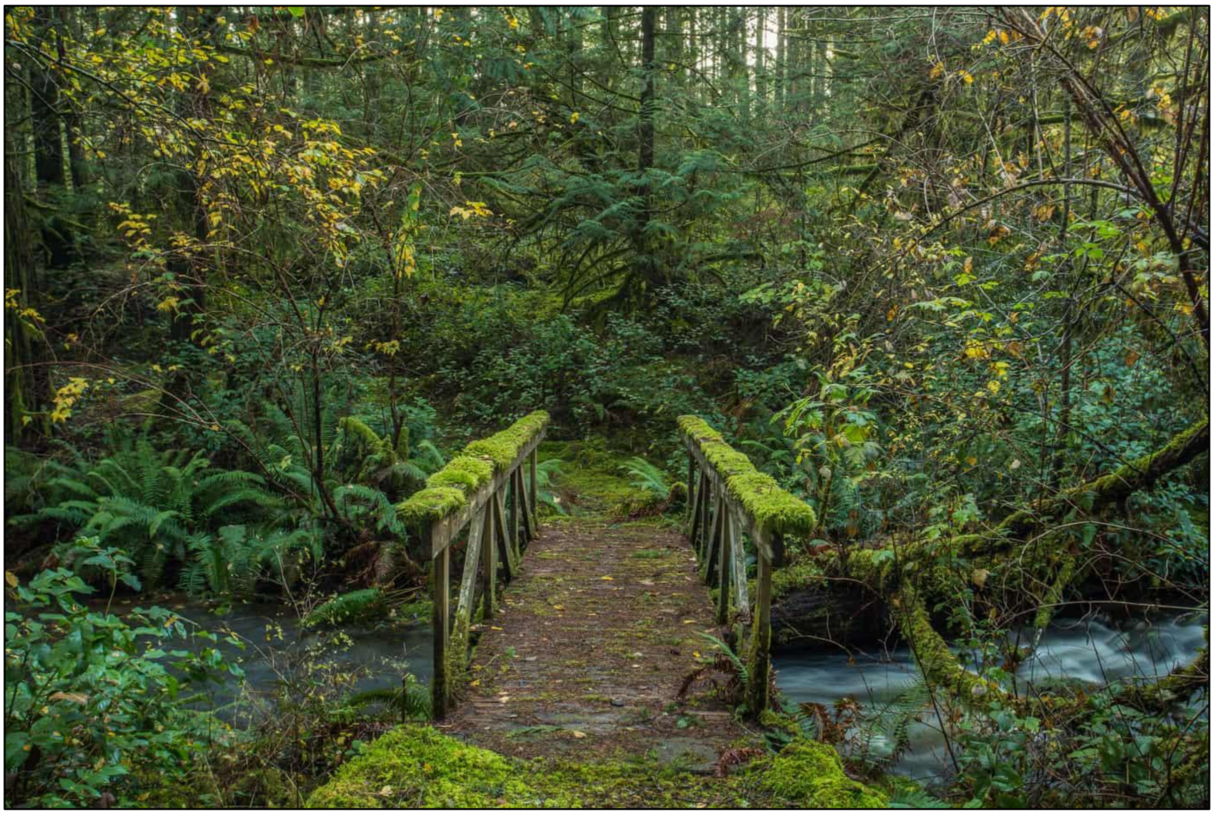
2022 MARY LAKE NATURE SANCTUARY

Research Project



Prepared for: Greater Victoria Greenbelt Society

By: East Island Environmental Consulting

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August 29th, 2022

E.I.E.C

East Island Environmental Consulting

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EXECUTIVE SUMMARY

This project was completed by East Island Environmental Consulting (EIEC) for the Greater Victoria Greenbelt Society's (GVGS) property, the Mary Lake Nature Sanctuary (MLNS). The property is located on approximately 17 hectares within the Highlands District, just north of Langford, British Columbia.

The first of two major components of this project involved the development of a procedure to survey the terrestrial broadleaf vegetation on the property. This procedure was then used to develop a baseline of terrestrial vegetation for the portion of the property to inventory, outline species presence/timelines, frequency, dominance, and importance. Importance in this study is defined as the sum of relative density, relative dominance and relative frequency as described in the Environment Canada *Terrestrial Vegetation Monitoring Protocol* (Roberts-Pichette et al., 1999). This procedural development and weekly terrestrial vegetation surveying was carried out from January - July 2022. This involved the division of the property into 23 transects, 30 m apart running from North to South. Transects 18-23 were selected to be surveyed using a GPS and flags marking 30 m increments along each. Weekly transect survey runs involved the use of a 1 x 1 m plot at the 30 m increments to catalog species present. Multiple resources were used for the identification of species, including previous reports, *Plants of Coastal British Columbia* field guide (Pojar & MacKinnon, 2004), iNaturalist (*n.d)*, and consultation with experienced individuals.

Results of the terrestrial vegetation survey included importance calculations which outlined Salal as one of the top three important species, followed by Morphospecies (MS), MS – Grass 1 and next MS – Grass 2. A general trend was followed throughout the results, where the same set of approximately seven species dominated the importance calculations. The average importance values of transect results indicated that transect 23 had the lowest importance, followed by transects 18 and 22, respectively. The highest average importance values of transects were from transect 20, followed by transects 19 then 21.

Plots located near areas with higher disturbance from increased traffic or proximity to anthropogenic influence (transects 18, 22 and 23) yielded lower average importance values overall than those further from disturbance.

The second major component involved the continuation of long-term water quality monitoring, which was established by Van Isle Eco Consulting (2019). This involved the amendment of the established water quality testing and sampling protocol for Mary Lake and its surrounding streams from previous recommendations. Weekly water quality monitoring included measurements of temperature, dissolved oxygen, pH, conductivity, and total dissolved solids. On two separate occasions throughout the sampling period, laboratory testing for nitrate, phosphate, alkalinity, and fecal coliforms was completed.

The temperature of the lake ranged from 6.7 °C to 20.7 °C and that of the surrounding creeks ranged from 5.9 °C to 19.4°C. Dissolved oxygen in the lake and surrounding streams ranged from 6.1 mg/L to 22.1 mg/L and decreased as the sampling period progressed. Conductivity values in the creeks measured between 119.6 µS/cm and 225 µS/cm and the lake sampling locations ranged from 115.8 µS/cm to 151.0 µS/cm. The average total dissolved solids (TDS) measurements at each location ranged from 91.2 mg/L to 118.5 mg/L. Laboratory analysis results showed most sampling locations had phosphate and nitrate concentrations below detectable limits; however, on April 5th, 2022, two of the lake samples taken at depth had phosphate concentrations of 0.060 ppm and Dead Deer Creek had a concentration of 1.292 ppm. On May 17th, 2022, North Earsman Creek was the only sample that had any detectable nitrate (0.386 ppm) and phosphate (0.043 ppm). The alkalinity of the lake and creeks ranged from 36.45 mg $CaCO_3$/L to 48.475 mg $CaCO_3$/L on April 5th, 2022, and 47.5 mg $CaCO_3$/L to 71.25 mg $CaCO_3$/L on May 17th, 2022. Fecal coliform concentrations were higher for April 5th, 2022, compared to May 17th, 2022, in most of the sampling locations. The apparent decrease of fecal coliforms was the result of a heavy rainfall event prior to sampling on April 5th, 2022. Water quality results were compared to standards and information referenced to the British Columbia Ministry of Environment and Climate Change Strategy (BC MoE, 2021), the United States Environmental Protection Agency (U.S EPA, 2012), and the Saskatchewan Research Council (Swain, 1982).

A comparison between 2022 data collected by EIEC and the last available data collected in 2019 by Van Isle Eco Consulting (2019), indicated there were similar trends in the lake and tributaries during each year. In 2022 there were slightly lower temperatures and conductivity, with higher dissolved oxygen (DO) concentrations.

Parameters of concern included phosphate and dissolved oxygen concentrations based on recommendations for healthy aquatic ecosystems outlined by the US EPA (2012) and the British Columbia Ministry of Environment (BC MoE) (2021). The measurable levels of phosphate present indicate eutrophic conditions in the lake and North Earsman Creek.

Recommendations for the Mary Lake Nature Sanctuary include a continuation of the terrestrial vegetative transect survey with amendments, invasive species control, and increased species survey scope with the potential to include bryophytes and aquatic species. Recommendations involving the routine water quality monitoring include the continuation of data collection with the amendments made by EIEC in 2022 as recommended by Van Isle Eco Consulting (2019), and additional laboratory procedure adjustments, including the Most Probable Number (MPN) technique for fecal coliform analyses, chemical / biological oxygen demand assays, dissolved oxygen management, and phosphate mitigation.

ACKNOWLEDGEMENTS

In conducting this research report, EIEC recognizes the lands which MLNS resides on. The property is located on the ancestral and traditional lands of the WSÁNEC (Saanich) Coast Salish Peoples. We acknowledge their historical relationship with the land and how it continues into perpetuity. We also recognize the imperative role of the ancestors of these people who served as past stewards of Mary Lake and the Highlands area.

A special thanks to Jonathan Moran and Sharon MacMillan for their guidance, comments, and advice. EIEC is appreciative to GVGS for supporting us through the duration of the project and allowing this research project to be conducted within the sanctuary property. With assistance from all parties, this has helped tremendously to improve this research report.

GLOSSARY OF TERMS

Alkalinity: The buffering capacity of a waterbody and its ability to neutralize both acids and bases.

Anthropogenic: Activities or events caused by humans.

Baseline: An initial set of values that future values can be used to compare against.

Biodiversity: Measure to describe the variance of the number of species present in an environment.

Bryophytes: A group of non-vascular plants that consist of mosses, liverworts, and hornworts (Crooks, 2021).

Conductivity: The measure of water's ability to conduct an electrical current

Density: "Average number of individuals of a species on a unit area basis", (Roberts-Pichette et al.,1999).

Dissolved Oxygen: The amount of oxygen that is present in the water, typically measured in mg/L.

Disturbance: A natural or anthropogenic interruption of a healthy functioning ecosystem or environment.

Dominance: "Area a species occupies in a stand (for trees, use basal area) on a unit area basis", (Roberts-Pichette et al.,1999).

Fecal Coliforms: A subset of total coliforms that are bacterium derived from the gut and feces of warm-blooded animals (BPWSP, 2017).

Frequency: "Distribution of a species through a stand, i.e., percentage of plots in the sample area in which a given species occurs.", (Roberts-Pichette et al.,1999).

Importance Value: "An index made up of Relative Density, Relative Dominance and Relative Frequency that profiles the structural role of a species in a stand. It is useful for making comparisons among stands in reference to species composition and stand structure.", (Roberts-Pichette et al.,1999).

iNaturalist: An online application that allows individuals to upload photos of vegetative species for professionals and artificial intelligence to identify.

Nitrates Substances containing the anion NO_3^- or the group $-NO_3$

pH Scale used to communicate the acidity or basicity of aqueous solutions, refers to potential of hydrogen. Ranges from 1-14 on a logarithmic scale of the Hydrogen ion concentration.

Phosphates Substances containing PO_4^{-3} or related ion groups (ex: $-OPO(OH)_2$).

Quadrat: A 1 m x 1 m square with a 10 cm grid inside to determine the frequency of species in a specific area.

Relative Density: "Density of one species relative to all species.", (Roberts-Pichette et al.,1999).

Relative Dominance: "Area a species occupies in a stand (for trees use basal area) on a unit area basis.", (Roberts-Pichette et al.,1999).

Relative Frequency: "Distribution of one species relative to all species.", (Roberts-Pichette et al.,1999).

Total Dissolved Solids: The measure of inorganic and organic substances dissolved in water.

Transect: A straight path along a set direction with intermittent observations or measurements along it.

Van Dorn: Tool used to collect water samples below surface depth.

ACRONYMS AND ABBREVIATIONS

BCMOE: British Columbia Ministry of Environment

BOD: Biochemical Oxygen Demand

COD: Chemical Oxygen Demand

CRD: Capital Regional District

DO: Dissolved Oxygen

EIEC: East Island Environmental Consulting

GPS: Global Positioning System

GVA: Greater Victoria Area

GVGS: Greater Victoria Greenbelt Society

MF: Membrane Filter Technique

m-FC: Membrane Fecal Coliform Agar

MLNS: Mary Lake Nature Sanctuary

MPN: Most Probable Number Technique

MS: Morphospecies

PID: Parcel Identifier

QC: Quality Control

Rf: Relative Frequency

Rdm: Relative Dominance

Rdn: Relative Density

TDS: Total Dissolved Solids

VIEC: Van Isle Eco Consulting

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1.0 Introduction

1.1 Project Overview

The 2022 Mary Lake Nature Sanctuary Research Project is a major project study undertaken by East Island Environmental Consulting. EIEC comprises four undergraduate students in Royal Road University's B.Sc. in Environmental Science program: Harrison Fawcett, Chantal Gendron, Braeden McKay, and Alexis Wilkes.

Since January 2022 EIEC has collaborated with their client, Greater Victoria Greenbelt Society (GVGS) at Mary Lake Nature Sanctuary (MLNS). In cooperation, both parties have agreed on a suitable project that (1) works to expand on pre-existing established databases provided by previous work groups, (2) provides useful qualitative and quantitative data that could be used by the property managers to track temporal changes, and (3) outlines strict survey protocols with embedded recommendations to ensure ease of project continuity.

Given the conditions EIEC has striven to comply with, it was determined that the group would develop and perform a baseline terrestrial vegetation survey on a portion of the MLNS property. The data would serve as a vegetative database for the client who may wish to consult with it prior to any development or modifications planned for the parcel of land. The existence of plants, their importance to the area and their conservation status are included in this study, which may dictate the degree of mitigation that needs to be implemented in land-disturbing activities. Additionally, a baseline terrestrial database and inventory can prove beneficial to compare against in the future to monitor local ecological changes due to both natural and anthropogenic impacts.

As part of the project, EIEC has continued Mary Lake's long-term water quality dataset. In conjunction with increased field and laboratory quantitative data analysis, the waterbody's physical and chemical properties can be used to predict its health and how it has changed over time.

1.2 Scope of Work

EIEC has focused the scope of work around the objectives mentioned below. The objectives were scheduled and completed within a condensed timeframe from January to August 2022. Project activities did not occur through May 31 to June 14, 2022.

1.2.1 Terrestrial Vegetation Baseline Survey Development

a. Subdivide the MLNS property into 23 transects spaced equally apart and include them in a GPS dataset to map their locations.
b. Create a standardized sampling protocol and appropriate timeframe that eliminates both scientific bias and ecosystem damage.
c. Produce sampling form templates that facilitate consistent and complete surveying amongst past, present, and future parties.
d. Employ the developed procedure to create a baseline terrestrial vegetation inventory for a portion of the MLNS property. Inventory includes species descriptions, conservation status according to the Government of British Columbia (2021) *Species and Ecosystem Explorer Tool* and measured qualitative and quantitative data,
e. Present measured data within a series of professional, clear, and concise tables and figures.
f. Attach an updated workbook with compiled data for future groups to utilize.

1.2.2 Continued Water Quality Monitoring

g. Amend the established water quality testing and sampling protocol for Mary Lake and its surrounding streams regarding previous recommendations.
h. Update the long-term dataset with weekly water quality measurements of temperature, dissolved oxygen, pH, conductivity, and total dissolved solids.

i. Provide nitrate, phosphate, alkalinity, and fecal coliform laboratory analysis at three-month intervals for a minimum of two occurrences.
j. Present measured water quality data in comparison to exiting data within a series of professional, clear, and concise tables and figures.
k. Attach an updated workbook with compiled data for future groups to utilize.

2.0 Site Setting & Background

2.1 Site History

Mary Lake Nature Sanctuary is located on 17 hectares within the Highlands district, just north of Langford, British Columbia. The area is part of the traditional territories of the W̱SÁNEĆ (Saanich) Coast Salish peoples whose ancestors played an integral role of stewards of the land (MLNS, 2022). Historically, Mary Lake was a place that was used by Tsartlip and other First Nations as a place needed to survive, find food, and medicine, and relax.

Not until 1887 was the land first acquired as private property from the crown (MLNS, 2022). Later, in 1935, a logger named Albert Reginald Manzer purchased the property and began light clearing of forest and agriculture on the property. At this time, Manzer had logged and cleared most old growth forest off the property, leaving it to regenerate naturally. In addition to the logging of the property, Manzer installed an earthen dam, forming part of what is now Mary Lake, named after his daughter.

In 1947, Gertrude Mabel Snider purchased the property and commenced its protection (MLNS, 2022). She had started conservation on the northern half of the property until 1963 when she sold the property to Peter and Hazel Brotherston. The Brotherstons were proud conservationists and spent time and money to safeguard the property. A larger house, a concrete dam and an adjacent fishpond and ladder were installed, which are still present.

The area was owned by the Brotherstons until 2016, when GVGS gained ownership (MLNS, 2022). GVGS is a registered non-profit society that promotes the preservation, acquisition, and protection of the parklands for the benefit of all. GVGS continues to support conservation efforts and safeguard the area's natural and cultural features for generations to come. This will require rezoning of the property so that GVGS can work with the Capital Region District (CRD) to add the property to an existing matrix of protected lands and parks in the Greater Victoria Area (GVA).

2.2 Site Overview

The study area is situated within the Highlands District municipality located in the GVA on Vancouver Island, British Columbia. The Nature Sanctuary is 25 km northwest of Downtown Victoria and 6 km north of Langford, along Millstream Road.

The civic address of the property is 1772 Millstream Rd, Victoria, BC N-48.499465, W-123.517944 (Google, 2022). MLNS has a Parcel Identifier (PID) of 003-346-668 (Land Title and Survey Authority of BC, 2022). According to the most recent District of Highlands zoning information, the municipality has zoned MLNS as Water 1 (W1) and Greenbelt B2 (GB2) (DoH, 2018). A figure illustrating the location and zoning of MLNS is below.

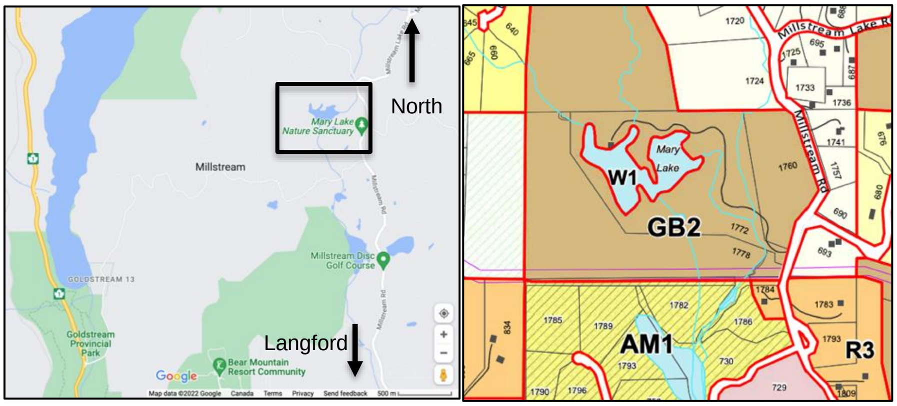


Figure 2 - Map obtained from Google Maps (2022) outlining the study area, Mary Lake Nature Sanctuary, relative to the City of Langford (left). District of Highlands zoning map describing the zoning characteristics of the Mary Lake property (DoH, 2018) (right).

The main feature of the 17-hectare property is the artificial waterbody in the center of the parcel, Mary Lake. The hydrology of the 3-hectare waterbody is affected by two streams: Dead Deer Creek in the northwest corner and Earsman Creek along the northern boundary. As water passes through Mary Lake, fluctuating levels of water flow southwards over the built concrete dam or through the fish ladder continuing downstream Earsman Creek South and eventually feeding into Millstream Creek and the Pacific Ocean.

2.3 Climate Background

To aid in the understanding of the project's results, it is crucial to observe historical climate records to identify changing trends. Increased frequency and intensity of severe weather systems, like heatwaves and rainstorms, and gradual temporal changes to regional environments, may have adverse effects on species composition within a given area.

For this season, EIEC decided to collect historical weather data from Environment and Climate Change Canada (2022) and observe differences in temperature and precipitation from current year conditions to climate characteristics in 2020, shown in the figures below.

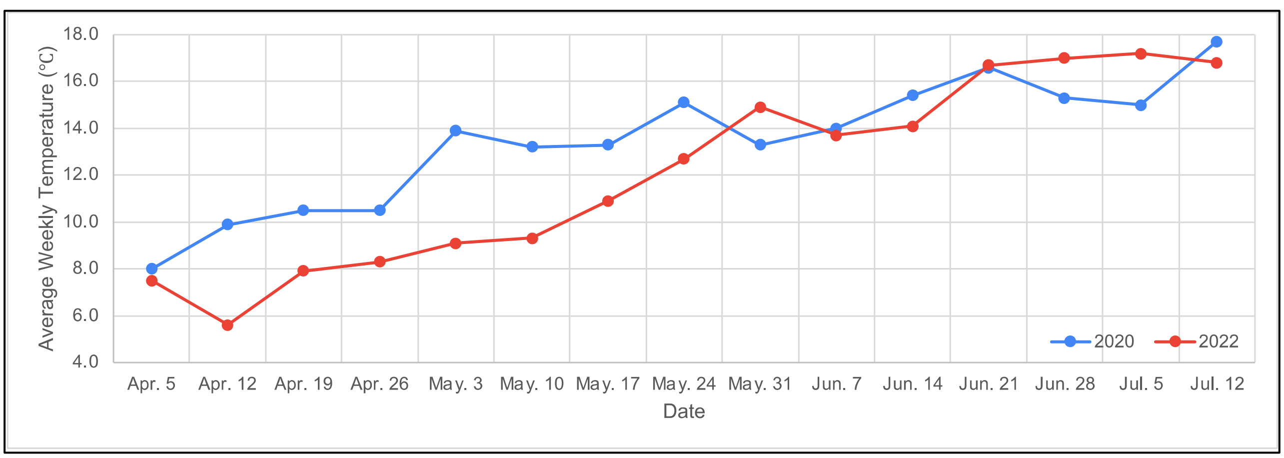


Figure 3 - Weekly temperature (°C) for Victoria, BC using the mean of the weekly minimum and maximum values over the period April 5 – July 12 of 2020 (blue) and 2022 (red). Historical weather information was obtained from the Victoria International Airport Station (Climate ID: 1018621) from the Environment and Climate Change Canada (2022) database.

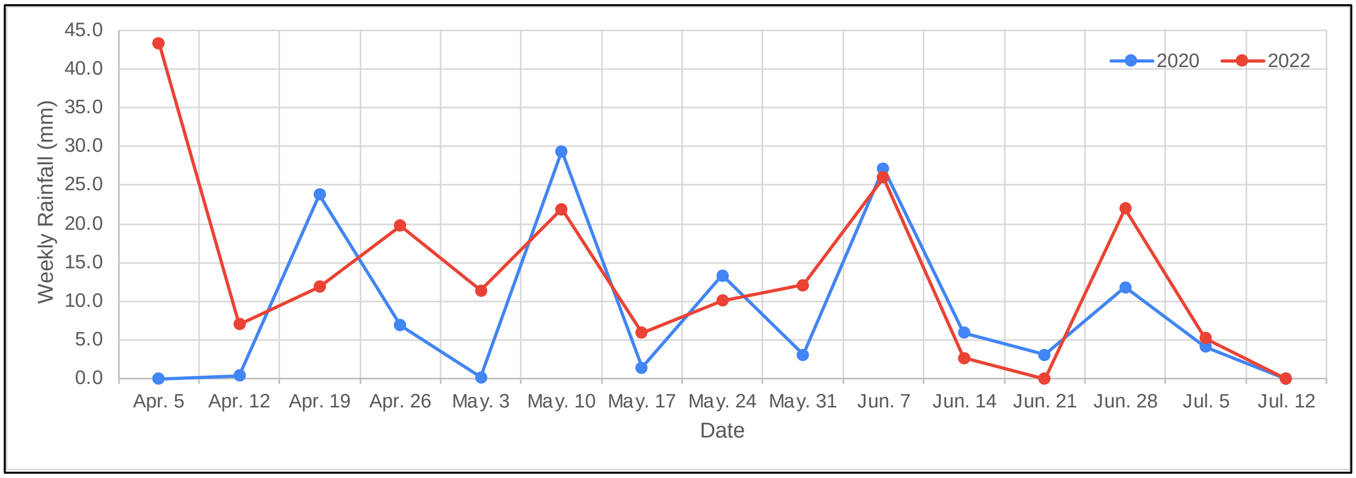


Figure 3 - Weekly precipitation (mm) for Victoria, BC over the period of April 5 – July 12 of 2020 (blue) and 2022 (red). Historical weather information was obtained from the Victoria International Airport Station (Climate ID: 1018621) from the Environment and Climate Change Canada (2022) database.

Figures 2 and 3 show that temperature and precipitation in 2022 were different than in 2020. Spring temperatures in 2020 were generally warmer than in 2022; however, by summer, temperatures stayed similar. Furthermore, the average weekly temperature dipped from 13.4 °C in 2020 to 12.1°C in 2022. As per the second figure, precipitation stayed consistent, although in early April, precipitation was higher in 2022. Looking at the larger picture for the spring and summer seasons, average precipitation rose from 8.7 mm in 2020 to 13.3 mm in 2022.

After a brief analysis of the climate data, this year's spring season has been cooler and wetter. This may result in effects related to the rate at which some plant species grow, bloom and fruit, and abundance of insects. These effects may lead to slowed productivity, late blooms and cascading trophic level impacts where insect-dependent birds may encounter insufficient food sources and have smaller body weights.

2.4 Water Quality Monitoring Background

At MLNS, a water quality monitoring plan was initially developed by Van Isle Eco Consulting (VIEC) in 2019. The plan outlined an extensive protocol to use field multiparameter probes to measure water temperature, dissolved oxygen, pH and conductivity at Mary Lake and its tributaries. The procedure included laboratory analysis of alkalinity, nitrate and phosphate concentrations, fecal coliform, and sediment heavy metal levels. This established water quality monitoring procedure has been conducted at Dead Deer Creek, Earsman Creek upstream and downstream of the lake (North and South respectively), at the dock, as well as three locations on the lake both at the surface and at a depth of 2 m.

Through the 2022 season, EIEC has continued the water quality monitoring work. However, the protocol was adjusted to meet the recommendations made by previous groups. Due to no significant heavy metal results, EIEC no longer pursued laboratory sediment analysis. Amendments to procedure included no turbidity readings using a Secchi disk, which were not taken due to insufficient water depth, and fecal coliform samples were less diluted by an order of ten. EIEC retained the sampling sites chemical parameters, with the addition of total dissolved solids, and the advanced laboratory analyses. While conducting this amended protocol, EIEC took weekly monitoring readings through April 5th – June 28th, 2022, apart from May 31st – June 14th, 2022. Also, laboratory analysis was performed twice at three-month intervals from the project start date.

Due to the water quality monitoring procedure established in 2019, an ongoing long-term data set already exists and chemical properties of the lake and its streams have been recorded. In subsequent sections of this report, newly-collected data will expand on the dataset to identify temporal trends in the water's chemical characteristics and what conditions MLNS might expect to see in the future.

2.5 Terrestrial Vegetation Background

Previous studies surrounding the vegetative baseline species on MLNS include:

2010: Report on vegetation present on site, collected by Hans Roemer PhD, Ecologist. The report consists of general observations of the site and identification of seven habitat types and includes a non-exhaustive list of species on site, referencing them to their respective habitat types, providing a sense of where to find a given species on the property (Roemer, H. 2010).

2019: *Mary Lake Nature Sanctuary – Lake Stewardship Project* produced a report from Van Isle Eco Consulting which included a brief description of several "uncommon and interesting plant species" (VIEC, 2019) including *Allotropa virgata* – Candystick, *Boschniakia hookeri* – Ground cones and *Utricularia* sp. – Bladderwort. Additional information on species on site is included in Appendix 2.0 of their report – an adapted table of organisms found in the habitats outlined by the 2010 Roemer report. Pertinent information for this report was limited to the prior sections of the 2019 document.

2021: *Mary Lake Nature Sanctuary Baseline Report* prepared by Keefer Ecological Services which provided sections on past ecological studies and highlighted the ecosystem mapping approach used both in the 2010 and 2019 reports, as well as an updated, non-exhaustive species inventory. Additionally, threats to the natural state of MLNS were outlined in Section 8 of that report. Previous research will aid in the identification of plant species identified in this report, as well as highlighting some potential variables which may influence the results of this study due to anthropogenic disturbances of the site (Keefer Ecological Services Ltd, 2021).

All three reports determined a dominant habitat type consisting of Douglas-fir trees at higher elevation and Western redcedar closer to riparian zones. Forest floor species are dominated primarily by Salal, Western sword fern, Dull Oregon-grape and others, which are identified below.

The purpose of studying the vegetative baseline composition of MLNS is to monitor and quantify changes over time. The data may be used to collect a snapshot of the vegetative inventory of the property at a given time, and compare it to years past and future, monitoring the biodiversity, species density, dominance, frequency, and importance over time. The data prove useful in determining the role that a given species performs in an area of the property, and how populations may fluctuate over time due to disturbance, development, ecosystem, or climate changes. By providing a baseline for the 2022 spring-summer vegetation inventory, future research projects and conservation organizations may be able to compare changes to the site in a quantifiable manner, instead of the traditional qualitative fashion.

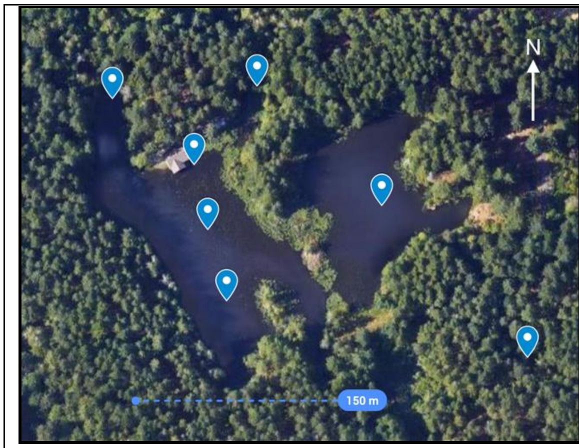
By assigning importance values to distinct species present on site, inferences may be made as to the roles these species play in the local ecology. It may additionally be used to monitor the spread of invasive species throughout the property or identify sensitive species requiring habitat protection. Finally, the data collected may be used to quantify which species make up the mature ecosystems across the nature sanctuary, proving useful for the long-term conservation of the area.

3.0 Investigation Methodology

3.1 Water Quality Monitoring

3.1.1 Field Water Quality Monitoring Protocol.

Routine weekly water quality monitoring was completed with an amended water quality monitoring plan initially developed by VIEC in 2019, incorporating their recommendations. Water quality monitoring included sampling at four lake locations as well as three surrounding stream locations. The locations and their coordinates can be seen in Table 1 below.



Location	GPS Coordinates
Lake 1	48.49942, -123.51866
Lake 2	48.49893, -123.51846
Lake 3	48.49958, -123.51688
Dock	48.49986, -123.5188
Dead Deer Creek	48.50031, -123.51964
North Earsman Creek	48.5004, -123.51816
South Earsman Creek	48.49855, -123.51538

Table 1: Map and coordinates of the Mary Lake Nature Sanctuary Research Project's water quality monitoring locations through Mary Lake and its surrounding streams.

All stream locations mentioned above had water quality measurements taken from a grab sample that was obtained from areas demonstrating running water. Still water would not accurately depict real readings of the active site's stream. As per the lake locations, water quality monitoring was achieved from both surface (0.0 m) and 2.0 m depth, apart from the dock location (surface only). To obtain these samples, water was collected using either a plastic beaker and/or a Van Dorn sampler. In collected grab samples, parameters such as temperature, DO, pH, conductivity, and TDS were measured with the Oakton PCTS Testr 50 and Cole-Parmer Dissolved Oxygen probes. These observations were noted along with the time, depth, and any other pertinent observations. To ensure consistency, the procedures for monitoring routine surface and depth samples are outlined below. A standardized form created by EIEC is attached in Appendix A.

3.1.1.1 Surface Samples.

1. Rinse beaker 3x with the sample water.
2. Just below surface level, scoop enough water into a 500 mL beaker, approximately ¾ full.
3. Measure temperature, pH, conductivity, and TDS with the Oakton PCTS Testr 50 probe.
4. Measure DO with the Cole-Parmer Temperature-corrected Dissolved Oxygen probe.
5. Record measurements on the water monitoring form provided by EIEC.
6. If performing lab analysis, fill a 1 L plastic bottle with sample water and label appropriately.

3.1.1.2 Deep Samples.

1. Rinse Van Dorn sampler 3x with the sample water, dipping it in and out of the lake.
2. Lower the Van Dorn sampler to 2 m below the lake surface. **Do not touch the bottom.**
3. Capture a sample volume of water, bring it up to the surface and empty into a 500 mL beaker that has been rinsed 3x with the sample water.
4. Measure temperature, pH, conductivity, and TDS with the Oakton PCTS Testr 50 probe.
5. Measure DO with the Cole-Parmer Temperature-corrected Dissolved Oxygen probe.
6. Record measurements on the water monitoring form provided by EIEC.
7. If performing lab analysis, fill a 1 L plastic bottle with sample water and label appropriately

3.1.2 Water Quality Laboratory Analysis.

Advanced water quality monitoring was performed twice, once on April 5th, 2022, and again on May 17th, 2022. This included laboratory analysis at all sample locations at the surface and at depths for locations that permit it except for fecal analysis. Fecal analysis assays did not include depth samples from the three lake locations.

For each of the samples, laboratory analysis was conducted to determine alkalinity, nitrate and phosphate concentrations, and the presence of fecal coliforms in the water. The procedures which EIEC utilized to conduct the four laboratory analyses were outlined by a *Laboratory Manual* provided by Noble, M. (2022). The methodology for the procedures is as follows:

3.1.2.1 Alkalinity.

1. Prepare 0.1 M NaOH and 0.1 M HCl solutions from NaOH and 1 M HCl, respectively. As well create three standards of potassium acid phthalate (KHP) solutions.
2. Standardize the 0.1 M NaOH against the primary KHP standard.
3. Standardize the 0.1 M HCl against the 0.1 M NaOH.
4. Calibrate the pH meter with the appropriate standards.
5. Measure 100 mL of a sample with a graduated cylinder into a 250 mL beaker.
6. Lower the pH probe into the sample.
7. Obtain a potentiometric curve by adding 0.1 M HCl by burette in 0.1 mL increments, stirring constantly with a magnetic stir bar and plate and recording the pH until a pH of 4.0 is reached. Record the pH after each increment of titrant.
8. Repeat steps 5 to 7 for each collected sample, ensuring enough standardized NaOH and HCl is available.
9. Record data in the Alkalinity Laboratory Analysis Form created by EIEC, provided in Appendix A.

3.1.2.2 Nitrates.

1. Using 50 ppm nitrate stock solution, prepare 50 mL of 0, 1, 2, 3, and 4 ppm. The 0 ppm standard equates to 50 mL of distilled water (DH_2O).
2. While simultaneously doing the same thing for each sample, measure 50 mL of each sample, standard and blank using a graduated cylinder into a 125 mL Erlenmeyer flask.
3. Add 1.0 mL of 1:4 diluted HCl to each flask and swirl.
4. Add 1.0 mL of sulfanilic acid reagent to each flask and swirl.
5. Add 1.0 mL of Zn/NaCl granular mixture to each flask and swirl for at least 7 minutes, in rotation.
6. Vacuum filter each sample from lowest to highest concentration while rinsing the Erlenmeyer flask with DH_2O while it's empty.
7. Add 1.0 mL of naphthylamine hydrochloride reagent to each filtered sample and swirl.
8. Let the flasks sit for a minimum of 5 minutes to allow colour development.
9. Measure the absorbances with a spectrophotometer at a wavelength of 520 nm, blanking the instrument with the 0 ppm solution.
10. Record data in the Nitrates Laboratory Analysis Form created by EIEC, provided in Appendix A.

3.1.2.3 Phosphates.

1. Using 100 ppm phosphate stock solution, prepare 50 mL of 0, 0.25, 0.5, 1.0, 2.0, and 3.0 ppm. The 0 ppm standard equates to 50 mL of distilled water (DH_2O).
2. While simultaneously doing the same thing for each sample, measure 50 mL of each sample, standard and distilled water (0 ppm) using a graduated cylinder into a 125 mL Erlenmeyer flask.
3. Add 2.0 mL of ammonium molybdate solution to each flask and swirl.
4. Add 4 drops of stannous chloride solution to each flask and swirl.
5. Let the flasks sit for 5 minutes to allow colour development.
6. Within 15 minutes, measure the absorbances with a spectrophotometer at a wavelength of 650 nm, blanking the instrument with unprocessed distilled water.
7. Record data in the Phosphates Laboratory Analysis Form created by EIEC, provided in Appendix A.

3.1.2.4 Fecal Coliforms.

1. For each sample, create 10^{-1} dilutions for each location using sterile equipment.
2. Create two m-FC plates per sample taken.
3. Label each plate with undiluted and diluted for each sample location.
4. Set up the membrane filter apparatus, ensuring sterility.
5. Filter 100 mL of the dilute sample through the funnel, shaking before and rinsing the sides of the glass funnel with approximately 30 mL of DH_2O.
6. Remove membrane filter from apparatus using sterile forceps and place grid side up on m-FC agar.
7. Invert plates and incubate for 24 hours at 44.5°C.
8. The next day, count all fecal coliform colonies (dark blue).
9. Record data in the Fecal Coliform Laboratory Analysis Form created by EIEC, provided in Appendix A.

3.2 Baseline Terrestrial Survey Protocol & Mapping

3.2.1 Transect spacing.

1. Transects run N-S to account for ecosystem variability with elevation change across MLNS, totaling 23 transects across the entirety of MLNS.

Reasoning: The transects were determined to run from North to South from one property line to the next on Mary Lake Nature Sanctuary Property to account for ecosystem variability with elevation change. This was determined based on recommendations of the document *Ecological Monitoring and Assessment Network: Terrestrial Vegetation Monitoring Protocols* (Roberts-Pichette et al., 1999). Recommendations for this include, “a series of quadrats arranged contiguously in a straight line.” (Roberts-Pichette et al., 1999).

2. A distance of 30 m between the transects was determined.

Reasoning: The value of 30 m was considered to decrease the ecological impact of each transect, while allowing for some degree of coverage across the entirety of the property. Additionally, it was recommended to have the start of transect locations at least 10 m apart (Roberts-Pichette et al., 1999).

3. Transects numbered 1-23 from West to East.

Transects number 18, 19, 20, 21, 22, 23 were selected to be sampled on a rotating weekly basis in groups of two to reduce ecological impact of sampling and monitoring. Average traverse time was 2-3 hours per transect, surveying two transects per day, only repeating the same transects at four-week intervals.

The mapping of the site consisted of compiling resources from both Open Street Map (Under MLNS recommendation) for property boundaries and Google Earth to determine study area and sampling locations. A 30 x 30 m grid was overlaid on the property and is provided below.

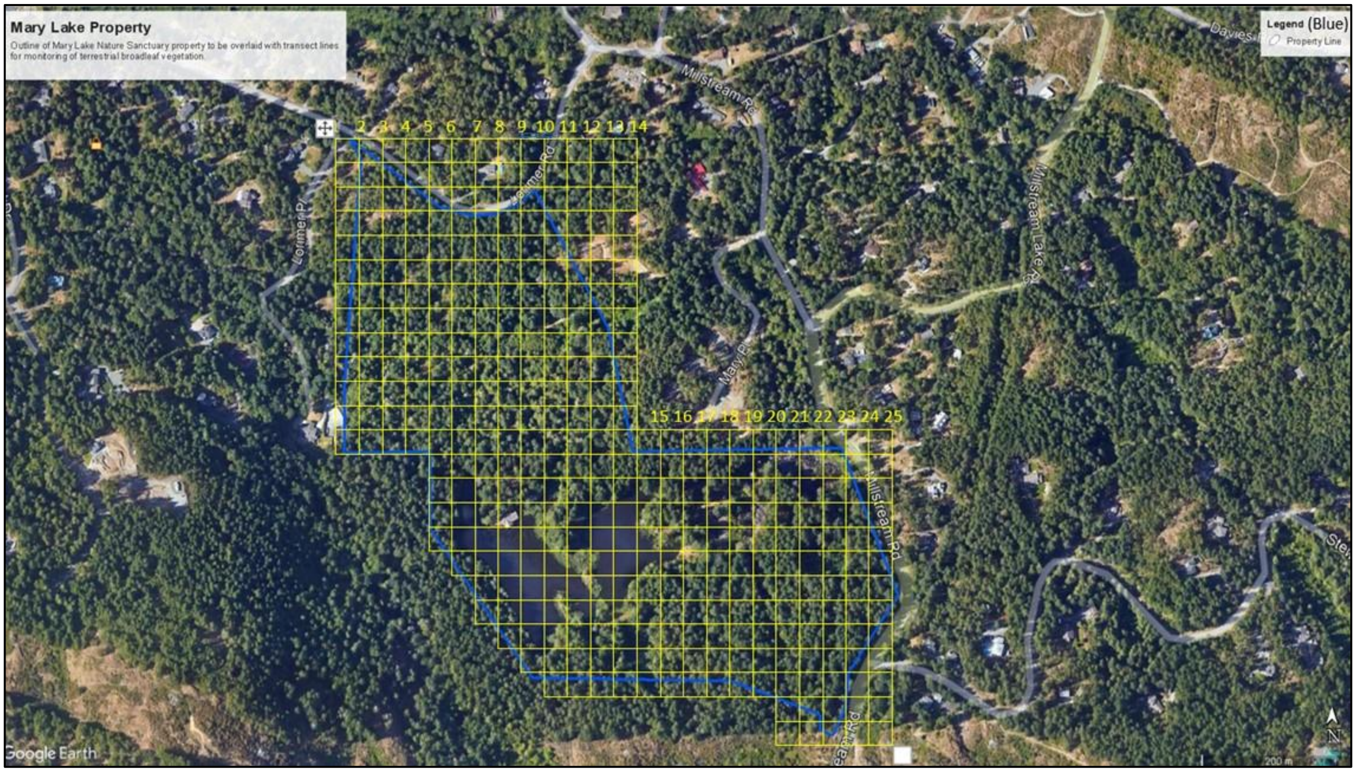


Figure 4 - Map of transect lines overlaid atop the MLNS property, numbered sequentially from West to East. Grid spacing is 30m by 30m. Produced using Google Earth Pro (Google, 2022).

3.2.2 Quadrat sampling protocol.

1. Every 30 m, quadrat sampling is conducted, filling out the predesignated form for consistency. Quadrats will be 1 m x 1 m. To remove bias, a coin flip will be performed to determine whether sampling of the left or right side of the transect will occur at any given location (in relation to facing North).

2. Upon completion of the sampling form, move to the next location designated on the GPS and repeat vegetative survey form.

3. If the transect path intersects a waterbody, the waterbody and its organisms will be omitted as aquatic species will not be recorded in the transect sampling plan.

4. If field species identification cannot be completed, during sampling, the following steps will be taken:
    - Create an appropriate morphospecies identifier for the species.
    - Capture a photo and small sample of the species.
    - Inquire about the species to a knowledgeable person

5. Data collection of transect samples consists of plant identification of all species present in each quadrat and documented on the provided form. Multiple resources may be used to identify species including previous reports, field guides, online applications including iNaturalist (n.d.), Plants of Coastal British

Columbia (Pojar & MacKinnon, 2004), and consultation with knowledgeable and experienced individuals. Any additional information will be included on the appropriate form, provided in Appendix A.

6. After returning from field, data will be input into Excel spreadsheets and calculations of density, dominance and frequency will be completed to determine relative ecological importance.

3.2.3 Materials required.

1x Garmin GPSMAP 64s handheld GPS
2x rechargeable AA batteries
72x flags for marking sampling locations (may change depending on transects under study)
1 x 1 m^2 sampling quadrat with a 10 cm x 10 cm grid
1x clipboard
1x clock or watch
Vegetation sampling forms

Transect	Northing	Easting
18	N 48.50059	W 123.51505
19	N 48.50059	W 123.51464
20	N 48.50059	W 123.51424
21	N 48.50059	W 123.51383
22	N 48.50059	W 123.51342
23	N 48.50059	W 123.51302

Table 2 - Coordinate list of transects under study (18-23), beginning from the north end of the property boundary, mark every 30 m for individual quadrat sampling locations until the south property boundary is met. Disregard sampling points which are submerged (may vary with precipitation events and seasonal changes).

3.3 Quality Control

Water quality monitoring quality control (QC) was provided initially via following experimental design provided from the 2019 research project by sampling from the same points, with the same equipment and performing the same laboratory analyses as previous studies. Monthly calibration of instrumentation prior to sampling (for both the Cole Parmer Temperature-corrected Dissolved Oxygen probe and the Oakton PCTS Tester 50) was conducted to ensure accurate readings throughout the study period. Proper sampling techniques were also performed to ensure that little to no contamination of samples occurred. These included rinsing of probes, samplers, and bottles between samples. Creek samples were taken with the bottles facing upstream and submerged. Lake water samples were collected with bottles fully submerged. Standardized sampling procedures and data forms were aimed to mitigate sampling event inconsistencies.

Terrestrial vegetation surveying QC was achieved by designing the sampling methodology following a stratified sampling plan. All sample locations were determined based on equal grid spacing of the property at distances apart to minimize impacts on adjacent sample locations. Additionally, bias was removed from sampling by relying on a coin flip to determine whether a sample would be recorded from the left or right side of the transect (in relation to facing North). Permanent marking of sample locations with flags was added to ensure the same relative area was being observed throughout the study period. Moreover, standardized forms were produced for data collection, to ensure that quality of data and procedure was followed, which may be continued by future research groups. Finally, non-biased interpretation of data was completed following Environment Canada *Terrestrial Vegetation Biodiversity Monitoring Protocol* (Roberts-Pichette et al., 1999, p. 26).

4.0 Results

4.1 In-Situ Water Quality Monitoring Results

The temperature of the lake and surrounding creeks increased as the season progressed. There was a large increase in water temperature throughout the sampling period. The coldest water body was Dead Deer Creek with an average temperature of 9.2 °C. At each location throughout the lake, the water sample was warmer at the surface than it was at depth. The creeks and streams were slightly colder than the samples taken from the lake (including samples taken beside the dock). Overall, the temperature of the lake ranged from 6.7 °C to 20.7 °C, whereas the creeks ranged from 5.9 °C to 19.4°C.

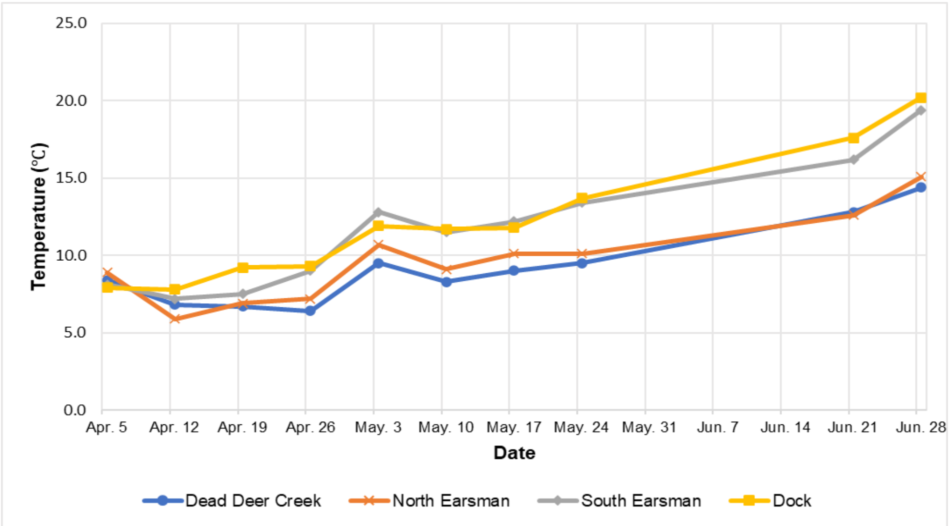


Figure 5 - Water temperature (℃) of Mary Lake Nature Sanctuary creeks and dock from April 5th – June 19th. Measurement taken with the Oakton PCTS Testr 50 probe to monitor seasonal variation.

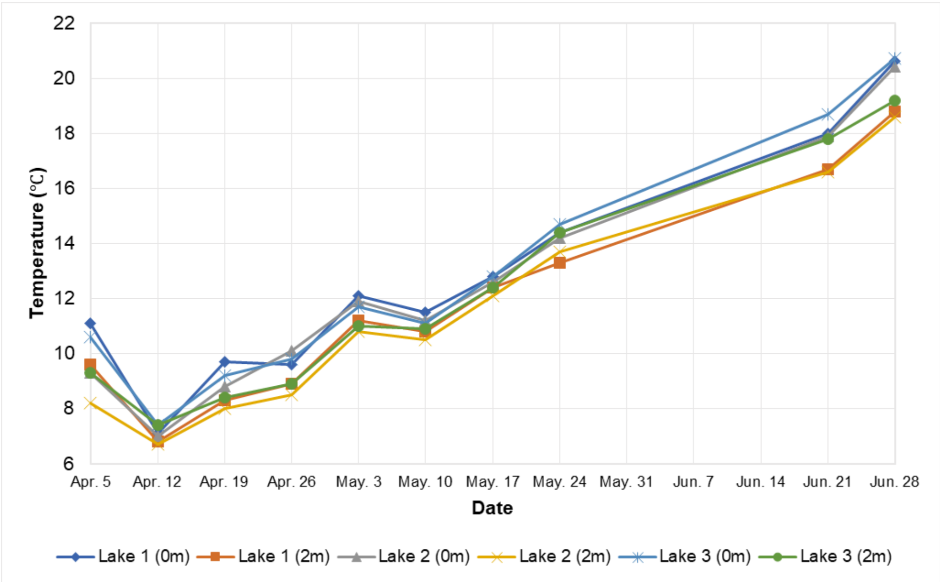


Figure 6 - Surface and depth water temperature (℃) of Mary Lake from April 5th - June 19th. Measurement taken with the Oakton PCTSTestr 50 Probe to monitor seasonal variation.

Dissolved oxygen (DO) ranged from 6.1 mg/L to 22.1 mg/L and decreased as the sampling period progressed. Similarly to temperature, there was slightly more variation in the measurements of the creek compared to the lake. The DO of the lake ranged from 6.3 mg/L to 20.6 mg/L, whereas the DO of the creeks ranged from 6.1 mg/L to 22.1 mg/L, with higher values being observed earlier in the year.

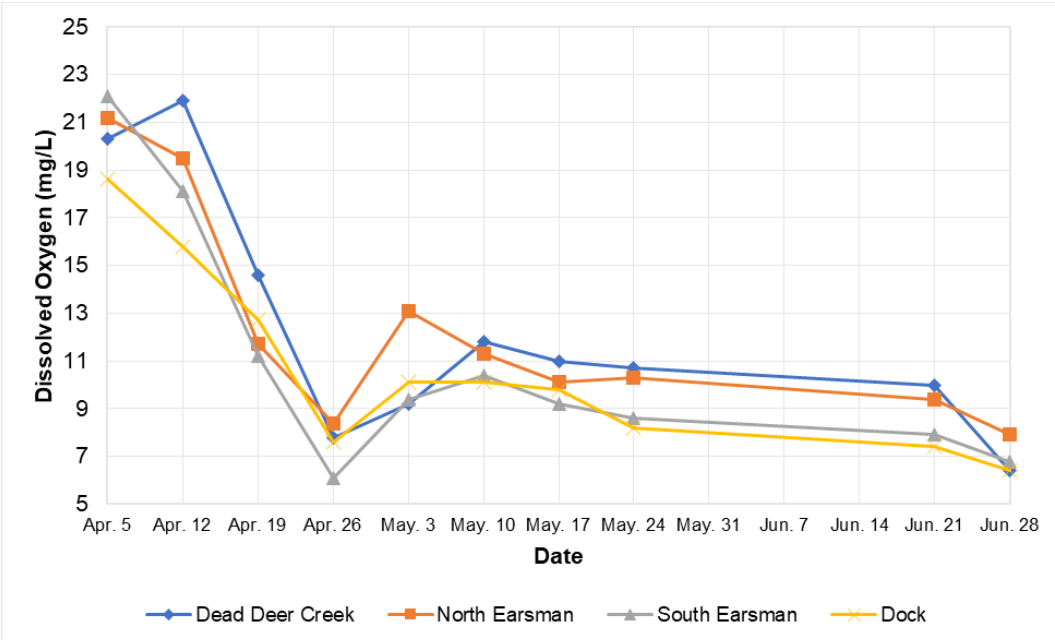


Figure 7 - Dissolved Oxygen (mg/L) of Mary Lake Nature Sanctuary creeks and dock from April 5th - June 28th Measurement taken with the Cole-Parmer DO Probe to monitor seasonal variation.

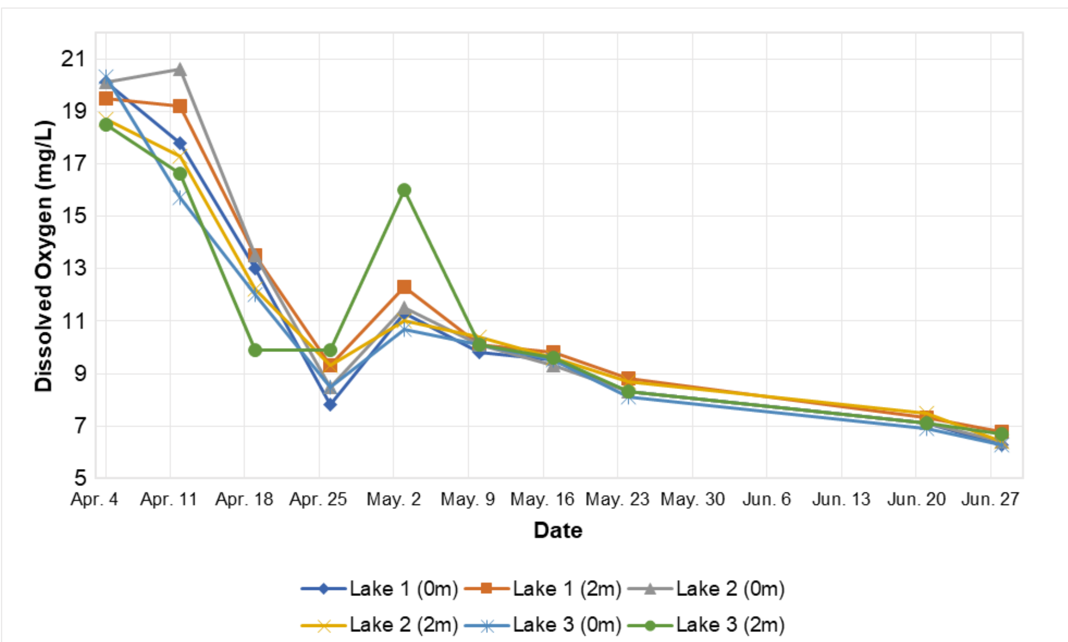


Figure 8 - Surface and depth dissolved oxygen (mg/L) of Mary Lake from April 5th - June 28th. Measurement taken with the Cole-Parmer DO Probe to monitor seasonal variation.

The most acidic measurement was taken from the lake at pH 7.38 and the most basic sample was taken from Dead Deer Creek at a pH of 8.76. All the pH measurements fell within this range, and there does not appear to be any trend in the change of pH with time.

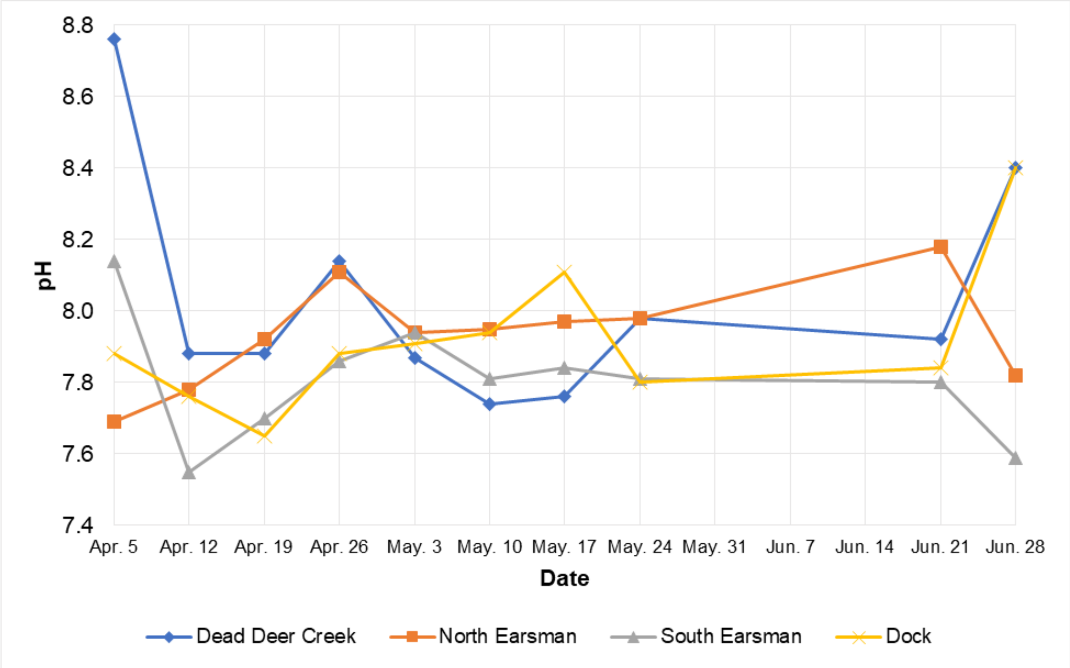


Figure 9 – Surface and depth pH of Mary Lake Nature Sanctuary creeks and dock from April 5th - June 28th. Measurement taken with the Oakton PCTSTestr 50 Probe to monitor seasonal variation.

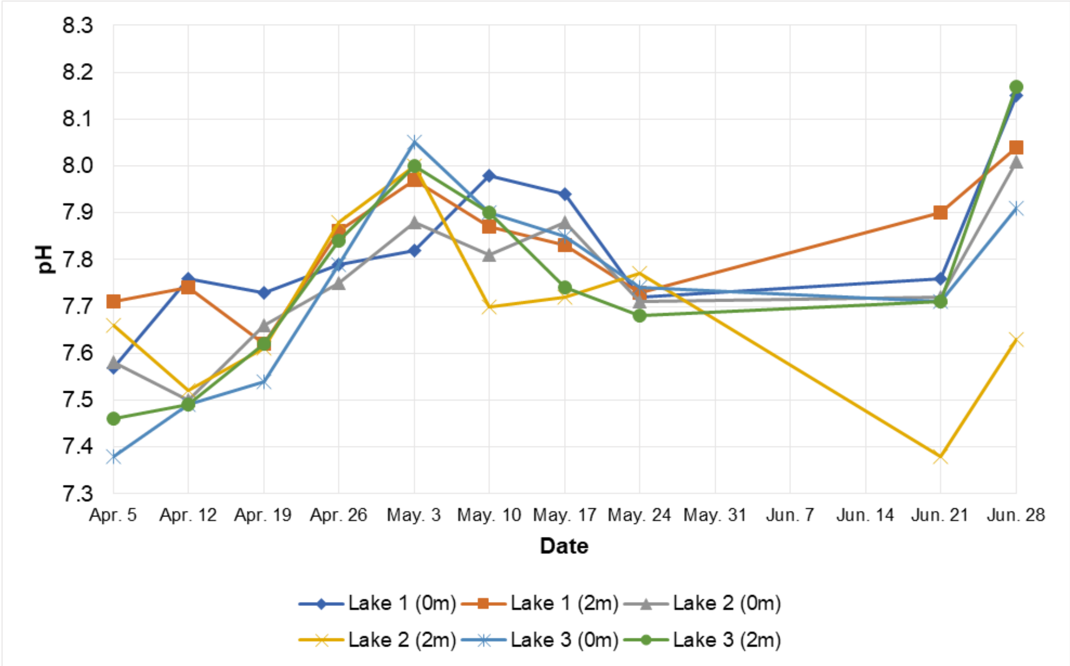


Figure 10 - Surface and depth pH of Mary Lake from April 5th - June 28th. Measurement taken with the Oakton PCTSTestr 50 Probe to monitor seasonal variation.

Conductivity measurements varied the most throughout the sampling period compared to the other parameters. The creeks measured between 119.6 µS/cm and 225 µS/cm and the lake samples ranged from 115.8 µS/cm to 151.0 µS/cm, with the exception of the measurement of 405.0 µS/cm taken at the dock on May 17th, which was determined to be an outlier and is not included in this graph. Conductivity increased with time, with less fluctuation in the lake compared to the creeks and little fluctuation between surface and depth samples.

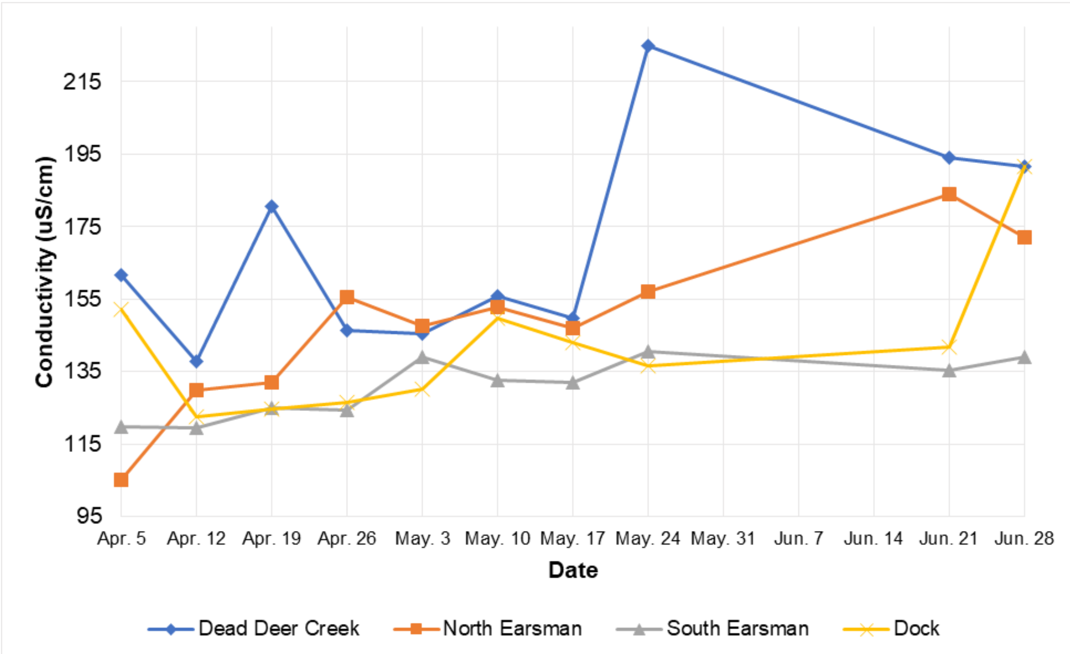


Figure 11 - Conductivity (uS/cm) of Mary Lake Nature Sanctuary creeks and dock from April 5th - June 28th. Measurement taken with the Oakton PCTSTestr 50 Probe to monitor seasonal variation.

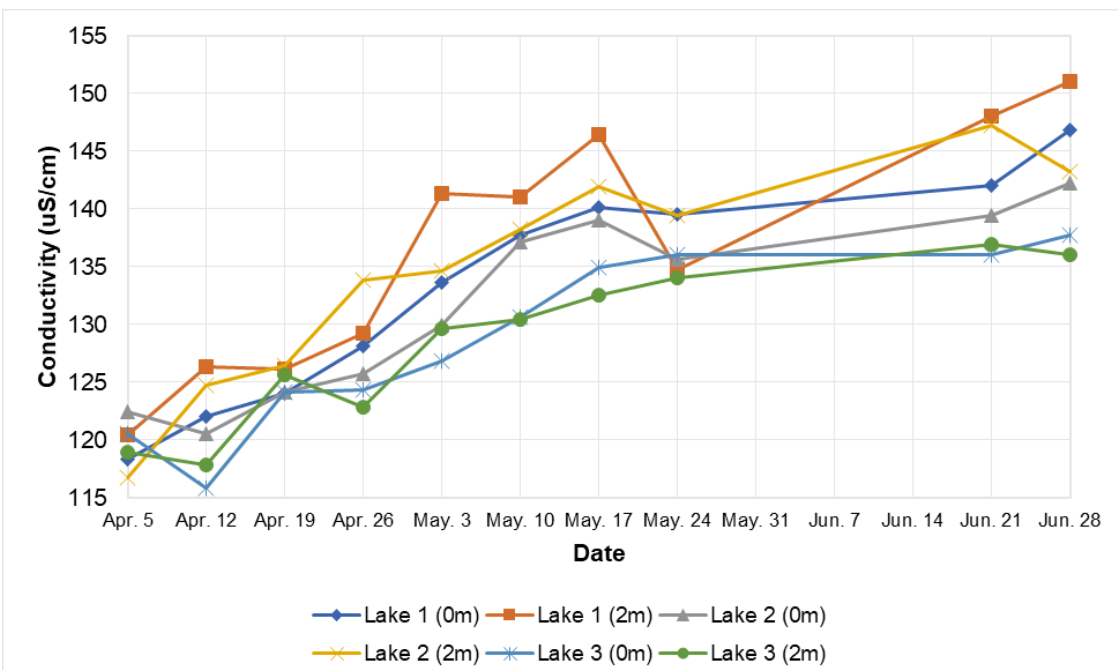


Figure 12 - Surface and depth Conductivity (uS/cm) of Mary Lake from April 5th - June 28th. Measurement taken with the Oakton PCTSTestr 50 Probe to monitor seasonal variation.

The average total dissolve solids (TDS) measurements at each location fell between 91.2 mg/L and 118.5 mg/L. The lake ranged from 81.6 mg/L to 107 mg/L, with the exception of the sample taken at the dock on May 17th which had a TDS measurement of 283.0 mg/L. The TDS of the creeks and lake increased with time.

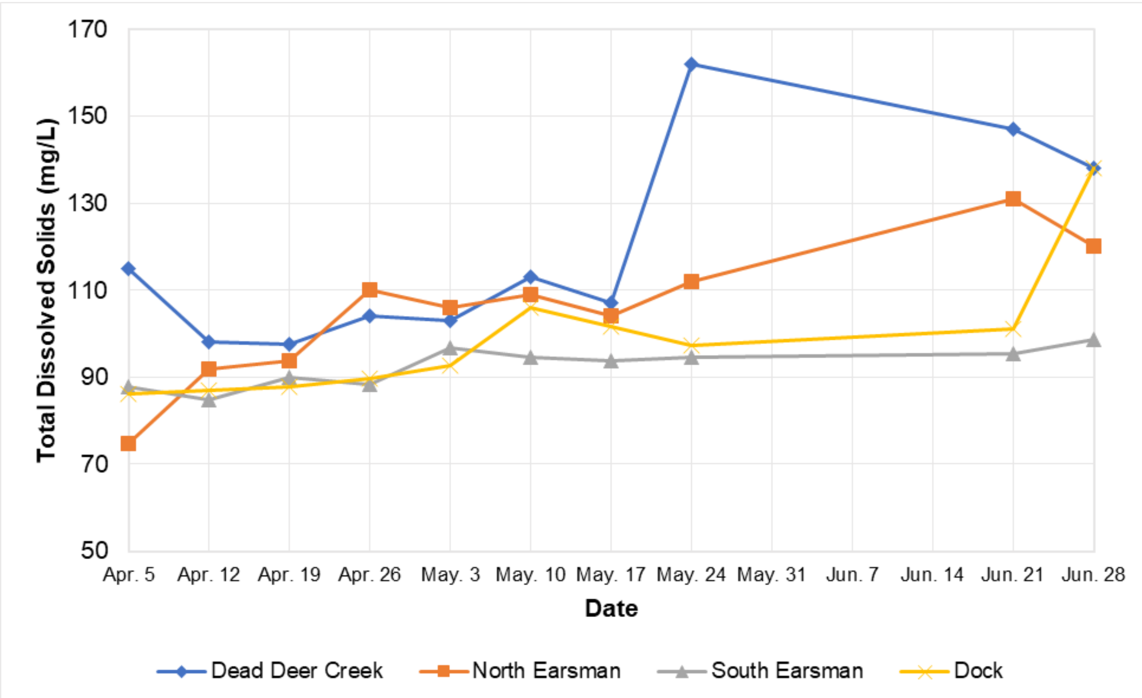


Figure 13 - Total Dissolved Solids (mg/L) of Mary Lake Nature Sanctuary creeks and dock from April 5th - June 28th. Measurement taken With the Oakton PCTSTestr 50 Probe to monitor seasonal variation.

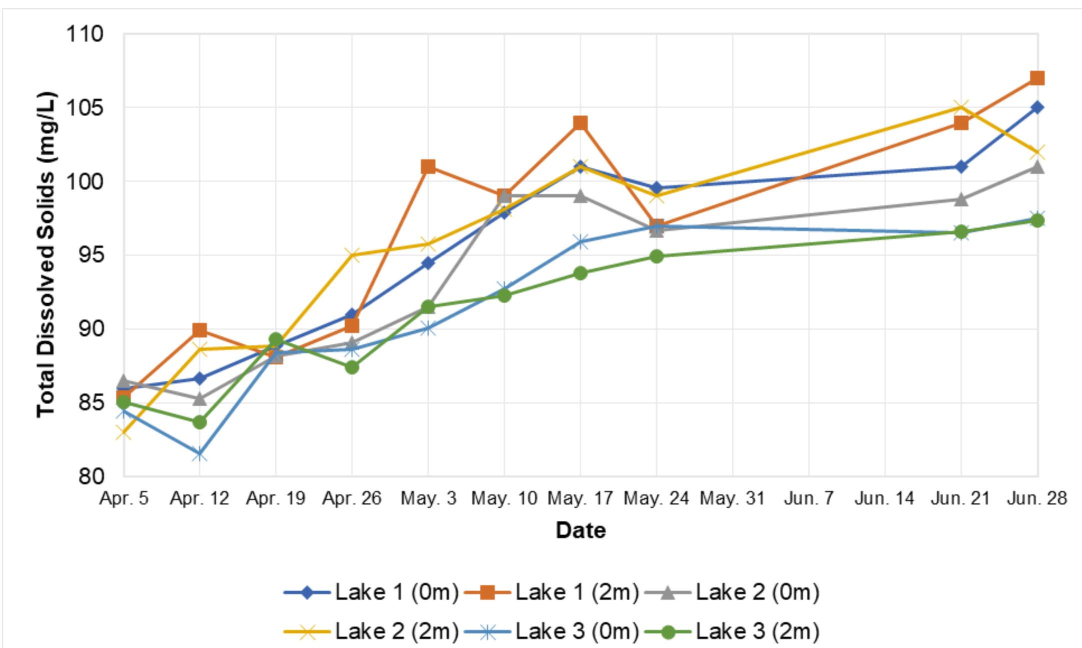


Figure 14 - Surface and depth Total Dissolved Solids (mg/L) of Mary Lake from April 5th - June 28th. Measurement taken with the Oakton PCTSTestr 50 Probe to monitor seasonal variation.

4.2 Laboratory Results

Fecal coliform concentrations were higher on April 5th compared to May 17th in most of the sampling locations due to rainfall prior to sampling. A 10^{-1} dilution of each sample was also plated and resulted in too low of a count to be considered significant.

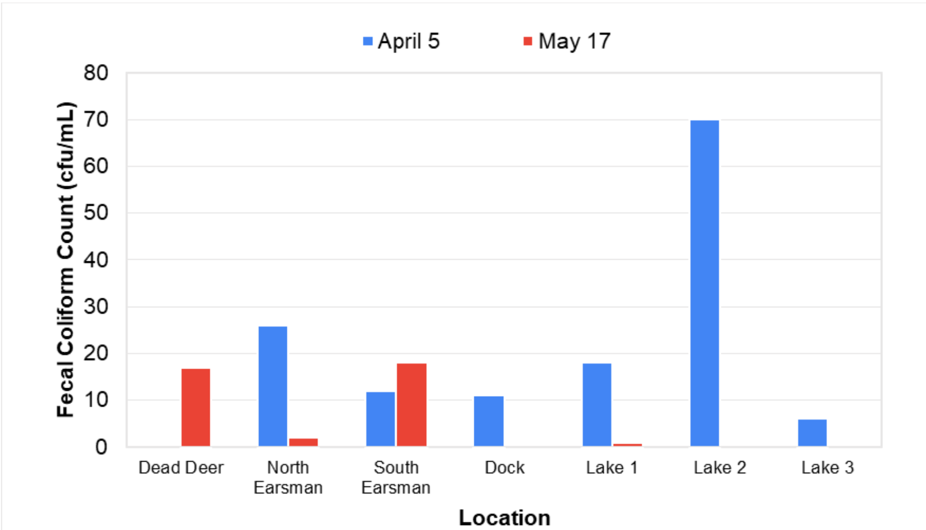


Figure 15 - Undiluted (100 mL) sample vacuum filtered through Millipore filter paper then plated on m-FC agar plates. Plates incubated for 24hrs at 37℃ on April 5th and May 17th, 2022, in order to determine fecal coliform concentration

The alkalinity of the lake and surrounding bodies ranged from 36.45 mg $CaCO_3$/L to 48.475 mg $CaCO_3$/L on April 5th and 47.5 mg $CaCO_3$/L to 71.25 mg $CaCO_3$/L on May 17th.The buffering capacity increased at each location between April 5th and May 17th.

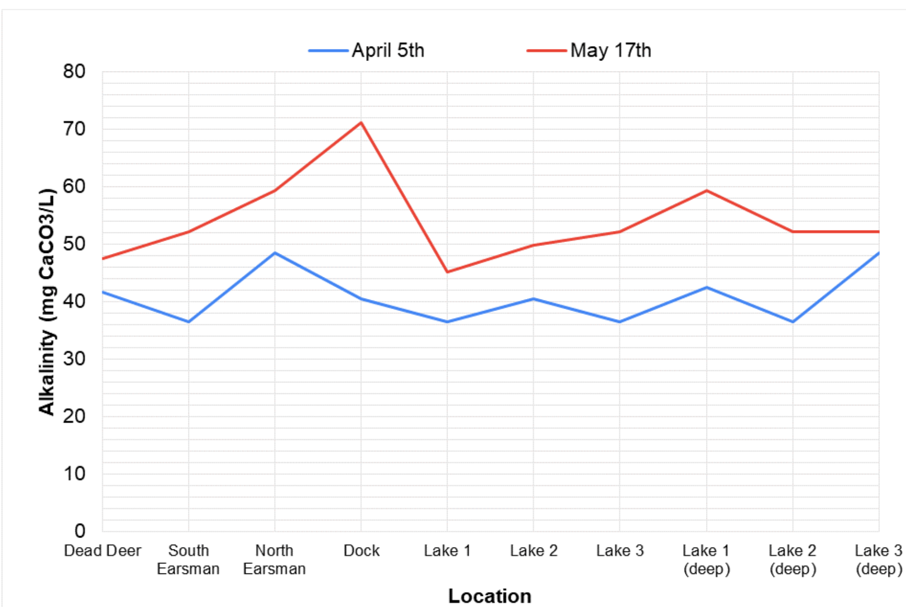


Figure 16 - Alkalinity of water from each sampling location determined through potentiometric titration against 0.1 M HCl on April 5th and May 17th, 2022.

Most of the sampling locations were determined to have phosphate and nitrate concentrations below detectable limits (Appendix B). On April 5th, two of the lake samples taken at depth had phosphate concentration of 0.060 ppm and Dead Deer Creek had a concentration of 1.292 ppm. On May 17th, North Earsman Creek was the only sample that had detectable levels of nitrate (0.386 ppm) and phosphate (0.043 ppm).

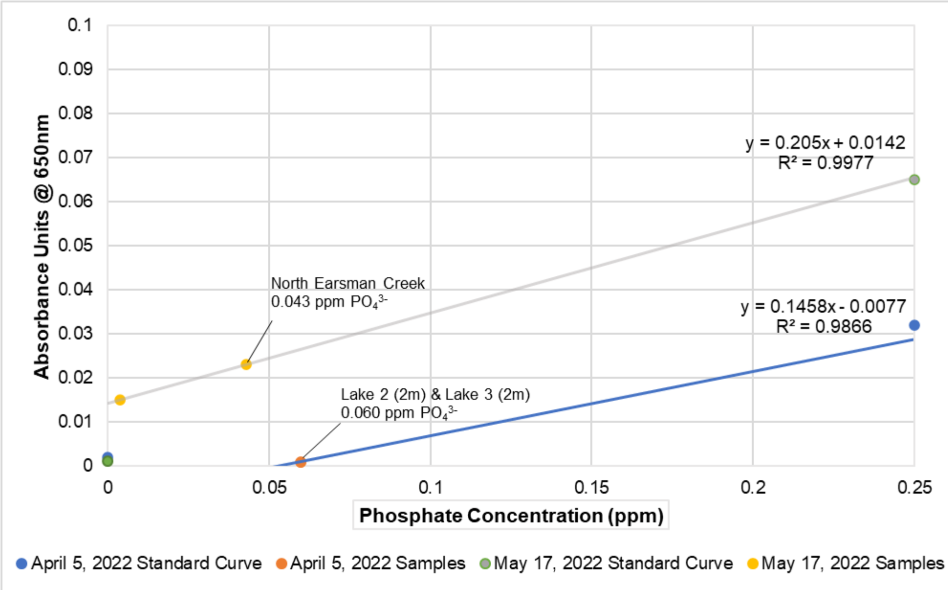


Figure 17 - Concentration of phosphate in water samples collected on April 5th and May 17th against the calibration curve (0, 0.25, 0.5, 1, 2 and 3 ppm PO_4^{3-} Standards) Analyzed at 650 nm on the Genesys10 UV-Vis Spectrophotometer #11.

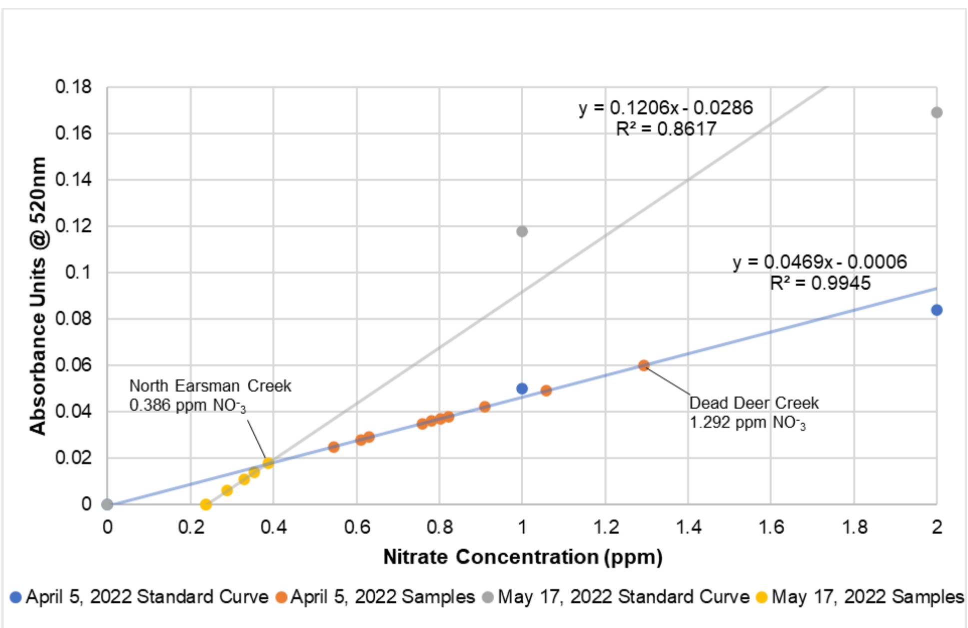


Figure 18 - Concentration of NO^-_3 in the April 5 & May 17, 2022, of water samples collected by EIEC against the calibration curve (0, 1, 2, 3 and 4 ppm NO^-_3 Standards). Analyzed at 520 nm on the Genesys10 UV-Vis #11.

4.3 Terrestrial Vegetation Survey Results

Results from the terrestrial vegetation transect survey showed that species composition (biodiversity), relative density, dominance and frequencies varied over time and between each transect across the study area (transects 18-23). Summation of relative values of each factor studied (density, dominance and frequency) produced an importance value for a given species within a given quadrat sample. Importance as defined in this study is the summation of relative numbers of a plant species in a given quadrat sample (relative density), relative values of the area occupied by a plant species in a sample (relative dominance) and the relative frequency, where relative refers to comparison of one species data against others from the same quadrat sample (except for frequency, which is compared against species reoccurrence across an entire transect).

Results from importance calculations identified salal as one of the top three important species in fifteen out of eighteen transects over the course of three months, closely followed by MS – Grass 1 with MS – Grass 2. A general trend was followed throughout the results, where the same set of roughly seven plants dominated the importance calculations throughout the study period as shown in the following plots. It should be noted that results indicating importance of 300 are due to a single plot consisting of a single species, without repeats along the same transect to dilute the importance of said species. Results like these should be classified as outliers, as one species occupying a single sampling point is not representative of the MLNS terrestrial ecosystems.

Following completion of terrestrial vegetation sampling and data compilation, average importance values for each transect over the entire study period were generated and plotted in Figure 40. Average importance over the study period for each transect in question (18-23) resulted in transect 23 having the lowest importance (46.20), followed by transects 18 (46.78) and 22 (53.37), respectively. The three highest importance transects were transect 20 (73.34), followed by transects 19 (72.85) and 21 (71.81), respectively.

Additionally, a total list of forest floor vegetation was compiled (excluding fungus, mosses, and lichens) with their respective risk statuses as identified in the BC Conservation Data Centre provided by the BC Ministry of Environment. This information can be found in Table 3.

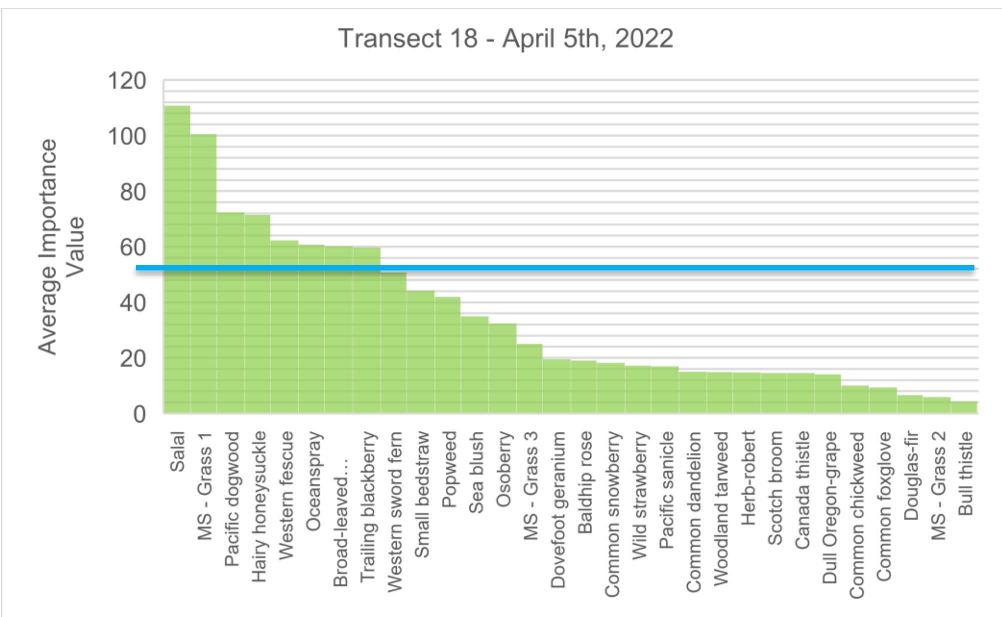


Figure 19 – Individual species average Importance values of transect 18 data collected at Mary Lake Nature Sanctuary on April 5th, 2022. First plot in transect starting at N48.50059, W123.51505, last plot starting at N48.49816, W123.51502. Average importance value of transect 18 on April 5th, 2022, displayed by the blue line (49.18).

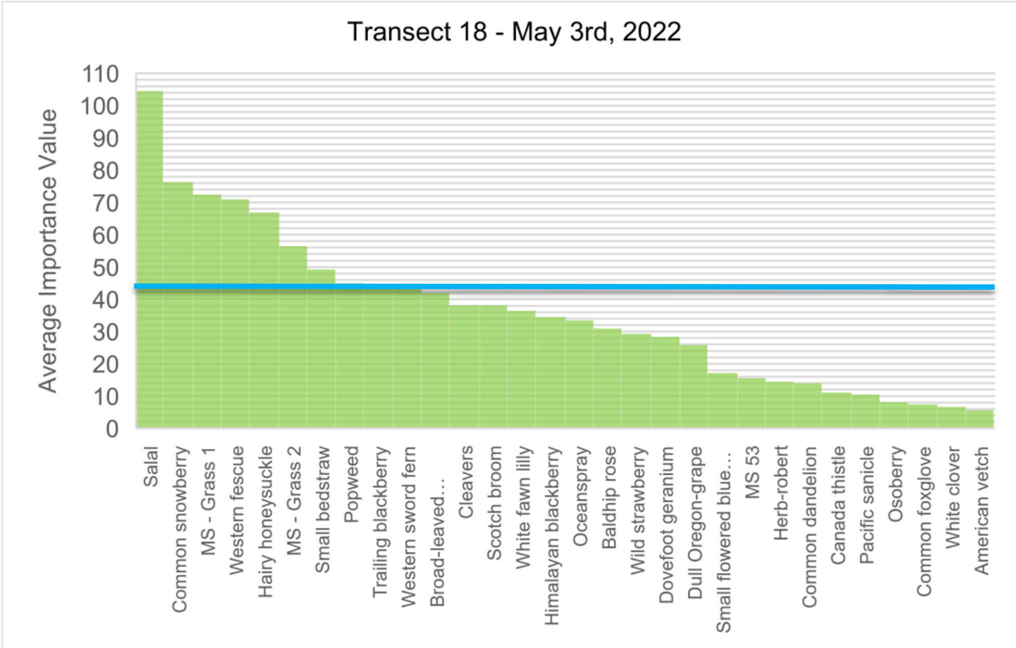


Figure 20 - Individual species average Importance values of transect 18 data collected at Mary Lake Nature Sanctuary on May 3rd, 2022. First plot in transect starting at N48.50059, W123.51505, last plot starting at N48.49816, W123.51502. Average importance value of transect 18 on May 3rd, 2022, displayed by the blue line (44.32).

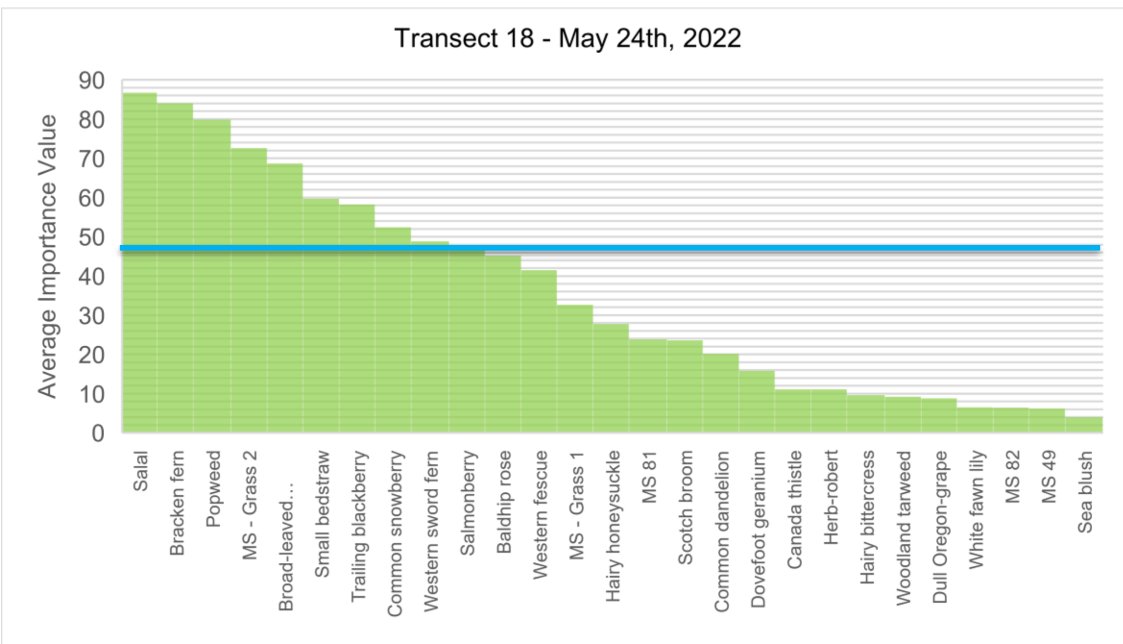


Figure 21 - Individual species average Importance values of transect 18 data collected at Mary Lake Nature Sanctuary on May 24th, 2022. First plot in transect starting at N48.50059, W123.51505, last plot starting at N48.49816, W123.51502. Average importance value of transect 18 on May 24th, 2022, displayed by the blue line (47.10).

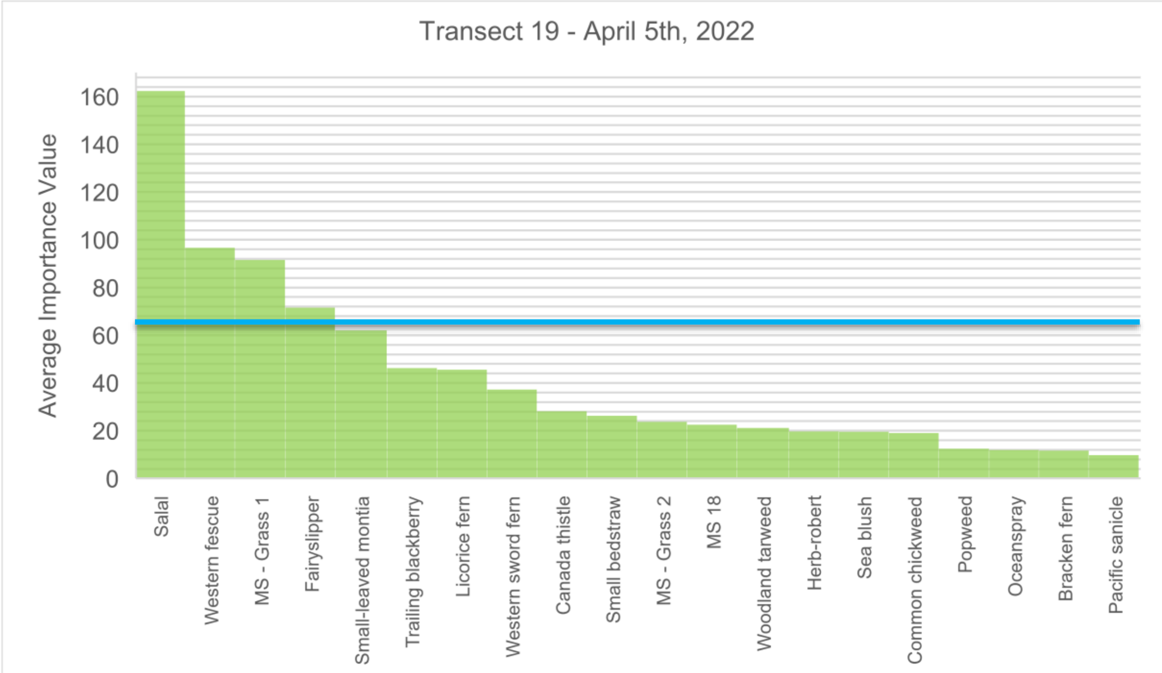


Figure 22 - Individual species average Importance values of transect 19 data collected at Mary Lake Nature Sanctuary on April 5th, 2022. First plot in transect starting at N48.50059, W123.51464, last plot starting at N48.49816, W123.51462. Average importance value of transect 19 on April 5th, 2022, displayed by the blue line (63.83).

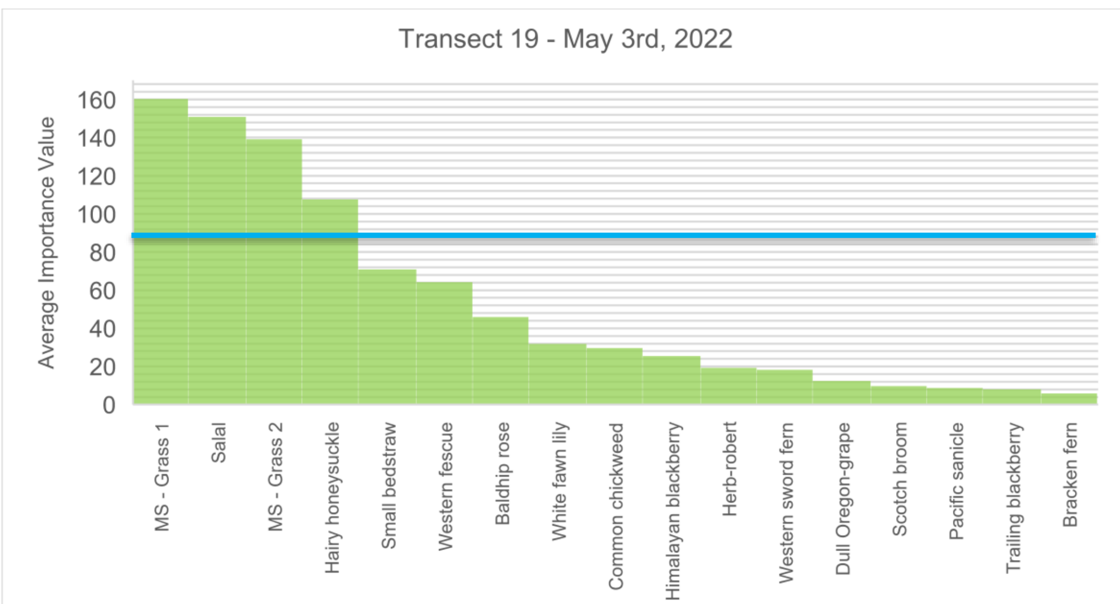


Figure 23 - Individual species average Importance values of transect 19 data collected at Mary Lake Nature Sanctuary on May 3rd, 2022. First plot in transect starting at N48.50059, W123.51464, last plot starting at N48.49816, W123.51462. Average importance value of transect 19 on May 3rd, 2022, displayed by the blue line (88.24).

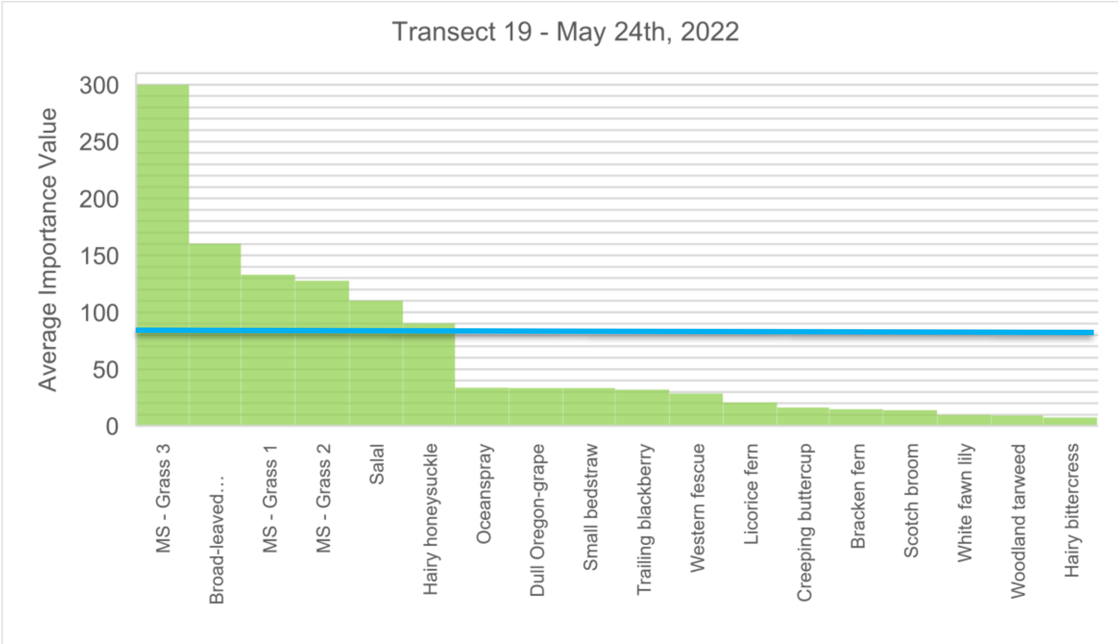


Figure 24 - Individual species average Importance values of transect 19 data collected at Mary Lake Nature Sanctuary on May 24th, 2022. First plot in transect starting at N48.50059, W123.51464, last plot starting at N48.49816, W123.51462. Average importance value of transect 19 on May 24th, 2022, displayed by the blue line (77.49).

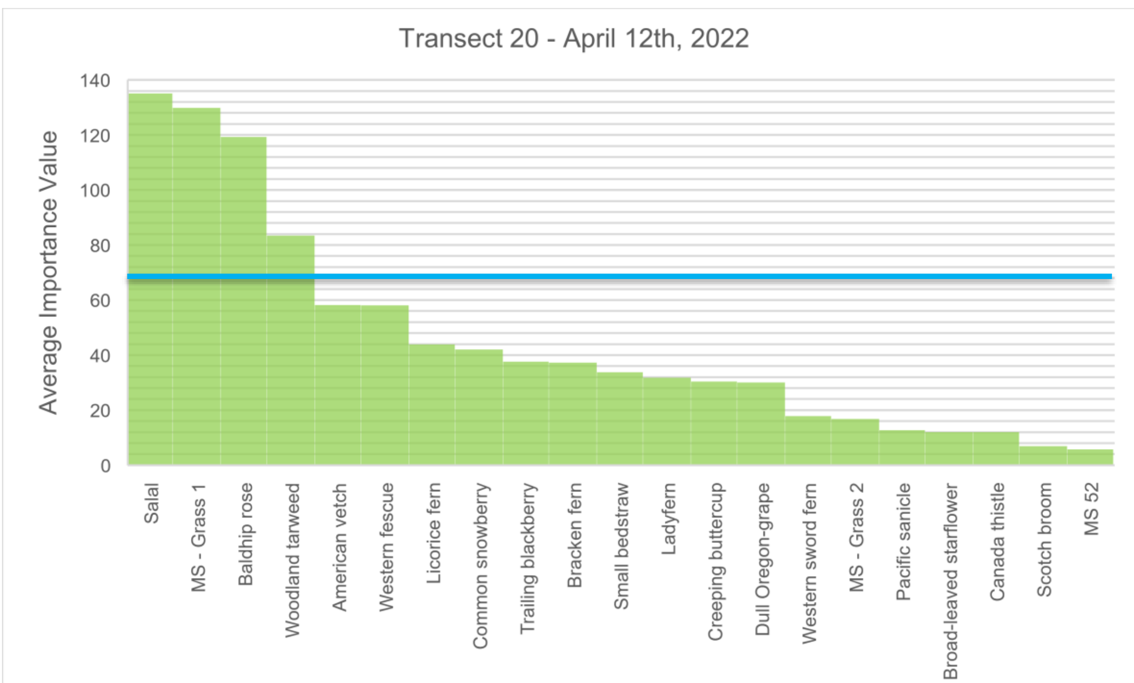


Figure 25 - Individual species average Importance values of transect 20 data collected at Mary Lake Nature Sanctuary on April 12th, 2022. First plot in transect starting at N48.50059, W123.51424, last plot starting at N48.49789, W123.51421. Average importance value of transect 20 on April 12th, 2022, displayed by the blue line (67.70).

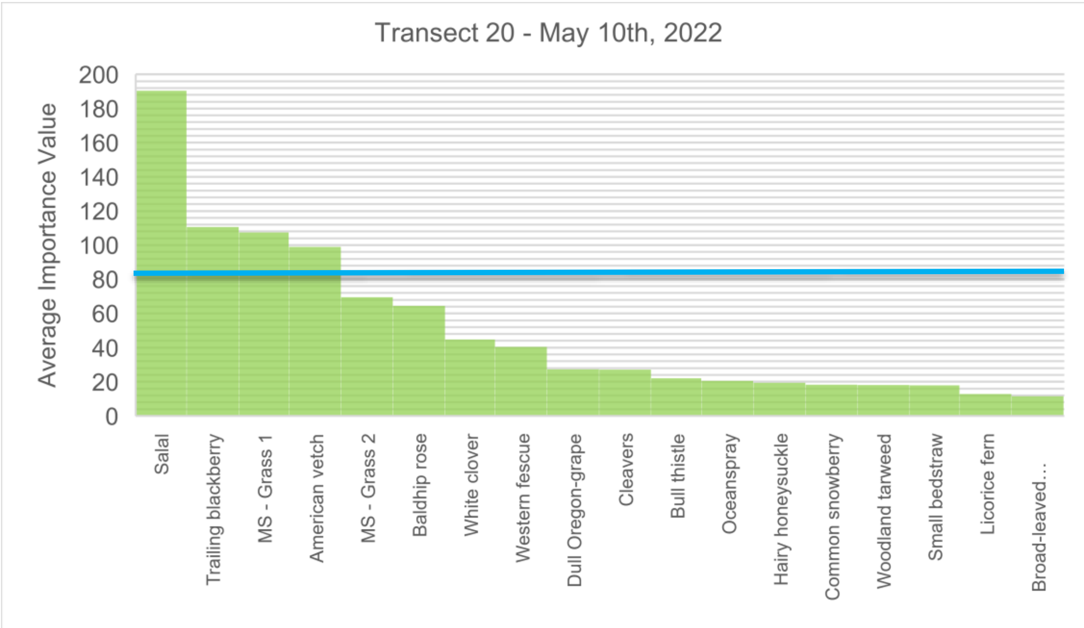


Figure 26 - Individual species average Importance values of transect 20 data collected at Mary Lake Nature Sanctuary on May 10th, 2022. First plot in transect starting at N48.50059, W123.51424, last plot starting at N48.49789, W123.51421. Average importance value of transect 20 on May 10th, 2022, displayed by the blue line (83.10).

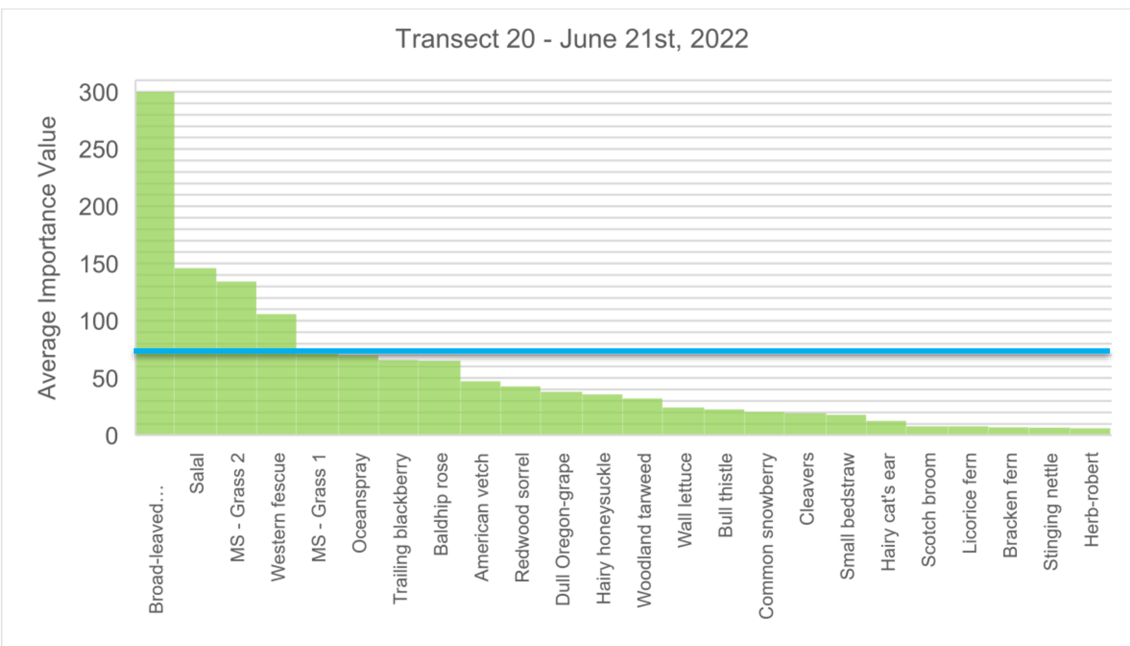


Figure 27 - Individual species average Importance values of transect 20 data collected at Mary Lake Nature Sanctuary on June 21st, 2022. First plot in transect starting at N48.50059, W123.51424, last plot starting at N48.49789, W123.51421. Average importance value of transect 20 on June 21st, 2022, displayed by the blue line (70.90).

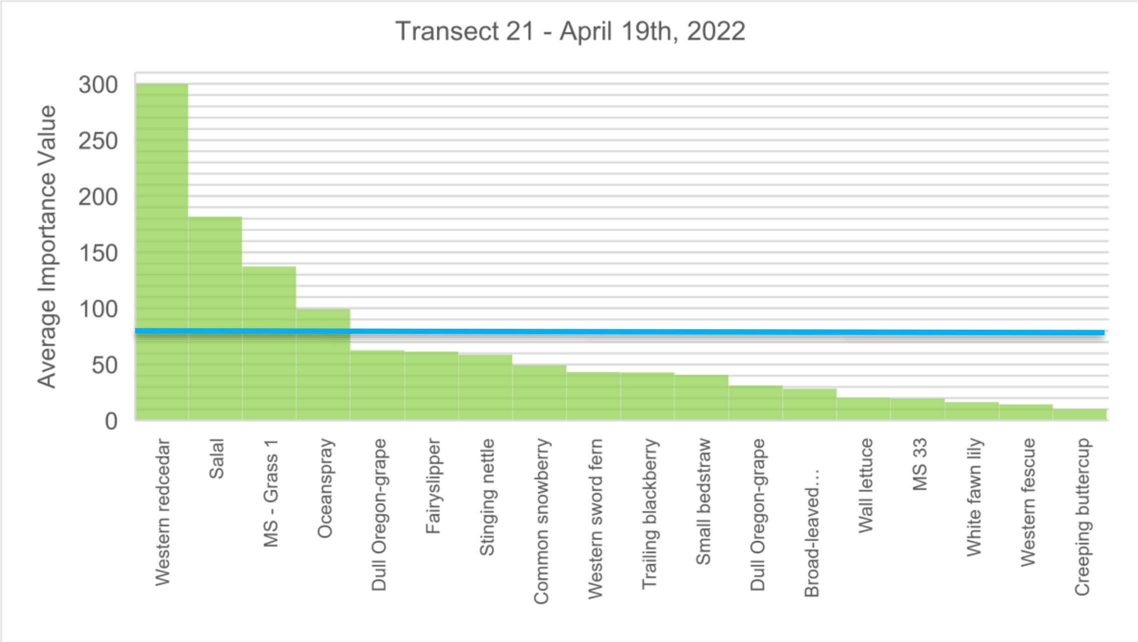


Figure 28 - Individual species average Importance values of transect 21 data collected at Mary Lake Nature Sanctuary on April 12th, 2022. First plot in transect starting at N48.50059, W123.51383, last plot starting at N48.49790, W123.51380. Average importance value of transect 21 on April 12th, 2022, displayed by the blue line (84.38).

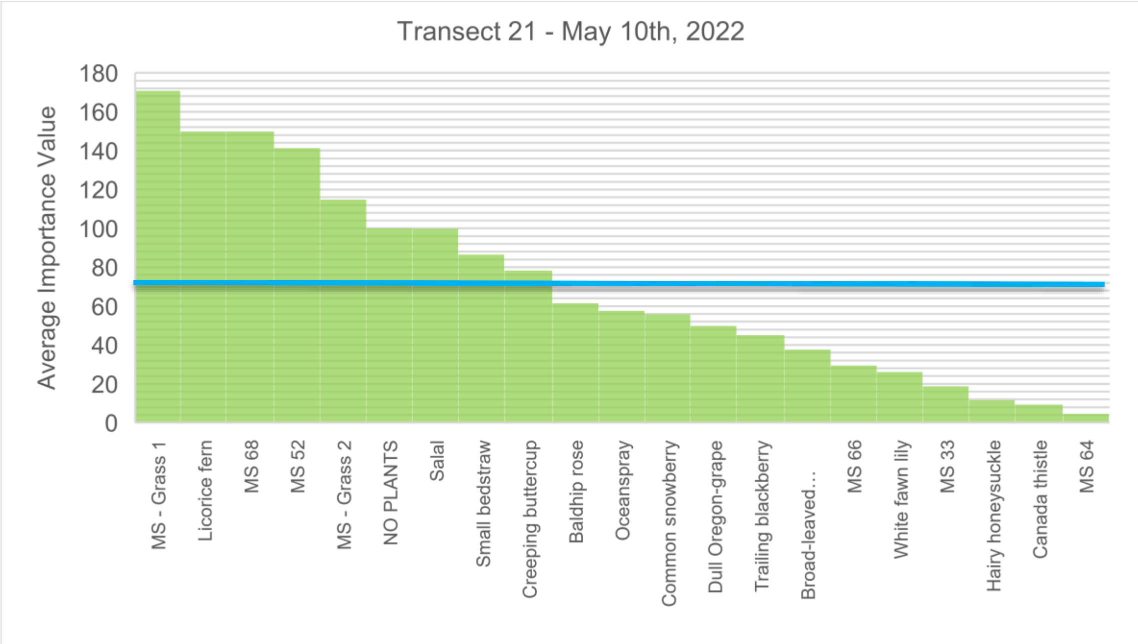


Figure 29 - Individual species average Importance values of transect 21 data collected at Mary Lake Nature Sanctuary on May 10th, 2022. First plot in transect starting at N48.50059, W123.51383, last plot starting at N48.49790, W123.51380. Average importance value of transect 21 on May 10th, 2022, displayed by the blue line (71.43).

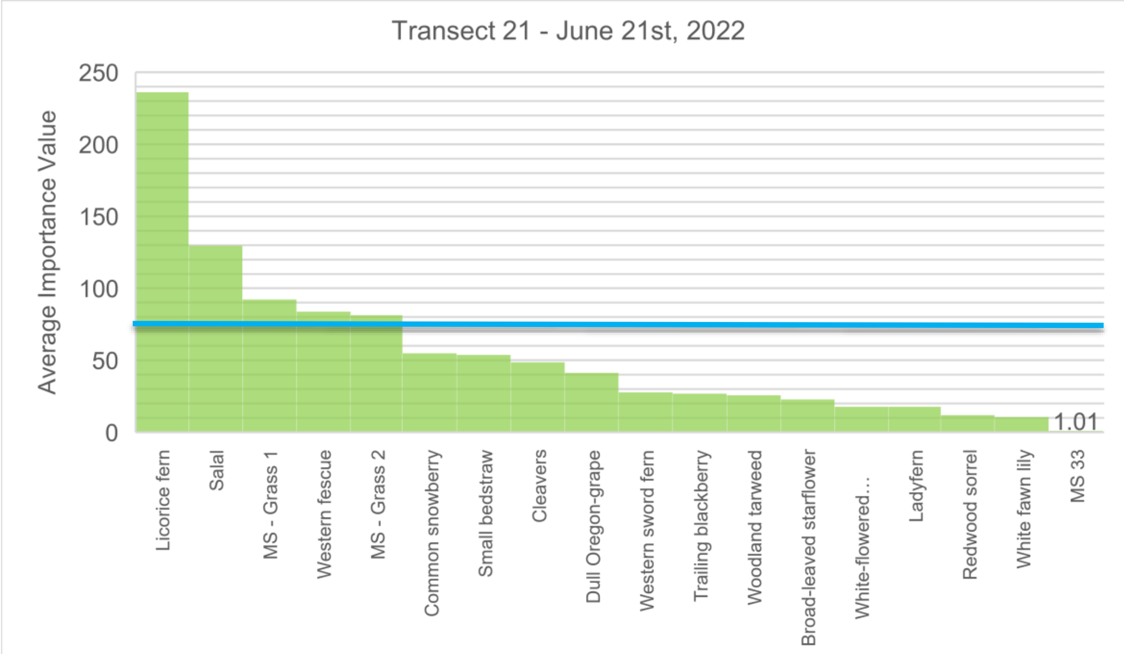


Figure 30 - Individual species average Importance values of transect 21 data collected at Mary Lake Nature Sanctuary on June 21st, 2022. First plot in transect starting at N48.50059, W123.51383, last plot starting at N48.49790, W123.51380. Average importance value of transect 21 on June 21st, 2022, displayed by the blue line (70.90).

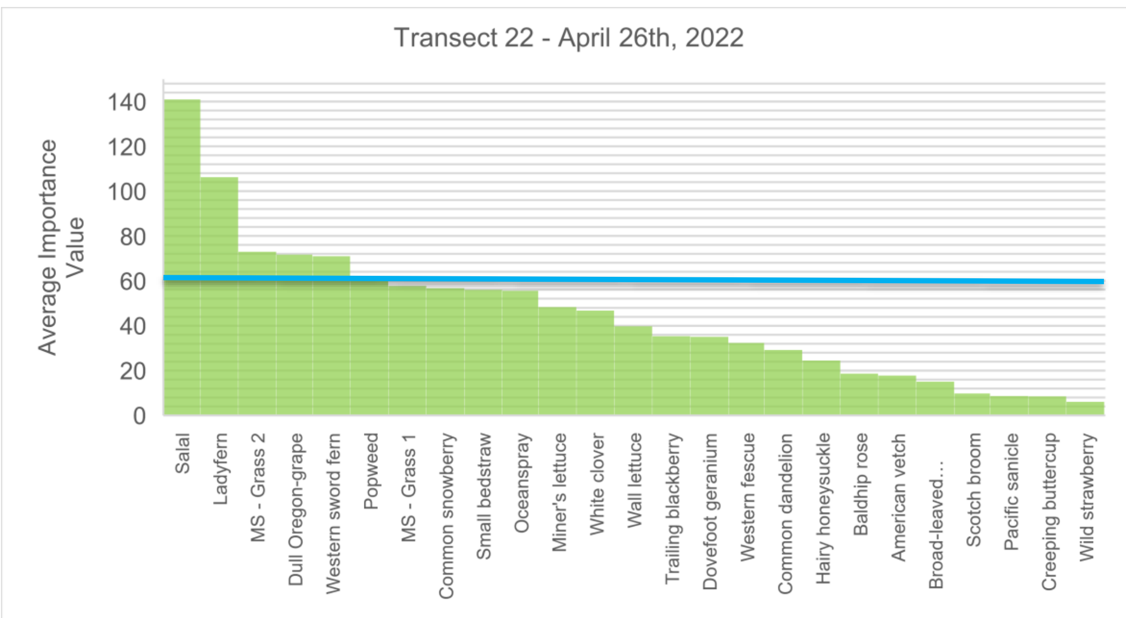


Figure 31 - Individual species average Importance values of transect 22 data collected at Mary Lake Nature Sanctuary on April 26th, 2022. First plot in transect starting at N48.50059, W123.51342, last plot starting at N48.49763, W123.51339. Average importance value of transect 22 on April 26th, 2022, displayed by the blue line (61.22).

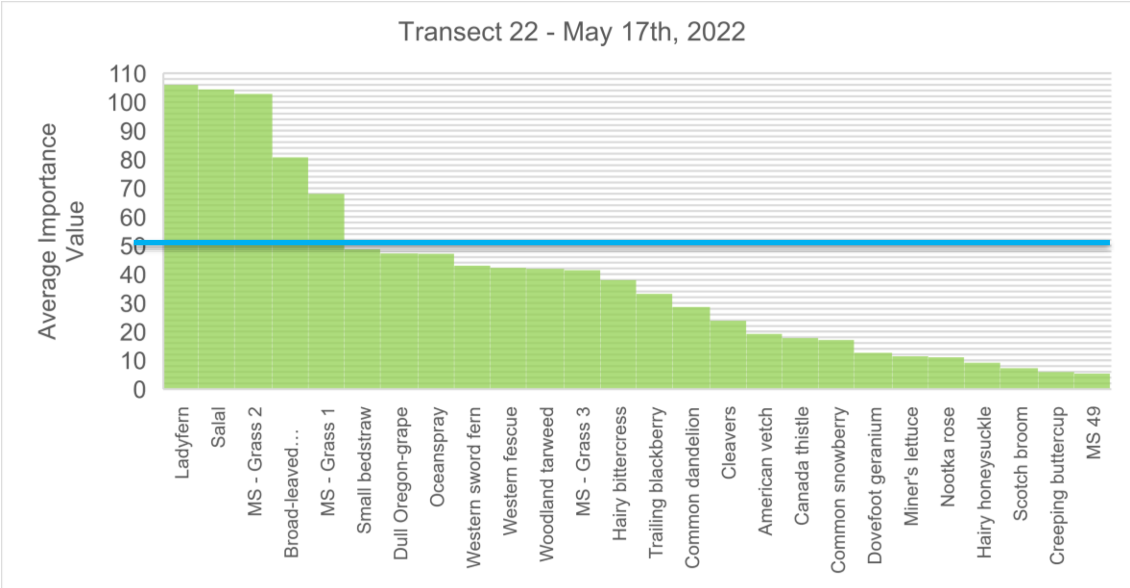


Figure 32 - Individual species average Importance values of transect 22 data collected at Mary Lake Nature Sanctuary on May 17th, 2022. First plot in transect starting at N48.50059, W123.51342, last plot starting at N48.49763, W123.51339. Average importance value of transect 22 on May 17th, 2022, displayed by the blue line (52.01).

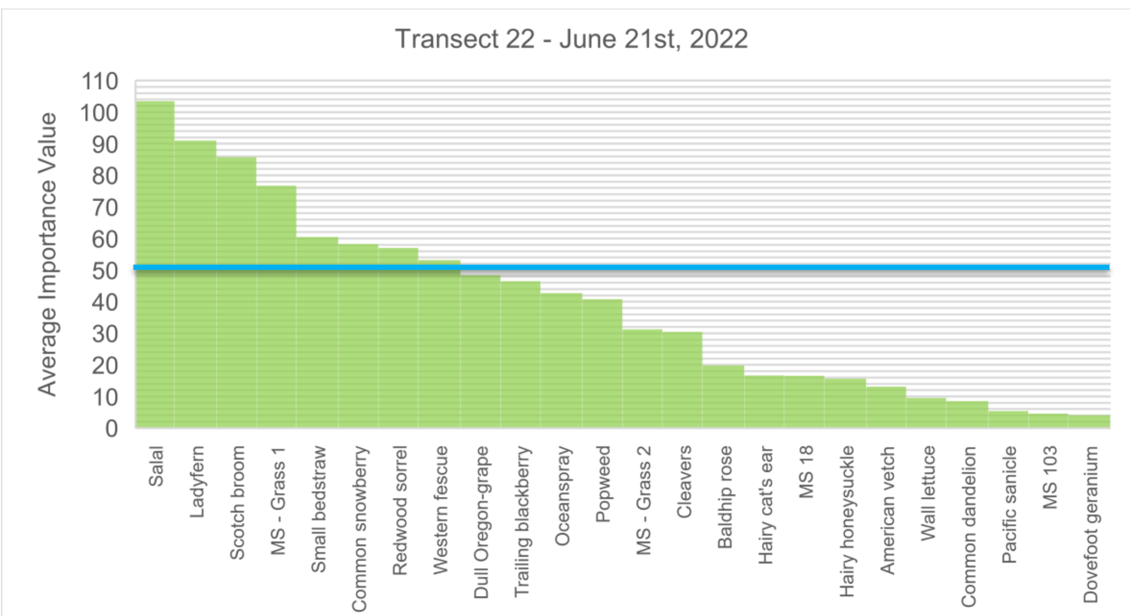


Figure 33 - Individual species average Importance values of transect 22 data collected at Mary Lake Nature Sanctuary on June 21st, 2022. First plot in transect starting at N48.50059, W123.51342, last plot starting at N48.49763, W123.51339. Average importance value of transect 22 on June 21st, 2022, displayed by the blue line (51.72).

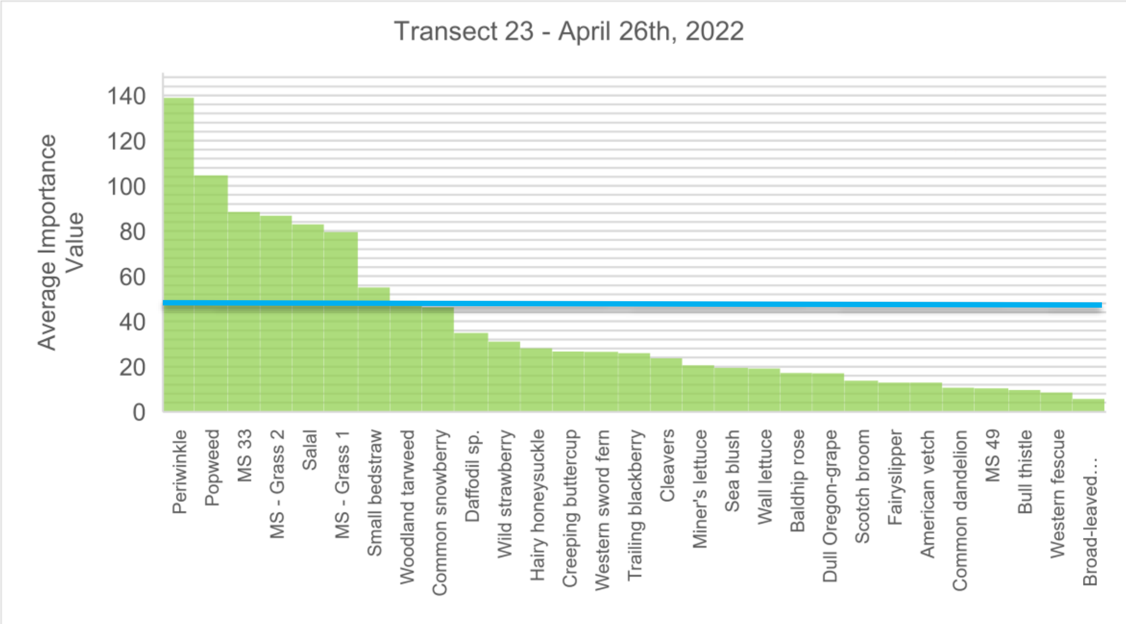


Figure 34 - Individual species average Importance values of transect 23 data collected at Mary Lake Nature Sanctuary on April 26th, 2022. First plot in transect starting at N48.50060, W123.51302, last plot starting at N48.49790, W123.51299. Average importance value of transect 23 on April 26th, 2022, displayed by the blue line (46.51).

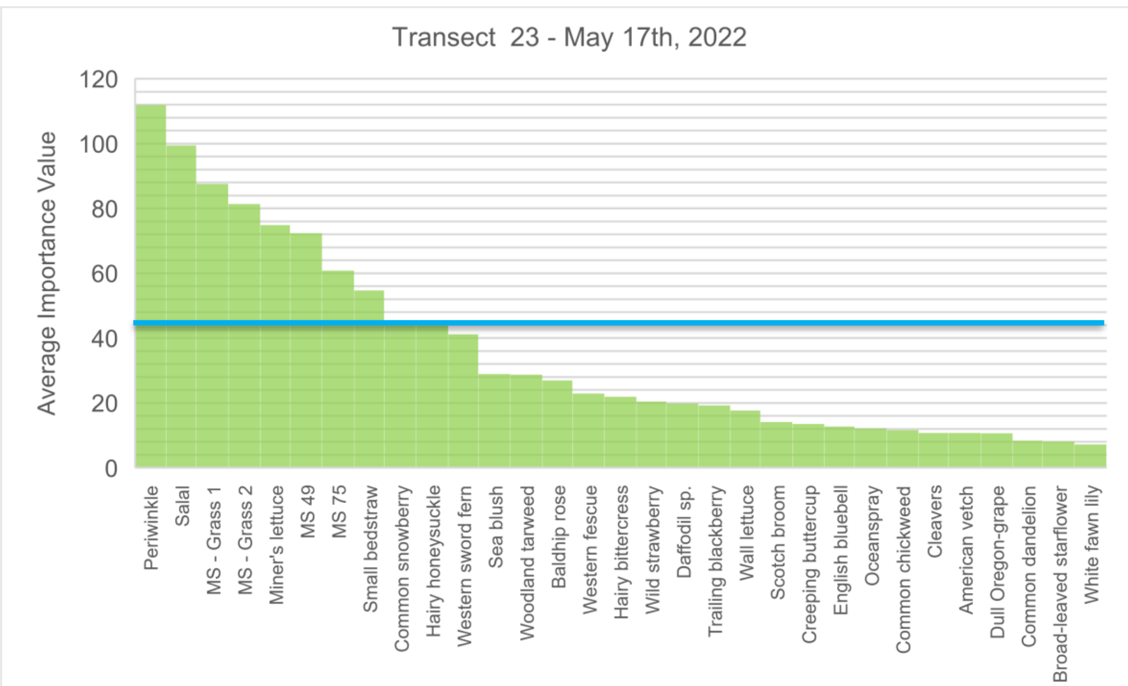


Figure 35 - Individual species average Importance values of transect 23 data collected at Mary Lake Nature Sanctuary on May 17th, 2022. First plot in transect starting at N48.50060, W123.51302, last plot starting at N48.49790, W123.51299. Average importance value of transect 23 on May 17th, 2022, displayed by the blue line (44.50).

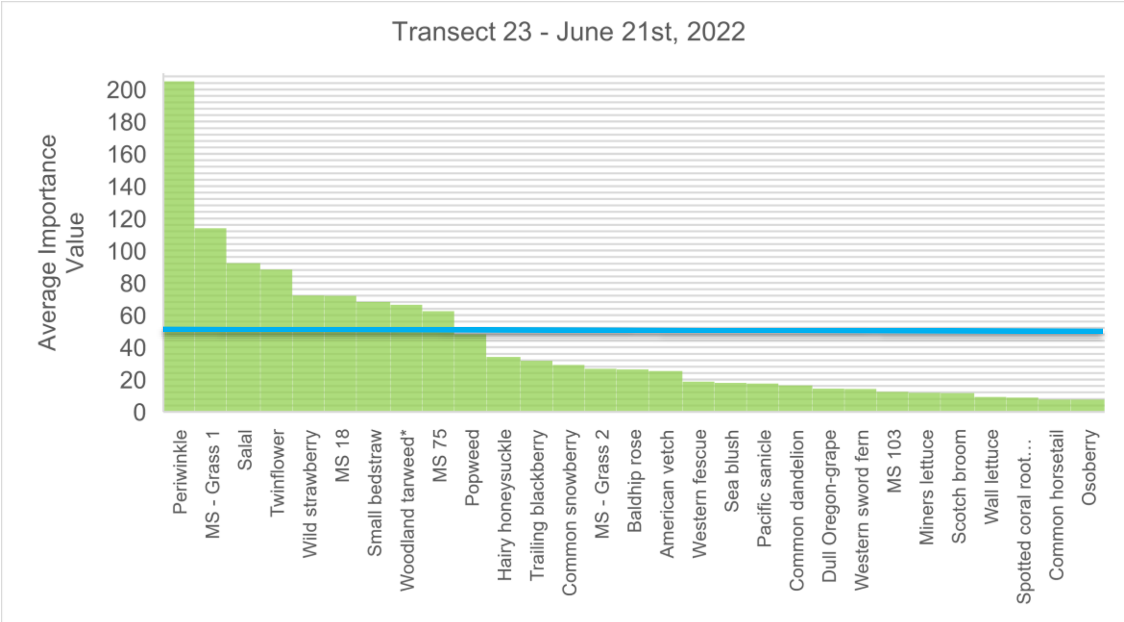


Figure 36 - Individual species average Importance values of transect 23 data collected at Mary Lake Nature Sanctuary on June 21st, 2022. First plot in transect starting at N48.50060, W123.51302, last plot starting at N48.49790, W123.51299. Average importance value of transect 23 on June 21st, 2022, displayed by the blue line (47.70).

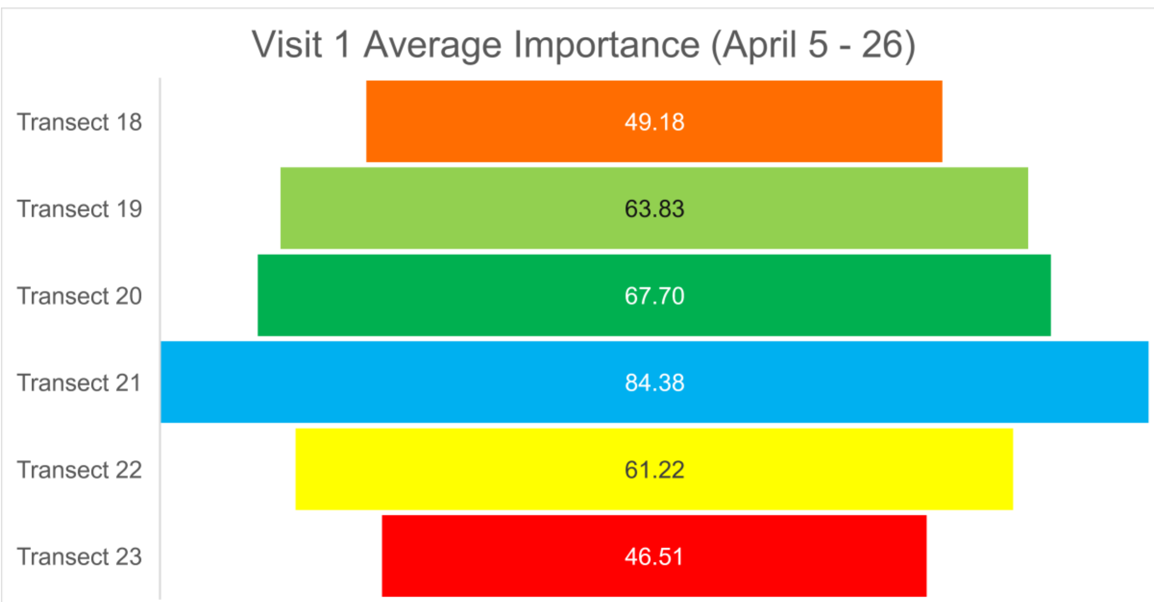


Figure 37 – Transects 18-23 overall average importance values of baseline vegetation transect survey data collected at Mary Lake Nature Sanctuary from April 5th – April 26th, 2022.

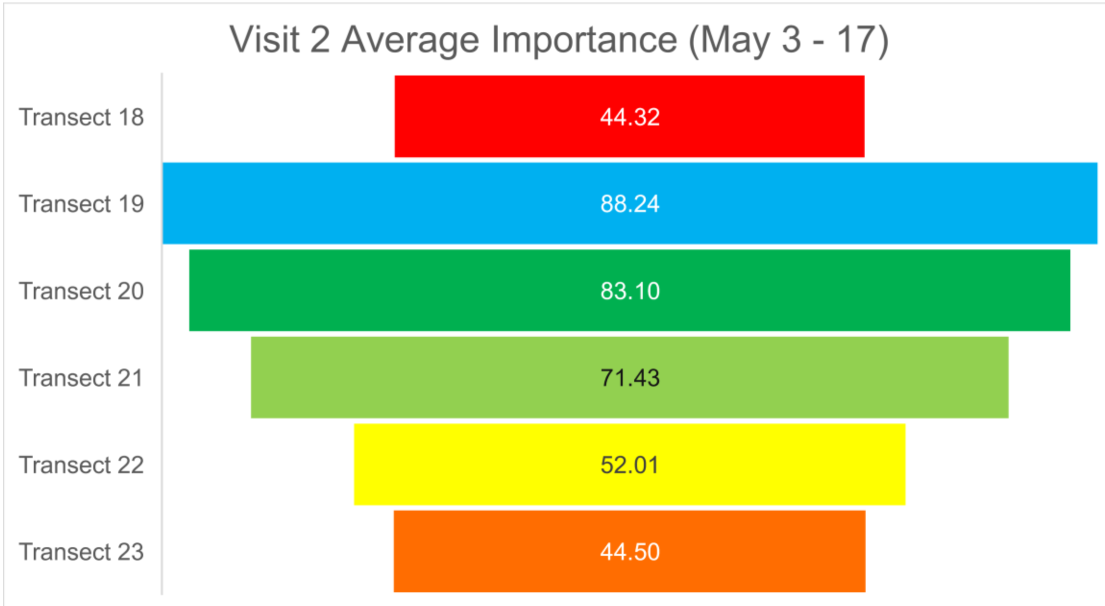


Figure 38 – Transects 18-23 overall average importance values of baseline vegetation transect survey data collected at Mary Lake Nature Sanctuary from May 3rd - May 17th, 2022.

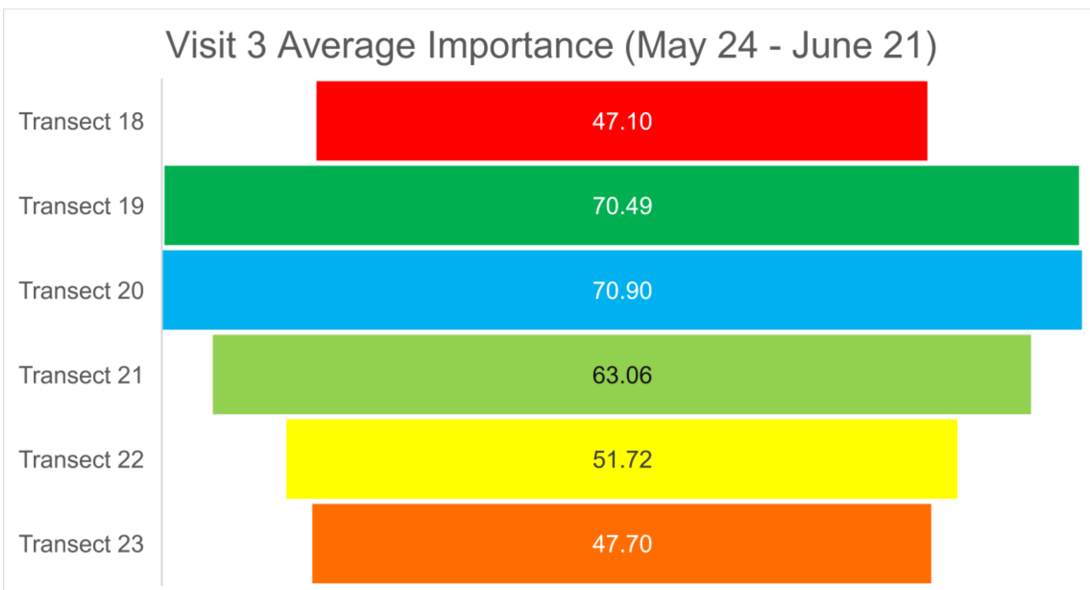


Figure 39 – Transects 18-23 overall average importance values of baseline vegetation transect survey data collected at Mary Lake Nature Sanctuary from May 24th - June 21st, 2022.

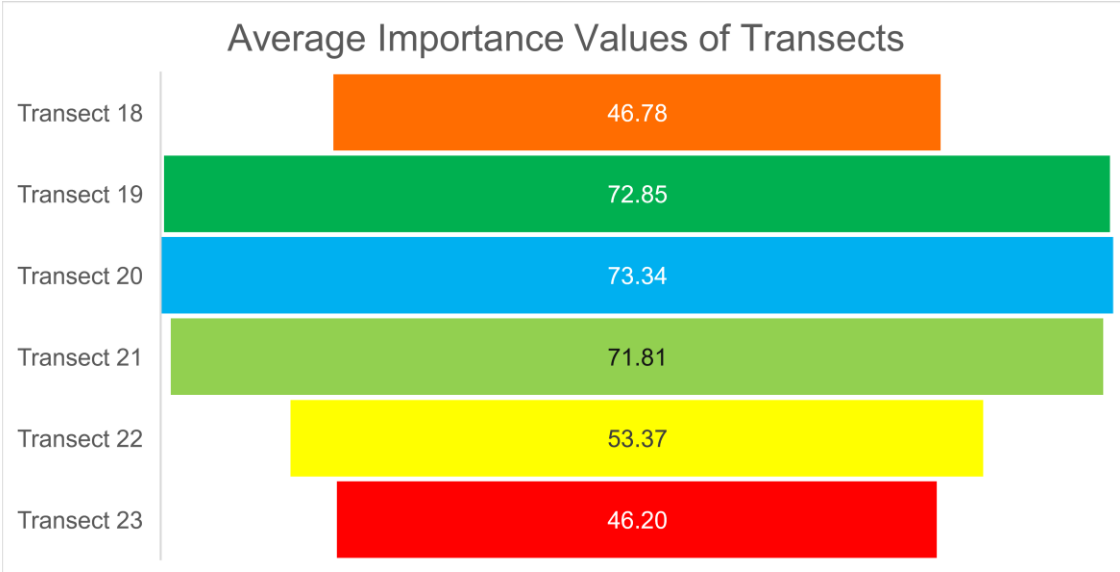


Figure 40 – Transects 18-23 overall average importance values of baseline vegetation transect survey data collected at Mary Lake Nature Sanctuary from April 5th – June 21st, 2022.

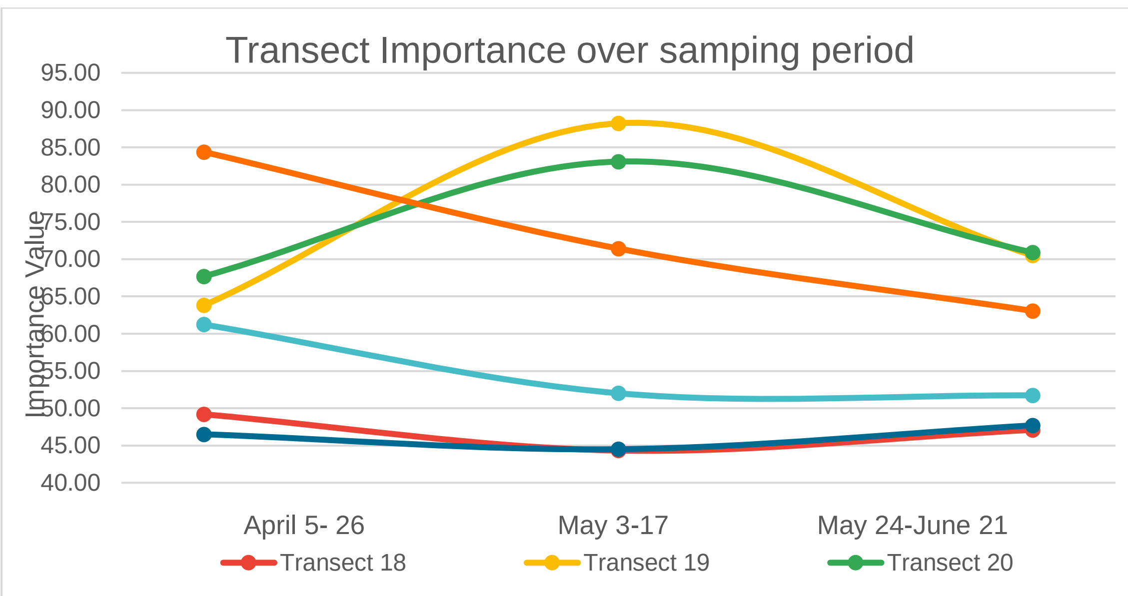


Figure 41 – Transects 18-23 average importance values of baseline vegetation transect survey data collected at Mary Lake Nature Sanctuary from April 5th – June 21st, 2022. Each data series is representative of a given transect over the study period.

Species	Risk	Species	Risk	Species	Risk
American vetch	Yellow	Oceanspray	Yellow	MS - Grass 1	N/A
Baldhip rose	Yellow	Osoberry	Yellow	MS - Grass 2	N/A
Bracken fern	Yellow	Pacific sanicle	Yellow	MS - Grass 3	N/A
Broad-leaved starflower	Yellow	Periwinkle	Exotic	MS18	N/A
Bull thistle	Exotic	Popweed	N/A	MS33	N/A
Canada thistle	Exotic	Redwood sorrel	Blue	MS49	N/A
Cleavers	Yellow	Salal	Yellow	MS52	N/A
Common chickweed	Exotic	Salmonberry	Yellow	MS58.1	N/A
Common dandelion	Exotic	Scotch broom	Exotic	MS65	N/A
Common horsetail	Yellow	Seablush	Yellow	MS66	N/A
Common snowberry	Yellow	Small bedstraw	Yellow	MS68	N/A
Creeping buttercup	Exotic	Small-leaved montia	Yellow	MS69	N/A
Daffodils	Exotic	Spotted coral root orchid	Yellow	MS75	N/A
Dovefoot geranium	Exotic	Stinging nettle	Exotic	MS81	N/A
Dull Oregon-grape	Yellow	Trailing blackberry	Yellow	MS100	N/A
English bluebell	Exotic	Twinflower	Yellow	MS103	N/A
Fairyslipper	Yellow	Wall lettuce	Exotic		
Hairy bittercress	Exotic	Western fescue	Yellow		
Hairy cat's ear	Exotic	Western redcedar	Yellow		
Hairy honeysuckle	Yellow	Western swordfern	Yellow		
Herb-robert	Exotic	White clover	Exotic	**Blue**: Special Concern	
Himalayan blackberry	Exotic	White fawn lily	Yellow	**Yellow**: Secure and not at risk of extinction	
Ladyfern	Yellow	White-flowered hawkweed	Yellow	**Exotic**: Non-indigenous species	
Licorice fern	Yellow	Wild strawberry	Yellow	**N/A**: Not applicable	
Miner's lettuce	Yellow	Woodland tarweed	Yellow		
Nootka rose	Yellow				
***Source**: B.C. Conservation Data Centre. 2022. BC Species and Ecosystems Explorer. B.C. Minist. of Environ. Victoria, B.C. Available: https://a100.gov.bc.ca/pub/eswp/*					

Table 3 - List of all species sampled during terrestrial transect surveying between April 5th, 2022. and June 21st, 2022, along transects eighteen to twenty-three, in reference to BC Conservation Datacenter information. Compiled on July 19th, 2022.

5.0 Discussion

5.1 Water Quality Monitoring Discussion

The sampling took place between April 5th and June 28th, 2022. Consequently, Mary Lake was undergoing spring turnover. Spring turnover occurs when snow and ice on the surface of a waterbody melts, which causes the temperature of the surface water to decrease relative to the deeper water. As a result, the warmer water at the bottom of the lake will have lower pressure compared to the surface, causing it to rise through the water column (Heidorn, 2005). Since this had occurred earlier in the season and surface and deep water had already mixed, less stratification would be evident at this point in the season.

Overall, water temperature increased from April - July due to an increase in seasonal temperatures, as shown in Figures 5 and 6. There was little variation in temperature between the sampling locations in the lake. The temperature of the three creeks changed across the property due to different light levels at each site. Conversely, the lake has a consistent lack of canopy overhead, resulting in even light distribution at the different sampling locations. Pure distilled water does not conduct electricity; therefore, conductivity in a water body is an indication that there are dissolved inorganics present. Thermal energy from the sun increases the overall energy within the water, allowing charged particles to move more freely, thus increasing the conductive capacity of the waterbody (U.S. EPA, 2022A).

A healthy freshwater body should have a DO concentration > 6.5 mg/L and be maintained at 80 to 120% saturation concentration (BC MoE, 2021). Saturation concentration is the number of molecules which can be dissolved in solution and is dependent on temperature. Without these levels of oxygen to supply the necessary biological oxygen demand (BOD) of the lake, aquatic species may perish. Furthermore, temperature and pressure can affect the levels of DO in each waterbody. When temperatures rise, DO drops; when pressure increases at greater depths, DO also decreases (Fondriest Environmental Inc., 2013).

Given the results of the routine water quality monitoring, most of the DO readings were recorded above the minimum threshold of 6.5 mg/L. It is important to note, however that the temperature is only an average of all the readings throughout the various locations (including at depth), and the atmospheric pressure on the day of the measurement was not recorded and would change the 80 – 120% saturation concentration. The blue cells in the Tables below fall below the recommended range and could start to cause negative health impacts. Regardless of temperature, dissolved oxygen should always be greater than 6.5 mg/L as this is the concentration at which smaller fish species may start to experience health issues and possibly die (ENR, n.d.). However, there would not be any negative effects at higher concentrations (BC MoE, 2021). This parameter may be of concern as the weather gets warmer and may have posed a threat to the health of the lake and surrounding creeks as the temperature continued to increase in the weeks following the sampling period (EPA, 2021).

Average temperature	Dissolved Oxygen in Lake (mg/L)	80 – 120% saturation at measured temperature (mg/L).
7.2	15.7 - 20.6	9.66 - 14.49
8.8	9.9 - 13.5	9.29 - 13.94
9.3	7.8 - 9.9	9.18 - 13.77
9.4	18.5 - 20.3	9.16 - 13.74
11.1	9.8 - 10.4	8.80 - 13.21
11.5	10.7 - 16	8.72 - 13.08
12.4	9.3 - 9.8	8.55 - 12.82
14.1	8.1 - 8.8	8.23 - 12.35
17.6	6.9 - 7.5	7.64 - 11.46
19.8	6.3 - 6.8	7.31 - 10.96

*Cells in blue represent when the dissolved oxygen concentration was at dangerously low levels.

Table 4 - Average Lake temperature, dissolved oxygen levels and percent saturation determined using U of MN Natural Resources Institute, 2015 of water over the study period (April 5th – June 28th, 2022).

Average temperature	Dissolved Oxygen in Lake (mg/L)	80 – 120% saturation at measured temperature (mg/L) (U of MN Natural Resources Institute, 2015).
6.6	18.1 - 21.9	9.81 - 14.71
7.0	11.2 - 14.6	9.71 - 14.57
7.5	6.1 - 8.4	9.59 - 14.39
8.5	20.3 - 22.1	9.36 - 14.04
9.6	10.4 - 11.8	9.12 - 13.68
10.4	9.2 - 11	8.95 - 13.42
11	9.2 - 13.1	8.82 - 13.24
11	8.6 - 10.7	8.82 - 13.24
13.9	7.9 - 10	8.27 - 12.4
16.3	6.4 - 7.9	7.85 - 11.77

*Cells in blue represent when the dissolved oxygen concentration was at dangerously low levels.

Table 5 - Average stream temperature, dissolved oxygen levels and present saturation of water over the study period (April 5th – June 28th, 2022)

The recommended pH for aquatic life in freshwater systems is between 6.5 and 8.5 (U.S. EPA, 2022_B). In Dead Deer Creek, on April 5th, 2022, one value fell outside this range. Dead Deer Creek is a shallow seasonal creek and runs over exposed rocks. This is important because the leaching from the exposed rock can increase amounts of inorganic solutes. These solutes would cause an increase in pH since rocks containing carbonates tend to be basic; however, the ions would not have large effects on ecosystem health due to the creek's shallowness and not being able to provide suitable habitat for fish species (U.S. EPA, 2022_B). The creek itself did not maintain a high pH for an extended period.

Within MLNS, there are a multitude of factors that can alter the pH of Mary Lake itself. The property is situated within the Coastal Douglas-fir biogeographic zone where the dominant tree species, Douglas-fir, has needles with a pH between 3.2 and 3.8 (Pokorny, 2017). Where needles can fall into waterbodies or tributary streams, they can decrease pH. Likewise, precipitation could also decrease pH, in part from acid rain. Atmospheric pollution can undergo both dry and wet deposition and cause waterbodies to become more acidic. Other influencing factors may involve the runoff from neighboring construction and industry or the diffusion of CO_2 into the water. Depending on the rate of CO_2 diffusion or introduced contaminants, pH may fluctuate both ways (Fondriest Environmental Inc., 2013).

Location	pH range between April 5th and June 28th	Average pH between April 5th and June 28th
Dead Deer	7.74 - 8.76	8.03
North Earsman	7.69 - 8.18	7.93
South Earsman	7.55 - 8.14	7.80
Dock	7.65 - 8.4	7.92
Lake 1	7.57 - 8.15	7.82
Lake 1 (depth)	7.62 - 8.04	7.83
Lake 2	7.50 - 8.01	7.75
Lake 2 (depth)	7.52 - 8.0	7.69
Lake 3	7.38 - 8.05	7.74
Lake 3 (depth)	7.46 - 8.17	7.76

Table 6 - pH range and average pH of each sample location (surface and depth for lake samples) throughout the study period (April 5th – June 28th, 2022).

There is not a required level of TDS to maintain aquatic life in freshwater systems; this includes all solutes in the water body, including inorganics and abiotic organics (B.C. MoE, 2021). Conductivity only represents the presence of inorganic solutes, since organic solutes do not conduct electricity.

Nitrate is a natural by-product of fish digestion. High nitrate concentrations indicate the presence of nitrifying bacteria as they convert atmospheric nitrogen into nitrate. In freshwater, long-term exposure to high nitrate concentrations can have negative health impacts on fish. In B.C., the recommended maximum nitrate concentration to maintain fish health is 32.8 mg/L and a 30-day average of 3.0 mg/L (Nordin, R. N., Pommen, L. W., 2009). Nitrate is used as a nutrient source by the aquatic vegetation; thus, falling below these levels may interfere with aquatic plant diversity and health. All the water bodies had nitrate concentrations below the maximum recommendation. Many of the locations did not contain detectable levels of nitrate.

Phosphate is a necessary nutrient in aquatic ecosystems; however, if levels of phosphorus are higher than levels needed for healthy freshwater systems (0.02 ppm), they can cause excess growth of algae since often they are limiting nutrients in the environment (CCME, 2004). Resulting algal growth can adversely affect a waterbody since it blocks incoming sunlight to other plants, limits oxygen generation and depletes overall dissolved oxygen in the aquatic environment, affecting biodiversity and species survival. Some sources of phosphorus could be derived from nearby construction, agriculture, and sewage. The concentrations of phosphate were below detection limits apart from North Earsman Creek and the Lake 2 and 3 samples taken at depth.

Alkalinity is the tendency to resist changes in pH upon the introduction of acidic compounds. This is an important parameter for the health of a water body because all living organisms have a preferred pH range. The Saskatchewan Research Council (Swain, 1987) classifies the sensitivity of a water body to changes in pH as (1) low (>20 mg/L), (2) medium (10-20 mg/L) and (3) high (<10 mg/L), which is measured as equivalence of Calcium Carbonate concentration. However, the measurement represents the concentration of other alkaline chemicals such as bicarbonate, limestone, phosphates, borates, silicates, and other alkaline minerals. The main source of alkaline minerals is leaching into the water from surrounding rocks. Additionally, alkaline materials from the use of concrete can introduce these substances through runoff into the hydrological cycle. Each location had low sensitivity to changes in pH as they were determined to have an alkalinity value > 20 mg/L and from April 5th to May 17th, 2022, the alkalinity increased. Further, the alkalinity tended to be greater in the deeper lake locations compared to the surface samples. This can be explained by the proximity of the deeper water to the rocks along the lakebed. Since the creeks ran over exposed rocks, one would suspect increased levels of alkaline materials from the leaching of rocks into the waterbody. However, there was no difference between alkalinity values of the creek or the lake samples.

Fecal coliforms are microorganisms that exist in the intestinal tract of warm-blooded organisms, and their concentration is an indicator of the potential for bacterial pathogenicity. Due to the animals present in the surrounding environment and increased runoff due to rainfall prior to sampling, there were detectable levels of fecal coliforms on April 5th, 2022, in all locations apart from Dead Deer Creek, while on May 17th, 2022, it had not rained prior to sampling, resulting in less runoff and lower levels of fecal coliforms. This lake is not used for drinking water or swimming; therefore, this is still below the recommended fecal coliform concentration for freshwater lakes, which is < 406 cfu/mL (New Hampshire Department of Environmental Services, 2019).

The data collected by VIEC in 2019 compared to the 2022 data indicated that the surface temperatures of the lake in 2019 were higher overall by 2-4°C between April and July. During the sampling periods in 2019 and 2022, the temperatures showed similar increases over the same timeframe. The largest difference in temperatures in 2019 and 2022 occurred in June: the temperature was 23.7°C on June 18th, 2019, and 18.1°C on June 21st, 2022. Similarly, in 2019 the average 2 m depth temperatures of the lake were higher by approximately 4-5°C from April - June. Average temperatures of the creeks in 2019 were higher than in 2022. Additionally, all tributaries were noted to dry up completely by the end of the season, according to VIEC’s findings.

Further, in comparison to VIEC 2019 data, the DO for the creeks on average was lower at the beginning (April) of the sampling period than in 2022 by almost 10 mg/L. During both 2019 & 2022, DO showed a steady decrease throughout the sampling period with only 1 mg/L difference seen between the years in June. Similarly, the average DO concentration of the lake was approximately 10 mg/L higher in 2022 than 2019 throughout, at the beginning of the sampling period (April). Additionally, the average DO concentrations of the lake surface and depths in 2019 and 2022 both showed a similar steady decline to approximately 7 mg/L by June. The observed difference in DO could be attributed to a wetter season and lower temperatures of the creeks in 2022. In 2019 the average pH data showed an increase into May and then a decrease until July. However, in 2022 the average pH data did not show any trends and fluctuated throughout. The differences in pH between these years could be due to several factors, although the largest factor is likely that the creeks feeding Mary Lake in 2019 had dried up by the end of the season. Finally, the average conductivity of the lake was slightly higher in 2019; however, in both years, conductivity increased overall throughout the lake locations from April to June.

5.2 Terrestrial Vegetation Transect Survey Discussion

While measures were taken to reduce the impact of the study on the ecosystem, repeated visits to sampling sites and the placing of sampling quadrats may have disturbed the areas under study, potentially impacting species composition. Additionally, the quality and significance of data relies on correct species identification. Using multiple resources may have introduced error through incorrect identification of species. Morphospecies designation and documentation also allowed for potentially incorrect identification and overlap with other morphospecies if samples were obtained from a species at different life stages. To overcome this, a list of morphospecies with attached photos has been provided in Appendix D. Data upload of field recordings may have also introduced error into interpretation and analyses, where misspelling of species during input may generate repeat values for density, dominance, frequency, and importance calculations.

5.2.1 **Relative Density.**

This refers to the number of individual plants of a single species compared to the sum of all individual plants counted within a quadrat sample, multiplied by 100 to generate a percentage value (Roberts-Pichette et al.,1999). This was calculated by counting individual plants of each species and comparing against the total sum of all plants in a quadrat. It should be noted that for species too numerous to count, every individual was granted a density value of 101 for consistency. A sample calculation is provided below:

$$Relative\ Density\ (Rdn) = \frac{Number\ of\ individual\ plants}{Sum\ of\ all\ individual\ plants} * 100$$

5.2.2 Relative Dominance.

This refers to the area occupied by a single species within a quadrat (m^2) compared to the total area occupied by all species within a plot (sum of all plant area occupied for each species (m^2)) (Roberts-Pichette et al.,1999). Data for relative dominance were collected using a quadrat sampler with a 10x10 grid of squares (each square was 10 cm x 10 cm). Squares were counted if any part of a plant of a given species was within it, generating a percent occupancy as seen in the field vegetation data forms in Appendix A. Overlap between species within squares does not affect the dominance of either, and will be documented as one percent occupancy for all species within a single square. Percent occupancy values were multiplied by 0.01 to convert from percent occupation to area in square meters. A sample calculation for relative dominance is included below:

$$Relative\ Dominance\ (Rdm) = \frac{Area\ occupied\ by\ one\ plant\ species\ (m^2)}{Total\ area\ occupied\ by\ all\ plant\ species\ (m^2)} * 100$$

5.2.3 Relative Frequency.

This refers to the distribution of one species across a single transect – where each occurrence of observing a species in a quadrat sample will be tallied and divided by the number of quadrats along a transect (Roberts-Pichette et al.,1999). This will generate a frequency value, which is then divided by the sum of all species frequencies within a given transect. A sample calculation for relative frequency is provided below.

$$Relative\ frequency\ (Rf) = \frac{\left(\frac{Number\ of\ occurences\ along\ a\ transect}{Number\ of\ quadrat\ samples\ in\ a\ transect}\right)}{Sum\ of\ all\ species\ frequencies} * 100$$

5.2.4 Importance Values.

These are the sum of all three above values, which indicate a single species' structural role within a transect and highlights the significance of a given species' presence throughout the year (Roberts-Pichette et al.,1999). Species with a low importance value will have been seen and documented in lower numbers, covering less area, and not observed as often elsewhere. Low importance values may be useful in identifying species that are not well-established within the MLNS, or more sensitive species which will require additional protection to ensure they are not extirpated, or those that do not serve as large a purpose as higher importance plants. For the purposes of monitoring the long-term health and conservation of MLNS terrestrial ecosystems, it is recommended that some species of lower importance which are native to the area, sensitive to disturbance, and desired by the conservation groups, be thoroughly studied for their continued relative density, dominance, and frequency.

The higher importance plants are those that have become well established in the MLNS ecosystem, serving as key species performing essential services and acting as important components of ecosystem. Higher importance values across a transect represent more mature and developed ecosystems in areas of less disturbance, as made evident by the average importance values of transects 19, 20, and 21, which are located deeper within the nature sanctuary. The higher density, dominance, and frequency which generate overall greater importance values hint to the species which have occupied a significant area of the nature sanctuary and are less likely to be affected by disturbance. Species such as salal, dull Oregon-grape, western sword fern and the MS grass species hold some of the highest importance values over the study period, where they have consistent and well-established populations. These high-importance plant species serve as strong indicators of the health of MLNS, where changes to their populations, densities, dominance, and frequencies over time may indicate larger changes to the ecology of the nature sanctuary.

Interestingly, plots near areas with higher disturbance yielded lower average importance values overall than those further away, reinforcing the negative impacts of anthropogenic activity on mature community development. This was made evident by the high species counts for these transects. While higher biodiversity is characteristic of healthy ecosystems, in the case of this analysis it is more representative of site disturbance. The species present within the disturbed sites (MS grass species, dandelions, pop weed, woodland tarweed etc.) are mostly characterized by short lifespans, high seeding rates and lower biomass than species from more established, mature communities. Transect 18 ran parallel to an access road leading to the nature house located on MLNS property, in the roadside ditch. While initial species counts were highest at the first two sampling locations along transect 18, other sampling locations had lower species counts, as they were dominated by species like salal and pacific dogwood - resulting in higher importance values for those species within the transect. Transects 22 and 23 were the closest samples to Millstream Road, which has high traffic, wildlife access, less dense canopy, and forest floor coverage.

Invasive species did have a presence on the property during the study period; however, they were found mostly in sites with greater disturbance and lacking mature community composition. Invasive species included pop weed, dandelion, scotch broom and Himalayan blackberry. The invasive species on site failed to establish themselves in deeper, more protected, and less disturbed areas. This signifies the potential capacity of MLNS to self-regulate species composition if disturbance can be limited. Removal of invasive species in more disturbed sites is recommended following integrated management strategies, specific to each species of concern. Should conservation of MLNS continue to be successful in the more protected areas of the property, it is hypothesized that the mature ecosystems will extend back into areas such as transects 18, 22, and 23, leading to the prolonged protection of these natural sites that are rapidly being lost to development.

6.0 Conclusion

The Mary Lake Nature Sanctuary Research Project was a major project study undertaken by East Island Environmental Consulting. Beginning in January 2022, the team comprised four undergraduate students at Royal Road University worked with Mary Lake Nature Sanctuary to produce a report outlying quantitative and qualitative data regarding a terrestrial vegetative survey and a pre-established water quality monitoring program outlined by previous Royal Roads University work group, Van Isle Eco Consulting.

Given the different stewards, owners, and land usage the property has had over its history, current owners are prioritizing the alignment of their values of conservation and preservation with the values of the W̱SÁNEĆ (Saanich) Coast Salish peoples. Given the zoning of the site and GVGS's objectives for MLNS, EIEC has worked to provide valuable information that will contribute to further understanding the characteristics of the waterbody, land, ecosystems, and species that exist within the 17-hectare property.

6.1 Water Quality Conclusion

EIEC had set out to expand on the established water quality monitoring plan, taking recommendations highlighted by previous sources and making the necessary changes. EIEC dismissed the need to sample sediments for heavy metals and take Secchi disc readings. Additionally, EIEC has added to the procedure by which TDS are now part of the weekly water monitoring protocol.

A comparison of water quality data collected in 2019 as outlined in the *Mary Lake Nature Sanctuary - Lake Stewardship Project* (VIEC, 2019), and 2022 data indicated that average temperatures of the lake and surrounding tributaries were 2 - 5°C higher in 2019 throughout the sampling periods for corresponding months. A comparison of 2019 and 2022 showed the average temperatures in the lake and surrounding creeks had similar trends of steady increase from April – July. In April 2022 the average DO was 10 mg/L higher in both the creeks and lake in comparison to 2019 at the beginning of the sampling period in April. However, average DO in both years for the lakes and creeks decreased to similar concentrations. The average pH in both years' data did not vary for both the creek and lake samples. The average conductivity in 2019 was higher than in 2022 for both the creeks and lakes; however, both showed a similar trend of increase throughout the sampling periods between April and July.

Parameters of concern for the health of the aquatic ecosystems included phosphate concentration and DO availability. These parameters were indicated to be a concern based on recommended ranges for healthy aquatic ecosystems outlined by the U.S EPA (2012) and the BC MoE (2021). The measurable levels of phosphate present indicate eutrophic conditions in the lake and North Earsman Creek. As both the ambient and water temperature increased, DO concentrations fell below levels sufficient to support aquatic life.

6.2 Terrestrial Vegetation Conclusion

The second half of the project focused on the terrestrial vegetation transect survey. The protocol involved a series of transects numbered 1 through 23 which ran North to South along the property and were spaced 30 m apart. An unbiased sampling protocol was completed to monitor plant species composition along the transects. 67 species were identified from transects 18 to 23 alone. Most species observed were classified as not-at-risk or exotic according to the BC Conservation Data Centre (Government of British Columbia, 2021). Only one species, redwood sorrel, was classified as having special concern. Included in the list of 67 total species are 16 morphospecies. These species were recorded as unique species by EIEC; however, their appropriate designations were not determined through the means of previously discussed resources.

Evident in the data collected from the six focused transects are varying levels of biodiversity. In transects closer to the interior of the property, aside from transect 18, less variance of species was seen as compared to those at the exterior of the property. Introduced species may have established themselves in the exterior transects due to the adjacent road. As for transect 18, which follows the same trend as transects 22 and 23, higher species diversity may be due to the property's access road; it travels along most of the transect and contributes to the migration of invading species. This alone could serve as the main reason why a lower variety of species in transects 19 through 21 was observed, compared to 18, 22, and 23. Continually low importance values in transects 18, 22, and 23 highlight that importance values are distributed amongst more diverse communities.

In conducting the terrestrial vegetation survey, species were identified and given an associated importance value. Species with high importance values were salal, dull Oregon-grape, western sword fern and the MS grass species. Furthermore, transects that had higher average importance values (19, 20, and 21), represent areas with more mature ecosystems that endured impacts from natural and anthropogenic disturbances.

The average importance values of the six studied transects were subject to changes throughout the seasons. Generally, importance values of the transects were highest at the start of the study period in April. As spring transitioned to summer, average importance values of all transects declined. EIEC predicts this event to be associated to the annual cycle of succession within the sanctuary and the extended cool temperatures endured in 2022. This led to delayed seeding, growing, and blooming of some plant species. The decreased averaged importance values are indicative of later blooming, increasing species variance, and decreasing the overall average importance value of each individual transect.

Going forward, monitoring terrestrial plant species may demonstrate changes occurring on site. Whether observations focus on species with low or high importance values, each can be valuable in monitoring long-term changes. Changes in low importance value species can indicate whether the presence of exotic, invasive species are increasing and further establishing themselves in the sanctuary. Subtle changes in species newly discovered in transects can have long-term impacts and affect the ecology of the established ecosystems. Due to this, changes in density, dominance, and frequencies of high importance value species are good indicators to how the overall ecosystem changes and whether invasive species are impacting the vegetative community of MLNS.

Changes to an area's natural plant species community can aid MLNS and the GVGS in determining future strategies to continue the conservation of the sanctuary. Monitoring can delineate areas of sensitivity to future development projects, determine whether invasive species removal is necessary, and guide future conservation efforts.

6.3 Recommendations

6.3.1 For Mary Lake Nature Sanctuary

1. Continue terrestrial vegetation transect survey
    a. EIEC suggests that MLNS continue the monitoring of terrestrial vegetation. Ideally, an appropriate schedule should encompass areas of the entire property and allow time for vegetative recovery between sampling areas, to mitigate anthropogenic disturbance from sampling.
2. Invasive Species Control
    a. Techniques can be implemented to slow and stop weed introduction and/or remove invasive species. Application of these practices should be directed to areas of high concern such as traffic corridors.
3. Continue the routine water quality monitoring
    a. To supply MLNS with a complete, accurate long-term water quality dataset, steps to maintain a weekly/bi-weekly monitoring schedule year-round on the lake and surrounding streams should be practiced.
4. Dissolved oxygen management
    a. To mediate the low concentrations of dissolved oxygen in the late spring and summer seasons, MLNS might investigate the potential implementation of pumping dissolved oxygen into Mary Lake or introducing vegetation. DO kept decreasing throughout the season, so monitoring should continue into September.
5. Mitigation of phosphate
    a. The Trophic State Index (TSI) classifies a water body as eutrophic, oligotrophic and mesotrophic based on the concentration of phosphorus, chlorophyll A and Secchi disk depth (Devi Prasad, Siddaraju, 2012). The trophic state was estimated using only the total phosphorus present but since the results indicated that the lake may be slightly eutrophic, further may be useful.
    b. Use chemical treatments that have proven to decrease phosphorus in natural bodies (Schindler et. Al, 2016).

6.3.2 For Future Research

1. Amend the terrestrial vegetation transect survey
    a. Remove the grid on the quadrat sampling form provided by EIEC and enlarge the species and density table.
    b. Define different stages of growth to be inputted in the species and density table.
2. Increase species survey scope
    a. Due to time constraints, EIEC was unable to include bryophytes and aquatic species in the survey. The inclusion of these species would provide a more complete survey to judge overall ecosystem health.
3. Adjust the routine water quality monitoring procedure
    a. Given the results of the fecal coliform analyses, EIEC suggests future laboratory tests to follow the Most Probable Number (MPN) Technique. This method is highly selective as compared to the current Membrane Filter (MF) technique and adopting the former will provide clearer results. If future studies preference the MF technique, EIEC suggests that colony morphologies taken for each distinct bacterium.
    b. A COD/BOD assay would provide supplementary information to how oxygen is used in the aquatic environment.
    c. Overall, more sensitive equipment with proper calibration schedules are better. The latest technology can aid in eliminating inconsistencies and detecting concentrations below thresholds of current field and laboratory technology.

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Appendix A Forms

E.I.E.C
East Island Environmental Consulting

MARY LAKE NATURE SANCTUARY WATER MONITORING FORM

NAMES(s): **DATE & TIME:**

EQUIPMENT:____

Sample ID	Depth (m)	pH	Conductivity (µS/cm)	Temperature (°C)	Dissolved O_2 (mg/L)	TDS (mg/L)	Time Sampled
"Dead Deer Creek"	Surface						
Earsman North	Surface						
Earsman South	Surface						
Dock	Surface						
Lake 1	Surface						
Lake 1	2 m						
Lake 2	Surface						
Lake 2	2 m						
Lake 3	Surface						
Lake 3	2 m						

Note: Surface is assumed at 0.0m

OBSERVATIONS: (Wildlife, Algal Growth, etc.)

____
____
____
____
____
____
____
____
____

Appendix A.1: Water monitoring form created by EIEC

Laboratory Analysis Form - Alkalinity

E.I.E.C
East Island Environmental Consulting

Date:____
Team Member(s):________________________________

Table 1 - KHP weights to standardize NaOH. Intended mass 0.5. Made with 75ml of distilled water.
Scale Information:

Standard	**Actual Mass**
Standard #1	
Standard #2	
Standard #3	

Table 2 – NaOH weights to standardize HCL. Intended mass 1.0 g made with 250 ml of distilled water.
Scale Information:

Standard	**Actual Mass**
Standard #1	
Standard #2	
Standard #3	

Table 3 - Standardization of 0.1 M NaOH against KHP using 3 drops phenolphthalein through volumetric titration.

Burette Reading (ml)	**Standard #1**	**Standard #2**	**Standard #3**
Final			
Initial			
Total Volume			
Observations:			

Table 4 - Standardization of 0.1 M HCL against 0.1 M NaOH using 3 drops phenolphthalein through volumetric titration.

Burette Reading (ml)	**Standard #1**	**Standard #2**	**Standard #3**
Final			
Initial			
Total Volume			
Observations:			

Table 5 - Potentiometric Titration using 100 ml samples using pH every addition of titrant added (0.1 ml) until pH of 4.0 reached.
Information of pH meter :

Sample	Burette Vol	pH	Sample	Burette Vol	pH

Appendix A.2: Alkalinity Laboratory Analysis Form created by EIEC

Laboratory Analysis Form - Nitrate

E.I.E.C
East Island Environmental Consulting

Date:___________________________

Team Member(s) Performing Analysis:____

Table 1 - Nitrate Absorbance Spectrophotometric Analysis. Measurements taken at 520 nm, using 0 ppm prepared solution to blank.
Spectrophotometer information:____

Sample/Solution	**Sample Depth**	**Reading @ 520 nm**	**Observations**
0 ppm (blank)			
1 ppm			
2 ppm			
3 ppm			
4 ppm			
Dead Deer Creek	Surface		
Earsman North	Surface		
Earsman South	Surface		
Dock	Surface		
Lake 1	Surface		
Lake 1			
Lake 2	Surface		
Lake 2			
Lake 3	Surface		
Lake 3			

Appendix A.3: Nitrates Laboratory Analysis Form created by EIEC

Laboratory Analysis Form - Phosphate

E.I.E.C
East Island Environmental Consulting

Date:___________________________

Team Member(s) Performing Analysis:____

Table 1 - Phosphate Absorbance Spectrophotometric Analysis. Measurements taken at 650 nm, using distilled water to blank.
Spectrophotometer information:____

Sample/Solution	Sample Depth	Reading @ 650 nm	Observations
0 ppm			
0.25 ppm			
0.5 ppm			
1 ppm			
2 ppm			
3 ppm			
Dead Deer Creek	Surface		
Earsman North	Surface		
Earsman South	Surface		
Dock	Surface		
Lake 1	Surface		
Lake 1			
Lake 2	Surface		
Lake 2			
Lake 3	Surface		
Lake 3			

Appendix A.4: Phosphates Laboratory Analysis Form created by EIEC

E.I.E.C
East Island Environmental Consulting

Laboratory Analysis Form - Fecal Coliforms

Date:______________________________

Team Member(s):____

Table 1: Fecal Coliform Analysis. Enumerated Fecal Colonies that Filtered Through Milipore Membrane Filter Paper and then Plated and Incubated on m-FC Plates for 24hrs at 44.5°C.

Sample ID	Dilution Factor	Colony Count Sample 1	Colony Count Sample 2	Average	Plate Observations
Dead Deer Creek	Undiluted				
Dead Deer Creek	10^{-1}				
Earsman North	Undiluted				
Earsman North	10^{-1}				
Earsman South	Undiluted				
Earsman South	10^{-1}				
Dock	Undiluted				
Dock	10^{-1}				
Lake 1	Undiluted				
Lake 1	10^{-1}				
Lake 2	Undiluted				
Lake 2	10^{-1}				
Lake 3	Undiluted				
Lake 3	10^{-1}				

Note: Lake 1, Lake 2 and Lake 3 samples taken at 2 m depth. Dead Deer Creek, Earsman North, Earsman South and Dock samples taken at surface.

Appendix A.5: Fecal Coliforms Laboratory Analysis Form created by EIEC

MARY LAKE NATURE SANCTUARY QUADRAT SAMPLING FORM

E.I.E.C
East Island Environmental Consulting

NAME(S):______________________________

DATE/TIME:____________________________

TRANSECT #:___________________________

PLOT # (L or R): ________________________

LOCAL TERRESTRIAL ECOLOGY WITHIN 10m:

(trees, shrubs, canopy cover, etc)

____

____

____

N

	A	B	C	D	E	F	G	H	I	J
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

GRID VIEW

SPECIES & DENSITY:

Species Name	Stage of growth	Individual Count	% Occupancy

Appendix A.6: Quadrat Sampling Form created by EIEC

Appendix B Water quality monitoring Data

Weekly water quality monitoring data

Dead Deer								
Parameter	pH		Conductivity (µS/cm)		Temp(°C)		DO (mg/L)	
Year	2019	2022	2019	2022	2019	2022	2019	2022
Min	7.47	7.74	191.6	137.9	8.5	6.4	8.4	6.4
Max	7.47	8.76	191.6	225.0	8.5	14.4	8.4	21.9
Average	7.47	8.03	191.6	168.8	8.5	9.2	8.4	12.4
South Earsman								
Parameter	pH		Conductivity (µS/cm)		Temp(°C)		DO (mg/L)	
Year	2019	2022	2019	2022	2019	2022	2019	2022
Min	7.11	7.55	162.1	119.6	10.8	7.2	7.5	6.1
Max	7.69	8.14	185.7	140.6	17.5	19.4	10.9	22.1
Average	7.47	7.80	174.4	130.8	14.8	11.7	9.4	11.0
North Earsman								
Parameter	pH		Conductivity (µS/cm)		Temp(°C)		DO (mg/L)	
Year	2019	2022	2019	2022	2019	2022	2019	2022
Min	7.64	7.69	184.9	105.1	8.3	5.9	9.0	7.9
Max	8.19	8.18	210.0	184	17.5	15.1	12.9	21.2
Average	7.97	7.93	197.9	148.3	13.22	9.7	10.7	12.3
Dock								
Parameter	pH		Conductivity (µS/cm)		Temp(°C)		DO (mg/L)	
Year	2019	2022	2019	2022	2019	2022	2019	2022
Min	7.59	7.55	167.4	122.5	11.3	7.8	6.5	6.4
Max	8.81	8.40	200.0	405.0	23.8	20.2	10.2	18.6
Average	7.88	7.90	183.8	168.1	18.6	12.1	8.8	10.7
Lake 1								
Parameter	pH		Conductivity (µS/cm)		Temp(°C)		DO (mg/L)	
Year	2019	2022	2019	2022	2019	2022	2019	2022
Min	7.54	7.57	167.0	118.3	12.0	7.1	5.4	6.3
Max	8.07	8.15	192.7	146.8	24.2	20.6	11.7	20.1
Average	7.67	7.82	180.0	133.2	18.7	12.7	8.1	11.1
Lake 1 (2 m depth)								

Parameter	pH		Conductivity (µS/cm)		Temp(°C)		DO (mg/L)	
Year	2019	2022	2019	2022	2019	2022	2019	2022
Min	7.36	7.62	171.8	120.4	13.0	6.8	6.1	6.8
Max	7.98	8.04	191.2	151.0	23.2	18.8	10.2	19.5
Average	7.60	7.83	180.0	136.4	18.6	11.7	8.0	11.7
Lake 2 (Surface)								
Parameter	pH		Conductivity (µS/cm)		Temp(°C)		DO (mg/L)	
Year	2019	2022	2019	2022	2019	2022	2019	2022
Min	7.48	7.50	167.6	120.5	12.6	7.0	5.8	6.4
Max	7.83	8.01	191.2	142.2	23.7	20.4	10.8	20.6
Average	7.59	7.75	180.0	131.6	18.5	12.3	8.1	11.5
Lake 2 (2 m depth)								
Parameter	pH		Conductivity (µS/cm)		Temp(°C)		DO (mg/L)	
Year	2019	2022	2019	2022	2019	2022	2019	2022
Min	7.31	7.38	167.4	116.7	11.7	6.7	6.5	6.4
Max	7.75	8.00	191.2	147.2	22.2	18.6	9	18.7
Average	7.54	7.69	180.4	134.6	17.9	11.4	7.5	11.1
Lake 3 (Surface)								
Parameter	pH		Conductivity (µS/cm)		Temp(°C)		DO (mg/L)	
Year	2019	2022	2019	2022	2019	2022	2019	2022
Min	7.39	7.38	162.1	115.8	13.4	7.4	4.7	6.3
Max	7.65	8.05	191.9	137.7	23	20.7	10.8	20.3
Average	7.52	7.74	177.9	128.7	19.0	12.7	7.6	10.8
Lake 3 (2 m depth)								
Parameter	pH		Conductivity (µS/cm)		Temp(°C)		DO (mg/L)	
Year	2019	2022	2019	2022	2019	2022	2019	2022
Min	7.36	7.46	163.2	117.8	12.7	7.4	5.8	6.7
Max	7.58	8.17	192.3	136.9	22.3	19.2	9.5	18.5
Average	7.45	7.74	180.4	128.5	18.4	12.0	7.3	11.3

Nitrate concentration lab results including absorption readings, nitrate concentrations generated from linear regression of standard solution concentrations for each sampling point. Results for April 5th and May 17th, 2022.

Sample ID 1 (April 5th, 2022)	Absorbance (520nm)	NO^{-3} (ppm) Concentration	Sample ID 2 (May 17th, 2022)	Absorbance (520nm)	NO^{-3} (ppm) Concentration
0 ppm	0	0.000	**0 ppm**	0	0.000
1 ppm	0.050	1.000	**1 ppm**	0.118	1.000
2 ppm	0.084	2.000	**2 ppm**	0.169	2.000
3 ppm	0.145	3.000	**3 ppm**	0.228	3.000
4 ppm	0.187	4.000	**4 ppm**	0.548	4.000
Dead Deer Creek	0.060	1.292	**Dead Deer Creek**	0.014	0.353
N. Earsman	0.042	0.908	**N. Earsman**	0.018	0.386
S. Earsman	0.028	0.610	**S. Earsman**	0.011	0.328
Dock	0.035	0.759	**Dock**	0.000	0.237
Lake 1 0m	0.049	1.058	**Lake 1 0m**	0.000	0.237
Lake 1 2m	0.038	0.823	**Lake 1 2m**	0.006	0.287
Lake 2 0m	0.036	0.780	**Lake 2 0m**	Below Detection	
Lake 2 2m	0.037	0.802	**Lake 2 2m**	Below Detection	
Lake 3 0m	0.029	0.631	**Lake 3 0m**	Below Detection	
Lake 3 2m	0.025	0.546	**Lake 3 2m**	Below Detection	

Total Dissolved Solids data collected throughout the study period (April 5th – June 28th, 2022) including minimum, maximum and average values for each sampling point.

Sample ID	Minimum (mg/L)	Maximum (mg/L)	Average
Dead Deer Creek	97.5	162.0	118.5
N. Earsman	84.9	98.7	105.2
S. Earsman	74.8	131.0	92.5
Dock	86.2	283.0	116.9
Lake 1 0m	86.0	105.0	95.2
Lake 1 2m	85.4	107.0	96.6
Lake 2 0m	85.3	101.0	93.5
Lake 2 2m	83.0	105.0	95.6
Lake 3 0m	81.6	97.5	91.3
Lake 3 2m	83.7	97.4	91.2

Alkalinity data from laboratory analysis completed for each sampling point, collected on April 5th and May 17th, 2022.

Sample ID	April 5th Alkalinity (mg/L)	May 17th Alkalinity (mg/L)
Dead Deer Creek	41.72	47.50
N. Earsman	48.48	59.38
S. Earsman	36.45	52.25
Dock	40.50	71.25
Lake 1 0m	36.45	45.13
Lake 1 2m	42.53	59.38
Lake 2 0m	40.50	49.88
Lake 2 2m	36.45	52.25
Lake 3 0m	36.45	52.25
Lake 3 2m	48.48	25.25

Phosphate data from laboratory analysis completed for each sampling point, collected on April 5th and May 17th, 2022. Absorbance readings taken using Genesys10 UV-Vis Spectrophotometer #11.

Sample ID 1 April 5th 2022	Absorbance (650nm)	PO_4^{-3} (ppm) concentration	Sample ID 2 May 17th 2022	Absorbance (650nm)	PO_4^{-3} (ppm) concentration
0.00 ppm	0.002	0.000	**0.00 ppm**	0.001	0.000
0.25 ppm	0.032	0.250	**0.25 ppm**	0.065	0.250
0.50 ppm	0.068	0.500	**0.50 ppm**	0.119	0.500
1.00 ppm	0.133	1.000	**1.00 ppm**	0.230	1.000
2.00 ppm	0.249	2.000	**2.00 ppm**	0.438	2.000
3.00 ppm	0.454	3.000	**3.00 ppm**	0.616	3.000
Dead Deer Creek	Below Detection		**Dead Deer Creek**	Below Detection	
N. Earsman	Below Detection		**N. Earsman**	0.023	0.043
S. Earsman	Below Detection		**S. Earsman**	Below Detection	
Dock	Below Detection		**Dock**	Below Detection	
Lake 1 0m	Below Detection		**Lake 1 0m**	0.002	Below Detection
Lake 1 2m	Below Detection		**Lake 1 2m**	Below Detection	
Lake 2 0m	Below Detection		**Lake 2 0m**	Below Detection	
Lake 2 2m	0.001	**Lake 2 2m**	**Lake 2 2m**	0.004	Below Detection
Lake 3 0m	Below Detection		**Lake 3 0m**	0.007	Below Detection
Lake 3 2m	0.001	**Lake 3 2m**	**Lake 3 2m**	0.015	0.004

Appendix C Embedded Vegetation Data spreadsheets

Below is the attached complete Excel workbook containing all terrestrial vegetation transect survey data collected throughout the study period by EIEC

MLNS Vegetation Data 2022.xlsx

Below are the files containing CSV UTF-8 (Comma Delimited) (.csv*) formatted data for each transect worksheet from the above file.

Transect 18.csv   Transect 19.csv   Transect 20.csv   Transect 21.csv   Transect 22.csv   Transect 23.csv

Appendix D Embedded Compiled Morphospecies List

Below is the complete compiled list of Morphospecies identified within this report

Compiled
Morphospecies List.d