quality and habitat impacts are few in comparison to the other locations, suggesting habitiat impairment or alternatively, some issues associated with sampling/handling (**Appendix A**).
- Giant diving beetle (*Graphoderus liberus*), Water scavenger beetle (*Tropisternus mixtus*), three Pigmy backswimmer species in the Pleidae family, and 9 species of Seed shrimps within the Cyprididae family, at the Blue Lake Road Beaver Pond & Sandy Bay location. This wetland habitat is well suited for Seed Shrimp and beetles which favour shallow, weedy habitats (**Appendix A**).
- Giant burrowing mayfly (*Hexagenia limbata*), Alderfly (*Sialis vagans*) and Giant floater clam (*Pyganodon grandis*) at the Salmon Lake Tributary & Salmon Lake Bay location. These burrowing species require soft substrates found at both sampling locations (**Appendix A**).
- Two Waterflea species in the Diplostraca order, Brown pionid beetle (*Orthosoma brunneum*), an unknown Biting midge (*Stilobezzia* sp.), 14 species of Non-biting midge in the Chironomidae family; and an unknown Dance fly (*Hemerodromia* sp.) at the Sovereign Lake Tributary & Mud Bay location (**Appendix A**). The groups generally favour slow moving with soft substrates found at Mud Bay. The Brown pionid beetle favours fallen woody debris with mature trees present at the Sovereign Lake Tributary location (**Appendix A**).

Diptera, particularly Chironomids (Midges) are a taxonomically and ecologically highly diverse group and often dominate all kinds of lotic and lentic ecosystems in terms of species diversity, accounting for over 50% of the total macroinvertebrate diversity and abundance in aquatic ecosystems, and are thus a key food resource for birds, bats, spiders, dragonflies, and fish, as well as being important indicators of freshwater ecosystem health (Theissinger, *et.al.* 2018). Chironomids (Midges) accounted for 231 of the 364 species detections in the six samples, roughly ~64% of the of the total species diversity (**Appendix E, Table E-1**). Macroinvertebrate species detections suggest a diverse lower trophic community, Including many zooplankton, beetle, mayfly, caddisfly, stonefly, shrimp, and other freshwater species, with representatives of many Families, Orders & Classes typical of creeks, wetlands, lake shorelines, and pelagic zones (**Appendix E, Table E-1**). The habitats sampled were well represented by the diverse macroinvertebrate communities detected in this assessment.

Fish

Of the 31 fish species detected, 22 were identified to species level (**Appendix E, Table E-2**). The most detected species were Pumpkinseed (*Lepomis gibbosus*), Largemouth Bass (*Micropterus salmoides*), and Creek Chub (*Semotilus atromaculatus*).

Northern Pearl Dace (*Margariscus nachtriebi*) was added to the list of species as this is the only *Margariscus* species with a distribution range in Northern Ontario (**Appendix E, Table E-2;** iNaturalist, 2023 and OFFLHD, 2023). Where a DNA sequence was associated with two possible species, distribution ranges were considered to infer which species the sequence was likely to be associated with (iNaturalist, 2023 and OFFLHD, 2023). Common Shiner (*Luxilus cornutus*) was more likely to occur in the sampling locations, when compared to the distribution range of Striped Shiner (*Luxilus chrysocephalus*), which was confirmed by the results of the vertebrate assay (**Appendix E, Table E-2;** iNaturalist, 2023 and OFFLHD, 2023). Similarly, Common Logperch (*Percina caprodes*) was more likely to occur in the sampling locations, when compared to the distribution range of Channel Darter (*Percina copelandi*; **Appendix E, Table E-2;** iNaturalist, 2023 and OFFLHD, 2023). *Notropis dorsalis* or Bigmouth Shiner was the only species to be considered to be mis-identified as an eDNA species detection. This species in only found on the west side of Lake Huron (**Appendix E, Table E-2;** iNaturalist, 2023 and OFFLHD, 2023). It is likely that this is a DNA sequence of another species of *Notropis* with a similar DNA barcode.

Fish species which were only detected at one of the various sampling locations, included the following species (**Appendix E, Table E-2**):

- An unknown Minnow (*Hybognathus* sp.), and Northern Pearl Dace (*Margariscus nachtriebi*) at the Broken Link Tributary and Sunny Point Bay location (**Appendix A**). The deep pool with followed by mixed gravels, pebbles, and sandy habitats in Broken Link Tributary suites these two fish species, which favour these habitats (**Appendix A**).
- Common logperch (*Percina caprodes*) at the Rankin Lake Tributary & Little Otter Bay location (**Appendix A**). The flow in Rankin Lake Tributary, with shallow and mixed moderate and slow flows suit this species, which favour these habitats (**Appendix A**).
- Northern redbelly dace (*Chrosomus eos*) and three unknown Cyprinids at the Link Property Tributary & Link Shore location. (**Appendix A**). These species favour the pools and weedy habitats found in these two locations.
- Hornyhead chub (*Nocomis biguttatus*) at the Blue Lake Road Beaver Pond & Sandy Bay location (**Appendix A**). This species has a very limited range within Northern Ontario; however it has been documented close to Bracebridge, and as far north as Parry Sound, North Bay & Sault Ste. Marie (iNaturalist 2023).
- Northern pike (*Esox lucius*) at the Sovereign Lake Tributary & Mud Bay location. The shallow and weedy habitats within Mud Bay are suited to young Northern Pike (**Appendix A**).

Amphibians

All 9 amphibian species detected were identified to species level (**Appendix E, Table E-3**). Red-spotted newt aka Eastern Newt (*Notophthalmus viridescens viridescens*) was added to the list of species as this is the only *Notophthalmus* species with a distribution range covering Northern Ontario (**Appendix E, Table E-3,** iNaturalist, 2023), which was confirmed by the results of the vertebrate assay. Additionally, *Ambystoma maculatum* or the Spotted Salamander, was also added to the list of species as this is the only other *Ambystoma* species with a distribution range covering Northern Ontario (**Appendix E, Table E-3**). Where a DNA sequence was associated with two possible species, distribution ranges were considered to infer which species the sequence was likely to be associated with (iNaturalist, 2023). The Gray Treefrog (*Dryophytes versicolor*) was more likely to occur in the sampling locations, when compared to the distribution range of Cope's Gray Treefrog (*Dryophytes chrysoscelis*; **Appendix E, Table E-3;** iNaturalist, 2023).

This however is not similar in the case of the Green Frog (*Lithobates clamitans*) and the American Bullfrog (*Lithobates catesbeianus*), which are both likely to occur within Otter Lake and have overlapping distribution ranges (**Appendix E, Table E-3;** iNaturalist, 2023). The DNA sequence was detected at all sampling locations and was the most common amphibian species detected (**Appendix E, Table E-3**). Green Frogs are mostly likely the species associated with the DNA sequence and were observed at three of the sampling locations during the sampling event (**Table 1**). However, American Bullfrog is also likely to occur at various locations around Otter Lake and Little Otter Lake.

Amphibian species which were only detected at one of the various sampling locations, included the following species (**Appendix E, Table E-3**):

- Red-spotted Newt or Eastern Newt (*Notophthalmus viridescens viridescens*), Gray treefrog (*Dryophytes versicolor*), Blue-spotted Salamander (*Ambystoma laterale*), and Spotted Salamander (*Ambystoma maculatum*) at the Blue Lake Road Beaver Pond & Sandy Bay location. This wetland habitat is well suited for amphibians which favour shallow, weedy habitats (**Appendix A**).
- Mudpuppy (*Necturus maculosus*) at the Salmon Lake Tributary & Salmon Lake Bay location. This species favours soft substrates, found at both sampling locations (**Appendix A**).
- Eastern Red-backed Salamander (*Plethodon cinereus*) at the Sovereign Lake Tributary & Mud Bay location. This species favours fallen woody debris with mature trees present at the Sovereign Lake Tributary location (**Appendix A**).

Reptiles

Only two Reptile species were detected using eDNA (**Table 2**). Both the Common Snapping Turtle (*Chelydra serpentina*) and the Midland Painted Turtle (*Chrysemys picta marginata*) were detected at the Blue Lake Road Beaver Pond & Sandy Bay location (**Appendix E, Table E-4**). Both Turtles are listed as Special Concern under Ontario provincial species at risk regulations (GOO, 2023), and the Midland Painted Turtle is also listed as Special Concern federally (GOC, 2023; **Appendix E, Table E-4**). This wetland habitat is well suited for Turtles which favour shallow, weedy habitats (**Appendix A**).

Birds

Only two Bird species were detected using eDNA (**Table 2**). The Wood duck (*Aix sponsa*) was detected at the Blue Lake Road Beaver Pond & Sandy Bay location, while an unidentified Passerine species was detected at the Broken Link Tributary and Sunny Point Bay location and at the Sovereign Lake Tributary & Mud Bay location (**Appendix E, Table E-5**).

Mammals

All 6 Mammal species were detected were identified to species level (**Appendix E, Table E-3**) North American River Otter (*Lontra canadensis*) was added to the list of species as this is the only *Lontra* species with a distribution range covering Otter Lake (**Appendix E, Table E-6**). Two DNA sequences for American Beaver (*Castor canadensis*) were detected, however this is the only species within this genus. At one-point numerous sub-species and distinct populations were distinguished, however substantial genetic mixing of populations has occurred because of the numerous reintroduction efforts intended to help the species recover following extirpation from many regions.

Mammal species which were only detected at one of the various sampling locations, included the following species (**Appendix E, Table E-6**):

- White-tailed Deer (*Odocoileus virginianus*) at the Broken Link Tributary and Sunny Point Bay location (**Appendix A**)
- North American River Otter (*Lontra canadensis*) and Muskrat (*Ondatra zibethicus*) at the Blue Lake Road Beaver Pond & Sandy Bay location (**Appendix A**)

- Northern Raccoon (*Procyon lotor*) at the Salmon Lake Tributary & Salmon Lake Bay location (**Appendix A**)
- Eastern Chipmunk (*Tamias striatus*) was detected at the Sovereign Lake Tributary & Mud Bay location (**Appendix A**)

The application of eDNA metabarcoding to assess the freshwater biodiversity at these locations in and around Otter Lake and Little Otter Lake indicated a presence of a diverse biological community. Some variation in community compositions and uniquely detected species occurrences at each of the sampling locations. Variation in habitat, local disturbances and other contribution factors are all considerations which may influence the biodiversity at the different locations. Over time, species richness, and community composition of the biodiversity at these locations can be monitored if eDNA sampling is conducted at regular intervals on a yearly and/or seasonal basis.

Recommendations

Future biodiversity monitoring assessments using eDNA metabarcoding are recommended and should be conducted at the same locations, and similar dates on an annual basis. Additionally, increasing the sampling locations along the shorelines of both Otter Lake and Little Otter Lake, as well as in deep waters of the pelagic zones, are likely to detect additional species which may be present but were not detected in the 2022 sample locations. The OLRA board and lake community should also consider additional sampling events during various seasons, to fully capture differences in resident and migratory species composition at various times of the year. Distinctions between the biodiversity compositions of creeks, wetlands and similar lake habitats is also recommended and should also be considered in future monitoring assessments.

Closure

The biodiversity assessment using eDNA metabarcoding technology at Otter Lake and Little Otter Lake in the summer of 2022 provided a comprehensive assessment of the diversity biological community in and around the lakes, with detections ranging from small zooplankton species to large mammal species. A wide range of aquatic, aquatic-dependant & terrestrial species were detected in the water samples. This data can be used as a baseline for future monitoring assessments. Combined with historical data, current monitoring surveys, citizen science (e.g., fish catch and iNaturalist data) and local First Nation traditional knowledge, eDNA is an effective measurement and monitoring tool. The residents and cottages around Otter Lake have the data and tools to effectively monitor biodiversity in and around Otter Lake. Should biodiversity change over time, informed decisions can be made using this data.

This report has been prepared and presented to the various Board members of OLRA. If there any questions or require additional clarification, please email Cameron von Bratt using the email address below.

Cameron von Bratt, *M.Sc., P.Biol.*
Freshwater Ecologist,

for and on behalf of the Company,

freshwater Ecosystem Services

G.S.T./H.S.T. Registration No. 788625747
Email: cameron@freshwaterecosystems.com

References

Barcode of life data (BOLD). 2023. Public Data Portal and Barcode Index Number (BIN) System. https://www.boldsystems.org/index.php/Public_BarcodeIndexNumber_Home. Accessed February 2023.

Committee on the Status of Endangered Wildlife in Canada (COSEWIC). Candidate Species Search. https://www.cosewic.ca/index.php/en-ca/reports/candidate-wildlife-species.html. Accessed February 2023.

Funk, W.C., Forsman, E.D., Johnson, M. Mullins, T.D. and Haig, S.M. 2010. Evidence for recent population bottlenecks in northern spotted owls (*Strix occidentalis caurina*). Conservation Genetics 11, 1013–1021 (2010). https://doi.org/10.1007/s10592-009-9946-5. Accessed February 2023.

Government of Canada (GOC). 2023. Federal Species at Risk Act Public Registry Search. https://species-registry.canada.ca/index-en.html#/species?sortBy=commonNameSort&sortDirection=asc&pageSize=10. Accessed February 2023.

Government of Ontario (GOO). 2023. Species at Risk in Ontario. Species at risk list https://www.ontario.ca/page/species-risk-ontario. Accessed February 2023.

iNaturalist. 2023. Species Search. https://www.inaturalist.org/observations. Accessed February 2023.

International Union for Conservation of Nature (IUCN). 2023. Red List of Threatened Species. https://www.iucnredlist.org/. Accessed February 2023.

National Centre for Biotechnology Information (NCBI). 2023. Nucleotide genetic reference database. https://www.ncbi.nlm.nih.gov/nucleotide/. Accessed February 2023.

Office of the Auditor General of Canada (OAG). 2022. Commissioner of the Environment and Sustainable Development. Biodiversity in Canada: Commitments and Trends. https://www.oag-bvg.gc.ca/internet/English/oth_202210_e_44128.html. Accessed February 2023.

Ontario Freshwater Fishes Life History Database (OFFLHD). 2023. Species Search. https://www.ontariofishes.ca/home.htm. Accessed February 2023.

Ruppert, K. M., Kline, R.J., and Rahman, S. 2019. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. Global Ecology and Conservation 17 (2019). https://doi.org/10.1016/j.gecco.2019.e00547. Accessed February 2023.

Theissinger K, Kästel A, Elbrecht V, Makkonen J, Michiels S, Schmidt S, Allgeier S, Leese F, Brühl C (2018) Using DNA metabarcoding for assessing chironomid diversity and community change in mosquito controlled temporary wetlands. Metabarcoding and Metagenomics 2: e21060. https://doi.org/10.3897/mbmg.2.21060. Accessed February 2023.

Appendix A

Site Photos

Appendix A – Broken Link Tributary & Sunny Point Bay Confluence

Photo A-1. Broken Link Tributary looking upstream (left) and downstream (right) of the culvert, 6 August 2022.
GPS Latitude: 45.269809 Longitude: -79.907262

Photo A-2. Sunny Point Bay looking upstream at the confluence with Broken Link Tributary, 6 August 2022.
GPS Latitude: 45.267358 Longitude: -79.907887

Appendix A – Rankin Lake Tributary & Little Otter Bay Confluence

Photo A-3. Rankin Lake Tributary looking upstream (left) and downstream (right) of the culvert, 6 August 2022.
GPS Latitude: 45.298555 Longitude: -79.930963

Photo A-4. Little Otter Bay looking upstream at the confluence with Rankin Lake Tributary, 6 August 2022.
GPS Latitude: 45.297647 Longitude: -79.936826

Appendix A – Link Property Tributary & Link Shore Confluence

Photo A-5. Link Property Tributary looking upstream (left) and downstream (right) of the culvert, 6 August 2022.
GPS Latitude: 45.272201 Longitude: -79.931471

Photo A-6. Link Shore looking upstream at the confluence with Link Property Tributary, 6 August 2022.
GPS Latitude: 45.266683 Longitude: -79.929358

Appendix A – Blue Lake Road Beaver Pond & Sand Bay Confluence

Photo A-7. Blue Lake Road Beaver Pond looking upstream (left) and downstream (right) of the culvert, 6 August 2022.
GPS Latitude: 45.292108 Longitude: -79.980526

Photo A-8. Sandy Bay looking upstream at the confluence with Blue Lake Road Beaver Pond, 6 August 2022.
GPS Latitude: 45.289983 Longitude: -79.978565

Appendix A – Salmon Lake Tributary & Salmon Lake Bay Confluence

Photo A-9. Salmon Lake Tributary looking upstream (left) and downstream (right) of the culvert, 6 August 2022.
GPS Latitude: 45.260713 Longitude: -79.966970

Photo A-10. Salmon Lake Bay looking upstream at the confluence with Salmon Lake Tributary, 6 August 2022.
GPS Latitude: 45.261357 Longitude: -79.968331

Appendix A – Sovereign Lake Tributary & Mud Bay Confluence

Photo A-11. Sovereign Lake Tributary & Mud Bay Confluence looking upstream (left) and downstream (right) of the culvert, 6 August 2022.
GPS Latitude: 45.260211 Longitude: -79.946364

Photo A-12. Mud Bay looking upstream at the confluence with Sovereign Lake Tributary, 6 August 2022.
GPS Latitude: 45.261627 Longitude: -79.946980

Appendix B

NatureMetrics Sampling Protocol: Standard Aquatic eDNA Kit

Appendix C

NatureMetrics Reports: Fish, Vertebrate & Macroinvertebrate Metabarcoding Results

Appendix D

NatureMetrics Data Tables: Fish, Vertebrate & Macroinvertebrate Metabarcoding Data

Appendix E

Data Interpretation Tables: Macroinvertebrates, Fish, Amphibians, Reptiles, Birds & Mammals

Table E-1: Macroinvertebrate biodiversity (i.e., diversity of macroinvertebrate species) detected using eDNA (**Appendix D**), in various sampling locations around Otter Lake, 6 August 2022

Phylum	Class	Class Common Name	Order	Family	Scientific Name (Genus)	Scientific Name (Genus & Species)	Common Name	Similarity to Species-level Sequence ID	Less than 3 Matches to Sequences Reference Database or Limited geographic occurrence record [1]	Broken Link Tributray & Sunny Point Bay	Rankin Lake Tributary & Little Otter Bay	Link Property Tributray & Link Shore	Blue Lake Road Beaver Pond & Sand Bay	Salmon Lake Tributray & Salmon Lake Bay	Sovereign Lake Tributary & Mud Bay
Annelida	Clitellata	Worms	Crassiclitellata	Lumbricidae	*Lumbricus*	*Lumbricus rubellus*	Red marsh worm*	100.00	Y	X					
Annelida	Clitellata	Worms	Enchytraeida	Enchytraeidae	*Cernosvitoviella*	sp.	Unknown worm sp.	-	Y	X					X
Annelida	Clitellata	Worms	Haplotaxida	Naididae	*Aulodrilus*	*Aulodrilus pluriseta*	Aquatic oligochaete worm	100.00	Y		X			X	
Annelida	Clitellata	Worms	Haplotaxida	Naididae	*Isochaetides*	*Isochaetides freyi*	Aquatic oligochaete worm	100.00	Y					X	
Annelida	Clitellata	Worms	Unknown	Unknown	Unknown	sp.	Unknown worm sp.	-	-	X					
Annelida	Clitellata	Worms	Unknown	Unknown	Unknown	sp.	Unknown worm sp.	-	-					X	X
Annelida	Clitellata	Worms	Unknown	Unknown	Unknown	sp.	Unknown worm sp.	-	-		X			X	X
Arthropoda	Arachnida	Spiders	Araneae	Unknown	Unknown	sp.	Unknown spider sp.	-	Y					X	
Arthropoda	Branchiopoda	Zooplankton (Water Fleas)	Diplostraca	Bosminidae	*Bosmina*	*Bosmina freyi*	Waterflea	100.00	Y						X
Arthropoda	Branchiopoda	Zooplankton (Water Fleas)	Diplostraca	Chydoridae	*Acroperus*	*Acroperus harpae*	Waterflea	100.00	-					X	
Arthropoda	Branchiopoda	Zooplankton (Water Fleas)	Diplostraca	Daphniidae	*Simocephalus*	sp.	Unknown waterflea sp.	-	-				X		
Arthropoda	Branchiopoda	Zooplankton (Water Fleas)	Diplostraca	Sididae	*Diaphanosoma*	sp.	Unknown waterflea sp.	-	-						X
Arthropoda	Collembola	Hexapods (Springtails)	Symphypleona	Dicyrtomidae	*Octoglena*	sp.	Unknown springtail sp.	-	-	X					
Arthropoda	Diplopoda	Millipedes	Polyzoniida	Hirudisomatidae	Unknown	sp.	Unknown millipede sp.	-	-	X	X				
Arthropoda	Hexanauplia	Zooplankton (Copepods)	Calanoida	Diaptomidae	*Leptodiaptomus*	*Leptodiaptomus minutus*	Copepod	100.00	-	X				X	
Arthropoda	Hexanauplia	Zooplankton (Copepods)	Calanoida	Diaptomidae	*Skistodiaptomus*	*Skistodiaptomus reighardi*	Copepod	100.00	-				X		X
Arthropoda	Hexanauplia	Zooplankton (Copepods)	Calanoida	Pontellidae	Unknown	sp.	Unknown copepod sp.	-	-	X	X			X	X
Arthropoda	Hexanauplia	Zooplankton (Copepods)	Cyclopoida	Cyclopidae	*Macrocyclops*	*Macrocyclops fuscus*	Copepod	99.30	Y				X		
Arthropoda	Hexanauplia	Zooplankton (Copepods)	Cyclopoida	Cyclopidae	*Macrocyclops*	sp.	Unknown copepod sp.	-	-				X		
Arthropoda	Hexanauplia	Zooplankton (Copepods)	Cyclopoida	Cyclopidae	Unknown	sp.	Unknown copepod sp.	-	-				X	X	
Arthropoda	Hexanauplia	Zooplankton (Copepods)	Cyclopoida	Cyclopidae	Unknown	sp.	Unknown copepod sp.	-	-			X			
Arthropoda	Hexanauplia	Zooplankton (Copepods)	Cyclopoida	Cyclopidae	Unknown	sp.	Unknown copepod sp.	-	-				X	X	
Arthropoda	Hexanauplia	Zooplankton (Copepods)	Cyclopoida	Cyclopidae	Unknown	sp.	Unknown copepod sp.	-	-				X	X	
Arthropoda	Hexanauplia	Zooplankton (Copepods)	Cyclopoida	Cyclopidae	Unknown	sp.	Unknown copepod sp.	-	Y					X	
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Carabidae	*Agonum*	sp.	Unknown beetle sp.	-	-		X				
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Carabidae	Unknown	sp.	Unknown beetle sp.	-	-	X					
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Carabidae	Unknown	sp.	Unknown beetle sp.	-	-	X					
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Cerambycidae	*Orthosoma*	*Orthosoma brunneum*	Brown pionid	100.00	Y						X
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Chrysomelidae	*Phyllobrotica*	sp.	Unknown beetle sp.	-	Y	X					
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Chrysomelidae	Unknown	sp.	Unknown beetle sp.	-	-			X	X	X	X
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Curculionidae	*Sciaphilus*	*Sciaphilus asperatus*	Strawberry root weevil*	100.00	-	X	X			X	
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Curculionidae	*Sciaphilus*	sp.	Unknown beetle sp.	-	-	X					
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Dytiscidae	*Graphoderus*	*Graphoderus liberus*	Giant diving beetle	100.00	-				X		
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Elateridae	*Hemicrepidius*	*Hemicrepidius memnonius*	Click beetle	100.00	-	X					
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Elateridae	*Pseudanostirus*	*Pseudanostirus hieroglyphicus*	Click beetle	100.00	-	X					
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Elmidae	*Dubiraphia*	sp.	Unknown beetle sp.	-	-		X			X	
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Elmidae	*Macronychus*	*Macronychus glabratus*	Riffle beetle	100.00	-		X				
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Elmidae	*Stenelmis*	sp.	Unknown beetle sp.	-	-					X	X
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Hydrophilidae	*Tropisternus*	*Tropisternus mixtus*	Water scavenger beetle	99.30	-				X		
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Lycidae	*Plateros*	*Plateros bispiculatus*	Net-winged beetle	100.00	-		X				
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Rutelidae	*Popillia*	*Popillia japonica*	Japanese beetle*	100.00	-				X		
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Scirtidae	*Prionocyphon*	*Prionocyphon limbatus*	Marsh beetle	98.59	-	X	X				
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Staphylinidae	*Dinothenarus*	*Dinothenarus badipes*	Large rove beetle	98.59	-	X					
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Staphylinidae	*Philonthus*	sp.	Unknown large rove beetle sp.	-	-	X					
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Tenebrionidae	*Isomira*	sp.	Unknown comb-clawed beetles sp.	-	-					X	
Arthropoda	Insecta	Insects (Beetles)	Coleoptera	Unknown	Unknown	sp.	Unknown beetle sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Anthomyiidae	*Pegomya*	sp.	Unknown root-maggot fly sp.	-	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Asilidae	*Neoitamus*	*Neoitamus flavofemoratus*	Robber fly	100.00	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Asilidae	*Tipulogaster*	*Tipulogaster glabrata*	Robber fly	99.30	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Calliphoridae	*Pollenia*	*Pollenia griseotomentosa*	Cluster fly	100.00	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Ceratopogonidae	*Atrichopogon*	sp.	Unknown biting midge sp.	-	-			X		X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Ceratopogonidae	*Atrichopogon*	sp.	Unknown biting midge sp.	-	-			X			
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Ceratopogonidae	*Bezzia*	sp.	Unknown biting midge sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Ceratopogonidae	*Bezzia*	sp.	Unknown biting midge sp.	-	-				X	X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Ceratopogonidae	*Bezzia*	sp.	Unknown biting midge sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Ceratopogonidae	*Bezzia*	sp.	Unknown biting midge sp.	-	-			X			
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Ceratopogonidae	*Bezzia*	sp.	Unknown biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Ceratopogonidae	*Palpomyia*	sp.	Unknown biting midge sp.	-	-				X	X	

Note: * Introduced non-native aquatic invasive species; Y – Yes; X - Presence (i.e., matching DNA sequence detected in sample); [1] There is lower support for this taxonomic identification as it is based on fewer than three matches to sequences in the reference database, and/or limited geographic occurrence records for the taxon; Orange - Unknown species with no known reference DNA sequence

Table E-1: Macroinvertebrate biodiversity (i.e., diversity of macroinvertebrate species) detected using eDNA (**Appendix D**), in various sampling locations around Otter Lake, 6 August 2022 (Continued)

Phylum	Class	Class Common Name	Order	Family	Scientific Name (Genus)	Scientific Name (Genus & Species)	Common Name	Similarity to Species-level Sequence ID	Less than 3 Matches to Sequences Reference Database or Limited geographic occurrence record [1]	Broken Link Tributray & Sunny Point Bay	Rankin Lake Tributary & Little Otter Bay	Link Property Tributray & Link Shore	Blue Lake Road Beaver Pond & Sand Bay	Salmon Lake Tributray & Salmon Lake Bay	Sovereign Lake Tributary & Mud Bay
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Ceratopogonidae	*Palpomyia*	sp.	Unknown biting midge sp.	-	-		X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Ceratopogonidae	*Stilobezzia*	sp.	Unknown biting midge sp.	-	-				X		
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Ceratopogonidae	*Stilobezzia*	sp.	Unknown biting midge sp.	-	-						X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Ceratopogonidae	Unknown	sp.	Unknown biting midge sp.	-	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chaoboridae	*Chaoborus*	*Chaoborus americanus*	Phantom midge	100.00	-				X		
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chaoboridae	*Chaoborus*	*Chaoborus flavicans*	Phantom midge	100.00	-	X		X	X	X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chaoboridae	*Chaoborus*	*Chaoborus punctipennis*	Phantom midge	100.00	-	X	X	X	X	X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chaoboridae	*Chaoborus*	sp.	Unknown phantom midge sp.	-	-				X		
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chaoboridae	*Chaoborus*	sp.	Unknown phantom midge sp.	-	-				X		
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chaoboridae	*Chaoborus*	sp.	Unknown phantom midge sp.	-	-			X	X		X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Ablabesmyia*	*Ablabesmyia annulata*	Non-biting midge sp. 1	100.00	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Ablabesmyia*	*Ablabesmyia annulata*	Non-biting midge sp. 2	98.59	Y			X			
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Ablabesmyia*	*Ablabesmyia aspera*	Non-biting midge	99.30	Y				X	X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Ablabesmyia*	*Ablabesmyia monilis*	Non-biting midge	100.00	-			X	X	X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Ablabesmyia*	sp.	Unknown non-biting midge sp.	-	-				X	X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Ablabesmyia*	sp.	Unknown non-biting midge sp.	-	-					X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Ablabesmyia*	sp.	Unknown non-biting midge sp.	-	-				X		
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Arctopelopia*	sp.	Unknown non-biting midge sp.	-	-	X	X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Chironomus*	*Chironomus bifurcatus*	Non-biting midge	100.00	-		X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Chironomus*	*Chironomus maturus*	Non-biting midge	100.00	-	X	X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Chironomus*	sp.	Unknown non-biting midge sp.	-	-	X	X			X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Chironomus*	sp.	Unknown non-biting midge sp.	-	-				X	X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Chironomus*	sp.	Unknown non-biting midge sp.	-	Y						X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Chironomus*	sp.	Unknown non-biting midge sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Chironomus*	sp.	Unknown non-biting midge sp.	-	-				X		
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Cladopelma*	sp.	Unknown non-biting midge sp.	-	-			X		X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Cladotanytarsus*	sp.	Unknown non-biting midge sp.	-	-			X	X	X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Cladotanytarsus*	sp.	Unknown non-biting midge sp.	-	-	X		X		X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Conchapelopia*	*Conchapelopia telema*	Non-biting midge	99.29	-	X					X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Conchapelopia*	sp.	Unknown non-biting midge sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Corynoneura*	sp.	Unknown non-biting midge sp.	-	-	X	X			X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Corynoneura*	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Corynoneura*	sp.	Unknown non-biting midge sp.	-	-		X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Corynoneura*	sp.	Unknown non-biting midge sp.	-	-	X	X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Corynoneura*	sp.	Unknown non-biting midge sp.	-	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Corynoneura*	sp.	Unknown non-biting midge sp.	-	-				X		X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Cricotopus*	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Cricotopus*	sp.	Unknown non-biting midge sp.	-	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Cryptochironomus*	sp.	Unknown non-biting midge sp.	-	-				X		X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Dicrotendipes*	*Dicrotendipes modestus*	Non-biting midge sp. 1	100.00	-			X		X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Dicrotendipes*	*Dicrotendipes modestus*	Non-biting midge sp. 2	100.00	-		X	X			X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Dicrotendipes*	*Dicrotendipes modestus*	Non-biting midge sp. 3	98.59	-		X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Dicrotendipes*	*Dicrotendipes tritomus*	Non-biting midge	100.00	-	X				X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Eukiefferiella*	*Eukiefferiella claripennis*	Non-biting midge	100.00	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Micropsectra*	*Micropsectra polita*	Non-biting midge sp. 1	100.00	-	X					X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Micropsectra*	*Micropsectra polita*	Non-biting midge sp. 2	100.00	-	X					X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Micropsectra*	*Micropsectra xantha*	Non-biting midge	100.00	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Micropsectra*	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Micropsectra*	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Micropsectra*	sp.	Unknown non-biting midge sp.	-	-	X					X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Microtendipes*	sp.	Unknown non-biting midge sp.	-	-			X			X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Nanocladius*	*Nanocladius balticus*	Non-biting midge	98.58	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Nanocladius*	*Nanocladius distinctus*	Non-biting midge	99.30	Y		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Nanocladius*	sp.	Unknown non-biting midge sp.	-	-		X			X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Nilotanypus*	*Nilotanypus fimbriatus*	Non-biting midge	100.00	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Orthocladius*	sp.	Unknown non-biting midge sp.	-	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Orthocladius*	sp.	Unknown non-biting midge sp.	-	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Parachironomus*	*Parachironomus tenuicaudatus*	Non-biting midge	100.00	-	X		X			X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Parachironomus*	sp.	Unknown non-biting midge sp.	-	-		X		X		X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Parachironomus*	sp.	Unknown non-biting midge sp.	-	-						X

Note: * Introduced non-native aquatic invasive species; Y – Yes; X - Presence (i.e., matching DNA sequence detected in sample); [1] There is lower support for this taxonomic identification as it is based on fewer than three matches to sequences in the reference database, and/or limited geographic occurrence records for the taxon; Orange - Unknown species with no known reference DNA sequence

Table E-1: Macroinvertebrate biodiversity (i.e., diversity of macroinvertebrate species) detected using eDNA (**Appendix D**), in various sampling locations around Otter Lake, 6 August 2022 (Continued)

Phylum	Class	Class Common Name	Order	Family	Scientific Name (Genus)	Scientific Name (Genus & Species)	Common Name	Similarity to Species-level Sequence ID	Less than 3 Matches to Sequences Reference Database or Limited geographic occurrence record [1]	Broken Link Tributray & Sunny Point Bay	Rankin Lake Tributary & Little Otter Bay	Link Property Tributray & Link Shore	Blue Lake Road Beaver Pond & Sand Bay	Salmon Lake Tributray & Salmon Lake Bay	Sovereign Lake Tributary & Mud Bay
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Parakiefferiella*	sp.	Unknown non-biting midge sp.	-	-	X	X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Parakiefferiella*	sp.	Unknown non-biting midge sp.	-	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Parametriocnemus*	sp.	Unknown non-biting midge sp.	-	-	X	X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Parametriocnemus*	sp.	Unknown non-biting midge sp.	-	-						X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Parametriocnemus*	sp.	Unknown non-biting midge sp.	-	-	X	X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Paratanytarsus*	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Paratendipes*	sp.	Unknown non-biting midge sp.	-	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Phaenopsectra*	sp.	Unknown non-biting midge sp.	-	-				X	X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Polypedilum*	*Polypedilum aviceps*	Non-biting midge	100.00	-	X	X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Polypedilum*	*Polypedilum convictum*	Non-biting midge	100.00	-		X			X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Polypedilum*	sp.	Unknown non-biting midge sp.	-	-			X	X	X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Polypedilum*	sp.	Unknown non-biting midge sp.	-	-		X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Polypedilum*	sp.	Unknown non-biting midge sp.	-	-				X		
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Polypedilum*	sp.	Unknown non-biting midge sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Polypedilum*	sp.	Unknown non-biting midge sp.	-	-				X		
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Procladius*	*Procladius denticulatus*	Non-biting midge	100.00	-	X	X	X		X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Procladius*	sp.	Unknown non-biting midge sp.	-	-		X	X			
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Procladius*	sp.	Unknown non-biting midge sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Procladius*	sp.	Unknown non-biting midge sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Psectrocladius*	*Psectrocladius simulans*	Non-biting midge	99.30	Y					X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Psectrocladius*	*Psectrocladius sordidellus*	Non-biting midge	100.00	-					X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Rheocricotopus*	*Rheocricotopus robacki*	Non-biting midge	100.00	-						X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Rheotanytarsus*	sp.	Unknown non-biting midge sp.	-	-	X	X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Rheotanytarsus*	sp.	Unknown non-biting midge sp.	-	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Rheotanytarsus*	sp.	Unknown non-biting midge sp.	-	-	X	X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Rheotanytarsus*	sp.	Unknown non-biting midge sp.	-	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Rheotanytarsus*	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Stempellinella*	*Stempellinella fimbriata*	Non-biting midge	100.00	-		X			X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Stempellinella*	sp.	Unknown non-biting midge sp.	-	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Stempellinella*	sp.	Unknown non-biting midge sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Stempellinella*	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Stempellinella*	sp.	Unknown non-biting midge sp.	-	-	X	X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Stempellinella*	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Stempellinella*	sp.	Unknown non-biting midge sp.	-	-					X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	*Tanytarsus aigos*	Non-biting midge	99.30	-	X			X	X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	*Tanytarsus glabrescens*	Non-biting midge sp. 1	100.00	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	*Tanytarsus glabrescens*	Non-biting midge sp. 2	100.00	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	*Tanytarsus mendax*	Non-biting midge	100.00	-		X	X		X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	-	X	X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	-		X		X	X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	-	X	X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	-		X			X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	Y		X		X	X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	-			X	X	X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	-	X	X		X		X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	-			X			X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	-				X	X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	-			X	X	X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	-	X	X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	-				X		
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	-			X			
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	-	X		X			
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	-		X				

Note: * Introduced non-native aquatic invasive species; Y – Yes; X - Presence (i.e., matching DNA sequence detected in sample); [1] There is lower support for this taxonomic identification as it is based on fewer than three matches to sequences in the reference database, and/or limited geographic occurrence records for the taxon; Orange - Unknown species with no known reference DNA sequence

Table E-1: Macroinvertebrate biodiversity (i.e., diversity of macroinvertebrate species) detected using eDNA (**Appendix D**), in various sampling locations around Otter Lake, 6 August 2022 (Continued)

Phylum	Class	Class Common Name	Order	Family	Scientific Name (Genus)	Scientific Name (Genus & Species)	Common Name	Similarity to Species-level Sequence ID	Less than 3 Matches to Sequences Reference Database or Limited geographic occurrence record [1]	Broken Link Tributray & Sunny Point Bay	Rankin Lake Tributary & Little Otter Bay	Link Property Tributray & Link Shore	Blue Lake Road Beaver Pond & Sand Bay	Salmon Lake Tributray & Salmon Lake Bay	Sovereign Lake Tributary & Mud Bay
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	-					X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	-					X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tanytarsus*	sp.	Unknown non-biting midge sp.	-	-					X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Thienemanniella*	*Thienemanniella xena*	Non-biting midge sp. 1	100.00	-	X	X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Thienemanniella*	*Thienemanniella xena*	Non-biting midge sp. 2	100.00	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Thienemannimyia*	sp.	Unknown non-biting midge sp.	-	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Thienemannimyia*	sp.	Unknown non-biting midge sp.	-	-		X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tribelos*	sp.	Unknown non-biting midge sp.	-	-		X			X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Tvetenia*	*Tvetenia paucunca*	Non-biting midge	100.00	-	X	X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Xylotopus*	sp.	Unknown non-biting midge sp.	-	-	X	X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	*Zavreliella*	*Zavreliella marmorata*	Non-biting midge	100.00	Y			X	X		
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X	X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-		X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X	X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X		X	X	X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-				X		X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-		X		X	X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-		X		X	X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X	X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-						X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X	X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X	X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-		X	X		X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X	X			X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-						X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	Y	X		X	X	X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X	X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-		X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-					X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-						X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X	X			X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X	X			X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-			X			
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-				X		
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-			X		X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X	X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-				X	X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-				X	X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-			X	X		X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X	X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-		X			X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-			X	X		
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-				X		
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					

Note: * Introduced non-native aquatic invasive species; Y – Yes; X - Presence (i.e., matching DNA sequence detected in sample); [1] There is lower support for this taxonomic identification as it is based on fewer than three matches to sequences in the reference database, and/or limited geographic occurrence records for the taxon; Orange - Unknown species with no known reference DNA sequence

Table E-1: Macroinvertebrate biodiversity (i.e., diversity of macroinvertebrate species) detected using eDNA (**Appendix D**), in various sampling locations around Otter Lake, 6 August 2022 (Continued)

Phylum	Class	Class Common Name	Order	Family	Scientific Name (Genus)	Scientific Name (Genus & Species)	Common Name	Similarity to Species-level Sequence ID	Less than 3 Matches to Sequences Reference Database or Limited geographic occurrence record [1]	Broken Link Tributray & Sunny Point Bay	Rankin Lake Tributary & Little Otter Bay	Link Property Tributray & Link Shore	Blue Lake Road Beaver Pond & Sand Bay	Salmon Lake Tributray & Salmon Lake Bay	Sovereign Lake Tributary & Mud Bay
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-						X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-					X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-				X		
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-		X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X	X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X	X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-				X	X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-				X		X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	Y					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-				X		
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-			X	X	X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	Y						X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-			X			
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-					X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-		X			X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-					X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-					X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-		X		X		
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-					X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-					X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-		X			X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-					X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-						X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-						X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X				X	

Note: * Introduced non-native aquatic invasive species; Y – Yes; X - Presence (i.e., matching DNA sequence detected in sample); [1] There is lower support for this taxonomic identification as it is based on fewer than three matches to sequences in the reference database, and/or limited geographic occurrence records for the taxon; Orange - Unknown species with no known reference DNA sequence

Table E-1: Macroinvertebrate biodiversity (i.e., diversity of macroinvertebrate species) detected using eDNA (**Appendix D**), in various sampling locations around Otter Lake, 6 August 2022 (Continued)

Phylum	Class	Class Common Name	Order	Family	Scientific Name (Genus)	Scientific Name (Genus & Species)	Common Name	Similarity to Species-level Sequence ID	Less than 3 Matches to Sequences Reference Database or Limited geographic occurrence record [1]	Broken Link Tributray & Sunny Point Bay	Rankin Lake Tributary & Little Otter Bay	Link Property Tributray & Link Shore	Blue Lake Road Beaver Pond & Sand Bay	Salmon Lake Tributray & Salmon Lake Bay	Sovereign Lake Tributary & Mud Bay
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Chironomidae	Unknown	sp.	Unknown non-biting midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Culicidae	*Aedes*	*Aedes excrucians*	Woodland snowmelt mosquito	100.00	-		X			X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Culicidae	*Anopheles*	sp.	Unknown mosquito sp.	-	-	X	X	X		X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Dixidae	Unknown	sp.	Unknown meniscus midge sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Dolichopodidae	*Gymnopternus*	sp.	Unknown long-legged fly sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Empididae	*Hemerodromia*	sp.	Unknown dance fly sp.	-	-		X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Limoniidae	*Geranomyia*	sp.	Unknown crane fly sp.	-	-						X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Limoniidae	*Molophilus*	sp.	Unknown crane fly sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Limoniidae	*Molophilus*	sp.	Unknown crane fly sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Limoniidae	*Molophilus*	sp.	Unknown crane fly sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Limoniidae	Unknown	sp.	Unknown crane fly sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Limoniidae	Unknown	sp.	Unknown crane fly sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Phoridae	*Megaselia*	*Megaselia arcticae*	Scuttle fly	100.00	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Ptychopteridae	*Bittacomorpha*	*Bittacomorpha clavipes*	Eastern phantom crane fly	100.00	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Simuliidae	*Simulium*	*Simulium tuberosum*	Twinn's black fly sp.1	100.00	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Simuliidae	*Simulium*	*Simulium tuberosum*	Twinn's black fly sp.2	100.00	Y		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Simuliidae	*Simulium*	sp.	Unknown black fly sp.	-	-	X	X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Simuliidae	*Simulium*	sp.	Unknown black fly sp.	-	-	X	X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Simuliidae	*Simulium*	sp.	Unknown black fly sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Tabanidae	*Chrysops*	*Chrysops carbonarius*	Deer fly	100.00	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Tabanidae	*Hybomitra*	*Hybomitra cincta*	Horse fly	100.00	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Tabanidae	Unknown	sp.	Unknown deer or horse fly sp.	-	-		X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Tabanidae	Unknown	sp.	Unknown deer or horse fly sp.	-	Y					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Tachinidae	*Parasetigena*	*Parasetigena silvestris*	Parasitic fly	100.00	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Tipulidae	*Tipula*	*Tipula abdominalis*	Giant crane fly	100.00	-	X	X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Tipulidae	*Tipula*	sp.	Unknown giant crane fly sp.	-	-	X	X				X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Tipulidae	*Tipula*	sp.	Unknown giant crane fly sp.	-	-	X	X				
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Tipulidae	Unknown	sp.	Unknown large crane fly sp.	-	-	X					
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Unknown	Unknown	sp.	Unknown fly sp.	-	-		X	X		X	X
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Unknown	Unknown	sp.	Unknown fly sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Unknown	Unknown	sp.	Unknown fly sp.	-	-					X	
Arthropoda	Insecta	Insects (Flies, Midges & Mosquitos)	Diptera	Unknown	Unknown	sp.	Unknown fly sp.	-	-		X				
Arthropoda	Insecta	Insects (Mayflies)	Ephemeroptera	Caenidae	*Caenis*	***Caenis diminuta*** /*Caenis youngi*	**Small square-gilled mayfly** / Young's small square-gilled mayfly	100.00	Y	X			X		X
Arthropoda	Insecta	Insects (Mayflies)	Ephemeroptera	Ephemerellidae	*Eurylophella*	*Eurylophella verisimilis*	Spiny crawler mayfly	100.00	-		X				
Arthropoda	Insecta	Insects (Mayflies)	Ephemeroptera	Ephemeridae	*Hexagenia*	*Hexagenia limbata*	Giant burrowing mayfly	100.00	Y					X	
Arthropoda	Insecta	Insects (Mayflies)	Ephemeroptera	Leptophlebiidae	*Leptophlebia*	***Leptophlebia cupida*** /*Leptophlebia nebulosa*	**Early brown spinner** / prong-gilled mayfly	100.00	-	X					
Arthropoda	Insecta	Insects (True Bugs)	Hemiptera	Nepidae	*Ranatra*	*Ranatra nigra*	Waterscorpion	99.30	-		X				
Arthropoda	Insecta	Insects (True Bugs)	Hemiptera	Pleidae	*Neoplea*	*Neoplea striola*	Pygmy backswimmer	100.00	-				X		
Arthropoda	Insecta	Insects (True Bugs)	Hemiptera	Pleidae	*Neoplea*	sp.	Unknown pygmy backswimmer sp.	-	-				X		
Arthropoda	Insecta	Insects (True Bugs)	Hemiptera	Pleidae	Unknown	sp.	Unknown pygmy backswimmer sp.	-	-				X		
Arthropoda	Insecta	Insects (Alderflies, Dobsonflies & Fishflies)	Megaloptera	Corydalidae	*Chauliodes*	*Chauliodes rastricornis*	Spring flyfish	100.00	-		X			X	
Arthropoda	Insecta	Insects (Alderflies, Dobsonflies & Fishflies)	Megaloptera	Corydalidae	*Nigronia*	*Nigronia serricornis*	Saw-combed fishfly	100.00	-		X				
Arthropoda	Insecta	Insects (Alderflies, Dobsonflies & Fishflies)	Megaloptera	Sialidae	*Sialis*	*Sialis vagans*	Alderfly	100.00	-					X	
Arthropoda	Insecta	Insects (Grasshoppers, Crickets & Katydids)	Orthoptera	Rhaphidophoridae	*Ceuthophilus*	*Ceuthophilus guttulosus*	Thomas' camel cricket	100.00	-	X					
Arthropoda	Insecta	Insects (Stoneflies)	Plecoptera	Leuctridae	*Leuctra*	*Leuctra tenuis*	Rolled-winged stonefly sp. 1	99.30	Y		X				
Arthropoda	Insecta	Insects (Stoneflies)	Plecoptera	Leuctridae	*Leuctra*	*Leuctra tenuis*	Rolled-winged stonefly sp. 2	98.59	-		X				
Arthropoda	Insecta	Insects (Stoneflies)	Plecoptera	Leuctridae	*Leuctra*	sp.	Unknown rolled-winged stonefly	-	-		X				
Arthropoda	Insecta	Insects (Stoneflies)	Plecoptera	Leuctridae	Unknown	sp.	Unknown rolled-winged stonefly	-	-	X					
Arthropoda	Insecta	Insects (Stoneflies)	Plecoptera	Nemouridae	*Amphinemura*	*Amphinemura delosa* /***Amphinemura nigritta***	Spring stonefly sp. 1 / **Little black forestfly**	100.00	Y		X				
Arthropoda	Insecta	Insects (Stoneflies)	Plecoptera	Nemouridae	*Soyedina*	*Soyedina vallicularia*	Valley forestfly	100.00	-	X					
Arthropoda	Insecta	Insects (Barklice, Booklice & Parasitic Lice)	Psocodea	Caeciliusidae	*Valenzuela*	sp.	Unknown lizard barklice sp.	-	-					X	
Arthropoda	Insecta	Insects (Caddisflies)	Trichoptera	Lepidostomatidae	*Lepidostoma*	*Lepidostoma griseum*	Bizarre caddisfly	100.00	-	X					
Arthropoda	Insecta	Insects (Caddisflies)	Trichoptera	Molannidae	Unknown	sp.	Unknown hood-casemaker caddisfly sp.	-	-	X					
Arthropoda	Insecta	Insects (Caddisflies)	Trichoptera	Philopotamidae	*Dolophilodes*	*Dolophilodes distinctus*	Fingernet caddisfly	100.00	-	X					
Arthropoda	Malacostraca	Crustaceans (Crayfish)	Decapoda	Cambaridae	*Faxonius*	*Faxonius propinquus*	Northern clearwater crayfish	100.00	-	X				X	X
Arthropoda	Ostracoda	Seed shrimps	Podocopida	Cyprididae	*Cypridopsis*	*Cypridopsis vidua*	Seed shrimp sp. 1	100.00	-				X		
Arthropoda	Ostracoda	Seed shrimps	Podocopida	Cyprididae	*Cypridopsis*	*Cypridopsis vidua*	Seed shrimp sp. 2	100.00	-				X		
Arthropoda	Ostracoda	Seed shrimps	Podocopida	Cyprididae	*Cypridopsis*	sp.	Unknown seed shrimp	-	-				X		
Arthropoda	Ostracoda	Seed shrimps	Podocopida	Cyprididae	*Cypridopsis*	sp.	Unknown seed shrimp	-	-				X		
Arthropoda	Ostracoda	Seed shrimps	Podocopida	Cyprididae	Unknown	sp.	Unknown seed shrimp	-	-				X		
Arthropoda	Ostracoda	Seed shrimps	Podocopida	Cyprididae	Unknown	sp.	Unknown seed shrimp	-	-				X		
Arthropoda	Ostracoda	Seed shrimps	Podocopida	Cyprididae	Unknown	sp.	Unknown seed shrimp	-	Y				X		
Arthropoda	Ostracoda	Seed shrimps	Podocopida	Cyprididae	Unknown	sp.	Unknown seed shrimp	-	Y				X		
Arthropoda	Ostracoda	Seed shrimps	Podocopida	Cyprididae	Unknown	sp.	Unknown seed shrimp	-	Y				X		
Arthropoda	Bivalvia	Bivalves	Unionida	Unionidae	*Pyganodon*	*Pyganodon grandis*	Giant floater	100.00	-					X	
Arthropoda	Eurotatoria	Zooplankton (Rotifers)	Ploima	Brachionidae	*Keratella*	*Keratella crassa*	Rotifer	98.61	Y		X		X		X
									Total Number of Taxa:	**132**	**121**	**42**	**76**	**123**	**115**

Note: * Introduced non-native aquatic invasive species; Y – Yes; X - Presence (i.e., matching DNA sequence detected in sample); [1] There is lower support for this taxonomic identification as it is based on fewer than three matches to sequences in the reference database, and/or limited geographic occurrence records for the taxon; **Bold** - Most likely species due to location & distribution range in Ontario; Orange - Unknown species with no known reference DNA sequence

Table E-2: Fish biodiversity (i.e., diversity of fish species) detected using eDNA (**Appendix D**), in various sampling locations around Otter Lake, 6 August 2022

Assay Used	Family	Genus	Species	Common Name	IUCN	SARA	SARO	COSEWIC	Similarity to Species-level Sequence ID	Less than 3 Matches to Sequences Reference Database or Limited geographic occurrence record [1]	Broken Link Tributray & Sunny Point Bay	Rankin Lake Tributary & Little Otter Bay	Link Property Tributray & Link Shore	Blue Lake Road Beaver Pond & Sand Bay	Salmon Lake Tributray & Salmon Lake Bay	Sovereign Lake Tributary & Mud Bay
Fish	Cyprinidae	*Chrosomus*	*Chrosomus eos*	Northern Redbelly Dace	Least Concern	-	-	-	99.43	Y			X			
	Cyprinidae	*Chrosomus*	*Chrosomus neogaeus*	Finescale Dace	Least Concern	-	-	-	99.43	Y	X		X			
	Cyprinidae	*Chrosomus*	sp.	Unknown Dace sp.	-	-	-	-	-	Y	X		X			
	Cyprinidae	*Gila*	sp.	Unknown Chub sp.	-	-	-	-	-	-	X		X			
	Cyprinidae	*Hybognathus*	sp.	Unknown Minnow sp.	-	-	-	-	-	Y	X					
	Cyprinidae	*Luxilus*	*Luxilus chrysocephalus* / ***Luxilus cornutus***	Striped Shiner / **Common Shiner**	Least Concern / **Least Concern**	Not at Risk	-	-	100.00	Y		X	X	X		X
	Cyprinidae	*Margariscus*	sp. (*Margariscus nachtriebi*)	Northern Pearl Dace	Least Concern	-	-	-	-	Y	X					
	Cyprinidae	*Notemigonus*	*Notemigonus crysoleucas*	Golden Shiner	Least Concern	-	-	-	100.00	-			X	X	X	X
	Cyprinidae	*Notropis*	*Notropis heterolepis*	Blacknose Shiner	Least Concern	-	-	-	100.00	Y	X		X	X		X
	Cyprinidae	*Pimephales*	*Pimephales notatus*	Bluntnose Minnow	Least Concern	Not at Risk	-	Not at Risk	99.43	-		X		X	X	X
	Cyprinidae	*Pimephales*	*Pimephales promelas*	Fathead Minnow	Least Concern	-	-	-	100.00	Y			X		X	
	Cyprinidae	*Rhinichthys*	sp.	Unknown Dace sp.	-	-	-	-	-	-	X	X		X		
	Cyprinidae	*Semotilus*	*Semotilus atromaculatus*	Creek Chub	Least Concern	-	-	-	100.00	-	X	X	X		X	X
	Cyprinidae	Unknown	sp.	Unknown Cyprinid sp.	-	-	-	-	-	-	X	X	X	X		X
	Cyprinidae	Unknown	sp.	Unknown Cyprinid sp.	-	-	-	-	-	-			X			
	Cyprinidae	Unknown	sp.	Unknown Cyprinid sp.	-	-	-	-	-	-			X			
	Cyprinidae	Unknown	sp.	Unknown Cyprinid sp.	-	-	-	-	-	-			X			
	Esocidae	*Esox*	*Esox lucius*	Northern Pike	Least Concern	-	-	-	100.00	-						X
	Umbridae	*Umbra*	*Umbra limi*	Central Mudminnow	Least Concern	-	-	-	100.00	-	X		X	X		X
	Centrarchidae	*Ambloplites*	*Ambloplites rupestris*	Rock Bass	Least Concern	-	-	-	100.00	-	X	X		X	X	X
	Centrarchidae	*Lepomis*	*Lepomis gibbosus*	Pumpkinseed	Least Concern	-	-	-	100.00	-	X	X	X	X	X	X
	Centrarchidae	*Micropterus*	*Micropterus dolomieu*	Smallmouth Bass	Least Concern	-	-	-	100.00	-	X	X			X	X
	Centrarchidae	*Micropterus*	*Micropterus salmoides*	Largemouth Bass	Least Concern	-	-	-	100.00	-	X	X	X	X	X	X
	Centrarchidae	*Pomoxis*	*Pomoxis nigromaculatus*	Black Crappie	Least Concern	-	-	-	100.00	-				X		X
	Percidae	*Etheostoma*	*Etheostoma exile*	Iowa Darter	Least Concern	-	-	-	100.00	Y		X		X	X	X
	Percidae	*Perca*	*Perca flavescens*	Yellow Perch	Least Concern	-	-	-	100.00	-		X		X	X	X
	Percidae	*Percina*	***Percina caprodes*** /*Percina copelandi*	**Common Logperch** / Channel Darter	**Least Concern** / Least Concern	- / Special Concern	- / Special Concern	- / Special Concern	99.41	Y		X				
	Ictaluridae	*Ameiurus*	*Ameiurus nebulosus*	Brown Bullhead	Least Concern	-	-	-	100.00	-	X			X	X	X
Vertebrates	Catostomidae	Catostomus	*Catostomus commersonii*	White Sucker	Least Concern	-	-	-	100.00	-			X			X
	Cyprinidae	Luxilus	*Luxilus cornutus*	**Common Shiner**	Least Concern	Not at Risk	-	-	100.00	-		X				X
	Cyprinidae	Nocomis	*Nocomis biguttatus*	Hornyhead Chub	Least Concern	Not at Risk	-	Not at Risk	100.00	Y				X		
	Cyprinidae	Notropis	*Notropis dorsalis*	Bigmouth Shiner / **Shiner sp.**	Least Concern	Not at Risk	-	Not at Risk	99.07	Y	X		X			X
										Total Number of Species:	**16**	**13**	**18**	**15**	**11**	**19**

Note: IUCN – International Union for Conservation of Nature's Red List (2023); SARA - Government of Canada's Federal Species-at-Risk Public Registry (GOC, 2023); SARO - Species at Risk in Ontario (GOO, 2023); COSEWIC - Committee on the Status of Endangered Wildlife in Canada (COSEWIC, 2023) ; Y – Yes; X – Presence (i.e., matching DNA sequence detected in sample); **Bold** - Most likely species due to location & distribution range in Ontario; Orange - Unknown species with no known reference DNA sequence; Green - Added as only likely species or sub-species of genus within area; Red - Unlikely species due to distribution range

Table E-3: Amphibian biodiversity (i.e., diversity of amphibian species) detected using eDNA (**Appendix D**), in various sampling locations around Otter Lake, 6 August 2022

Assay Used	Family	Genus	Species	Common Name	IUCN	SARA	SARO	COSEWIC	Similarity to Species-level Sequence ID	Less than 3 Matches to Sequences Reference Database or Limited geographic occurrence record [1]	Broken Link Tributray & Sunny Point Bay	Rankin Lake Tributary & Little Otter Bay	Link Property Tributray & Link Shore	Blue Lake Road Beaver Pond & Sand Bay	Salmon Lake Tributray & Salmon Lake Bay	Sovereign Lake Tributary & Mud Bay
Fish	Plethodontidae	*Plethodon*	*Plethodon cinereus*	Eastern Red-backed Salamander	Least Concern	-	-	Candidate (Group 2 priority)	99.36	Y						X
	Salamandridae	*Notophthalmus*	sp. (*Notophthalmus viridescens viridescens*)	Red-Spotted Newt / Eastern Newt	Least Concern	-	-	-	-	Y				X		
Vertebrates	Hylidae	*Dryophytes*	*Dryophytes chrysoscelis* / ***Dryophytes versicolor***	Cope's Gray Treefrog / **Gray Treefrog**	Least Concern / **Least Concern**	-	-	Candidate (Group 2 priority) / Candidate (Group 2 priority)	100.00	-				X		
	Ranidae	*Lithobates*	*Lithobates catesbeianus* / ***Lithobates clamitans***	American Bullfrog / **Green Frog**	Least Concern / **Least Concern**	-	-	Candidate (Group 3 priority)	100.00	-	X	X	X	X	X	X
	Ranidae	*Lithobates*	*Lithobates pipiens*	Northern Leopard Frog	Least Concern	Not at Risk	-	Onatrio populations - Not at Risk	100.00	-	X				X	
	Ambystomatidae	*Ambystoma*	*Ambystoma laterale*	Blue-Spotted Salamander	Least Concern	Not at Risk	-	Candidate (Group 3 priority)	100.00	Y				X		
	Ambystomatidae	*Ambystoma*	sp. (*Ambystoma maculatum*)	Spotted Salamander	Least Concern	-	-	Candidate (Group 3 priority)	-	Y				X		
	Plethodontidae	*Eurycea*	*Eurycea bislineata*	Northern Two-Lined Salamander	Least Concern	-	-	Candidate (Group 3 priority)	100.00	-	X	X				
	Proteidae	*Necturus*	*Necturus maculosus*	Mudpuppy	Least Concern	Not at Risk	-	-	100.00	Y					X	
	Salamandridae	*Notophthalmus*	*Notophthalmus viridescens viridescens*	Red-Spotted Newt / Eastern Newt	Least Concern	-	-	Candidate (Group 3 priority)	100.00	-				X		
										Total Number of Species:	**3**	**2**	**1**	**6**	**3**	**2**

Note: IUCN – International Union for Conservation of Nature's Red List (2023); SARA - Government of Canada's Federal Species-at-Risk Public Registry (GOC, 2023); SARO - Species at Risk in Ontario (GOO, 2023); COSEWIC - Committee on the Status of Endangered Wildlife in Canada (COSEWIC, 2023) ; Y – Yes; X – Presence (i.e., matching DNA sequence detected in sample); **Bold** - Most likely species due to location & distribution range in Ontario; Orange - Unknown species with no known reference DNA sequence; Green - Added as only likely species or sub-species of genus within area

Table E-4: Reptile biodiversity (i.e., diversity of reptile species) detected using eDNA (**Appendix D**), in various sampling locations around Otter Lake, 6 August 2022

Assay Used	Family	Genus	Species	Common Name	IUCN	SARA	SARO	COSEWIC	Similarity to Species-level Sequence ID	Less than 3 Matches to Sequences Reference Database or Limited geographic occurrence record [1]	Broken Link Tributray & Sunny Point Bay	Rankin Lake Tributary & Little Otter Bay	Link Property Tributray & Link Shore	Blue Lake Road Beaver Pond & Sand Bay	Salmon Lake Tributray & Salmon Lake Bay	Sovereign Lake Tributary & Mud Bay
Vertebrates	Chelydridae	*Chelydra*	*Chelydra serpentina*	Common Snapping Turtle	Least Concern	-	Special Concern	-	100.00	-				X		
	Emydidae	*Chrysemys*	*Chrysemys picta (Chrysemys picta marginata)*	Midland Painted Turtle	Least Concern	Special Concern	Special Concern	Special Concern	100.00	Y				X		
										Total Number of Species:	**0**	**0**	**0**	**2**	**0**	**0**

Note: IUCN – International Union for Conservation of Nature's Red List (2023); SARA - Government of Canada's Federal Species-at-Risk Public Registry (GOC, 2023); SARO - Species at Risk in Ontario (GOO, 2023); COSEWIC - Committee on the Status of Endangered Wildlife in Canada (COSEWIC, 2023) ; Y – Yes; X – Presence (i.e., matching DNA sequence detected in sample); Green - Added as only likely species or sub-species of genus within area

Table E-5: Bird biodiversity (i.e., diversity of bird species) detected using eDNA (**Appendix D**), in various sampling locations around Otter Lake, 6 August 2022

Assay Used	Family	Genus	Species	Common Name	IUCN	SARA	SARO	COSEWIC	Similarity to Species-level Sequence ID	Less than 3 Matches to Sequences Reference Database or Limited geographic occurrence record [1]	Broken Link Tributray & Sunny Point Bay	Rankin Lake Tributary & Little Otter Bay	Link Property Tributray & Link Shore	Blue Lake Road Beaver Pond & Sand Bay	Salmon Lake Tributray & Salmon Lake Bay	Sovereign Lake Tributary & Mud Bay
Vertebrates	Anatidae	*Aix*	Aix sponsa	Wood Duck	Least Concern	-	-	Candidate (Group 2 priority) / Candidate (Group 2 priority	100.00	Y				X		
	Passerine (Bird family)	Unknown	sp.	Unknown Passerine sp.	-	-	-	-	-	-	X					X
										Total Number of Species:	**1**	**0**	**0**	**1**	**0**	**1**

Note: IUCN – International Union for Conservation of Nature's Red List (2023); SARA - Government of Canada's Federal Species-at-Risk Public Registry (GOC, 2023); SARO - Species at Risk in Ontario (GOO, 2023); COSEWIC - Committee on the Status of Endangered Wildlife in Canada (COSEWIC, 2023) ; Y – Yes; X – Presence (i.e., matching DNA sequence detected in sample); Orange - Unknown species with no known reference DNA sequence

Table E-6: Mammal biodiversity (i.e., diversity of mammal species) detected using eDNA (**Appendix D**), in various sampling locations around Otter Lake, 6 August 2022

Assay Used	Family	Genus	Species	Common Name	IUCN	SARA	SARO	COSEWIC	Similarity to Species-level Sequence ID	Less than 3 Matches to Sequences Reference Database or Limited geographic occurrence record [1]	Broken Link Tributray & Sunny Point Bay	Rankin Lake Tributary & Little Otter Bay	Link Property Tributray & Link Shore	Blue Lake Road Beaver Pond & Sand Bay	Salmon Lake Tributray & Salmon Lake Bay	Sovereign Lake Tributary & Mud Bay
Vertebrates	Cervidae	*Odocoileus*	*Odocoileus hemionus* / ***Odocoileus virginianus***	Mule Deer / **White-Tailed Deer**	Least Concern / **Least Concern**	-	-	-	100.00	Y	X					
	Mustelidae	*Lontra*	sp. *(Lontra canadensis)*	North American River Otter	Least Concern	-	-	-	-	Y				X		
	Procyonidae	*Procyon*	*Procyon lotor*	Northern Raccoon	Least Concern	-	-	-	100.00	-					X	
	Castoridae	*Castor*	*Castor canadensis*	American Beaver	Least Concern	-	-	-	100.00	-				X	X	X
	Castoridae	*Castor*	*Castor canadensis*	American Beaver	Least Concern	-	-	-	-	-				X		
	Cricetidae	*Ondatra*	*Ondatra zibethicus*	Muskrat	Least Concern	-	-	-	100.00	-				X		
	Sciuridae	*Tamias*	*Tamias striatus*	Eastern Chipmunk	Least Concern	-	-	-	100.00	-						X
										Total Number of Species:	**1**	**0**	**0**	**4**	**2**	**2**

Note: IUCN – International Union for Conservation of Nature's Red List (2023); SARA - Government of Canada's Federal Species-at-Risk Public Registry (GOC, 2023); SARO - Species at Risk in Ontario (GOO, 2023); COSEWIC - Committee on the Status of Endangered Wildlife in Canada (COSEWIC, 2023) ; Y – Yes; X – Presence (i.e., matching DNA sequence detected in sample); **Bold** - Most likely species due to location & distribution range in Ontario; Orange - Unknown species with no known reference DNA sequence; Green - Added as only likely species or sub-species of genus within area