



# **Nuclear Innovation Institute 2023 Annual Public Health and Environment Research Report**

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Prepared by the Nuclear Innovation Institute

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## Introduction

The Nuclear Innovation Institute (NII) is an independent, not-for-profit organization with a unique role as a connector, industry voice and product manager. NII's vision is one of a Canadian landscape that celebrates an innovative nuclear industry as an integral part of the clean energy future.

Environment@NII is home to the Nuclear Innovation Institute's projects assessing the impact of energy generation on human health and the environment.

Delivering actionable intelligence from leading-edge researchers focused on fostering a clean and healthy environment, Environment@NII's focus is three-fold:

- **On the future of energy** – advancing knowledge and practices in the nuclear industry to help the world transition to a clean energy future
- **On the future of health** – accelerating research and advocacy for medical isotopes, from improved cancer diagnoses and treatments to expanded use in food production and industrial safety
- **On the future environment** – researching the impact of the nuclear fuel life cycle on our water, land and air while also supporting efforts to fight climate change.

Located in Saugeen Shores, Ontario, NII is supported by an engaged group of Founding Members: Bruce Power, AtkinsRéalis, BWXT Canada, Cameco, E.S. Fox Ltd., Kinectrics and the Town of Saugeen Shores, as well as Supporters: ATS Industrial Automation, Framatome and Sargent & Lundy.

### **The Annual Public Health and Environment Research Report**

NII supports public health and environment research through direct research funding from Bruce Power. NII support allows researchers to succeed in applying for competitive, peer-reviewed funding from federal and provincial agencies. The receipt of these matching funding grants demonstrates the scientific rigor of the academic research that Bruce Power is supporting.

This report presents the research progress for the NII's diverse public health and environment programs and provides an update on research plans for 2023.



# Public Health Research Programs

## Sub-natural background radiation exposure (2023)

The primary aim of this project is to investigate the biological effects of sub-natural background radiation exposure. All living systems have evolved and adapted in the presence of ionizing natural background radiation. The research group has previously demonstrated that exposure to low-doses of ionizing radiation, at levels slightly above background, can have a beneficial effect on living organisms through up-regulation of repair pathways and immune function. The hypothesis is that removal of background radiation will be detrimental to biological systems.

This work is being investigated through experiments conducted in SNOLAB, where the two kilometers of overhead rock effectively shields out cosmic radiation to achieve one of the lowest background dose rates in the world. Long-term experiments are being conducted using several different model systems, including human cells and yeast.

This project builds on the previous Ultra-Low Dose research program (2016-2023). Funding for the Ultra-Low Dose research project ended in 2022, culminating in the end of the seven-year research project. Given the importance of this research to the understanding of the fundamental effects of ionizing radiation on biological life, the Environment@NII group has decided to continue funding SNOLAB research for another five years.

This Ultra-Low Dose program explored the effect of ultra-low levels of background radiation on Lake Whitefish embryo survival and development. Lake Whitefish embryos were chosen due to availability, to be used as a multi-cellular organism for comparison with cell studies. Single-cell models grown in typical ambient radiation and under ultra-low dose SNOLAB conditions were evaluated for markers of cancer development including mutation frequency, chromosomal aberrations and differentiation. Part of the aim of this research is to more accurately describe dose effects at natural and slightly elevated levels, testing the linear-no-threshold model of risk.

## Facilities, Equipment and Methodology

The following sections outline the experimental tools used to perform the sub-natural background radiation exposure and ultra-low-dose research.

### Low-Radon Specialized Tissue Culture Incubator

Due to radiological decay in the surrounding rock, underground radon levels are higher than on the surface (approximately 130 Bq/m<sup>3</sup> underground compared to 5 Bq/m<sup>3</sup> on surface). A low-radon specialized tissue culture incubator was designed in 2017 to



reduce radon levels below natural background and allow researchers to achieve much lower radiation levels underground compared to the surface.

SNOLAB management reviewed and approved the technical design of the underground laboratory for the Researching the Effects of the Presence and Absence of Ionizing Radiation (REPAIR) project. Researchers worked with a design team at SNOLAB to build a low-radon specialized tissue culture incubator that will be used for culturing human cells. Construction and installation were completed for both the low-radon glovebox and all equipment required for the underground laboratory (Figure 1). The low-radon specialized tissue culture incubator will enable researchers to culture cells in an ultra-low background radiation environment to better understand fundamental mechanisms of low-dose radiation exposure.

In 2019, training and testing of the specialized tissue culture incubator monitoring systems was completed. This included work to quantify and calculate the levels of natural background radiation (NBR) components (i.e., radon, gamma, neutrons) in the SNOLAB, both in the underground REPAIR laboratory and within the custom low-radon glovebox, and at the above ground laboratory. This work demonstrated that the low-radon specialized tissue culture incubator successfully reduced the major background radiation components of gamma, neutron and radon radiological contaminants to a sub-NBR environment. Methodology for the transportation and growth of cell lines in the underground laboratory was tested in preparation for longer-term experiments.

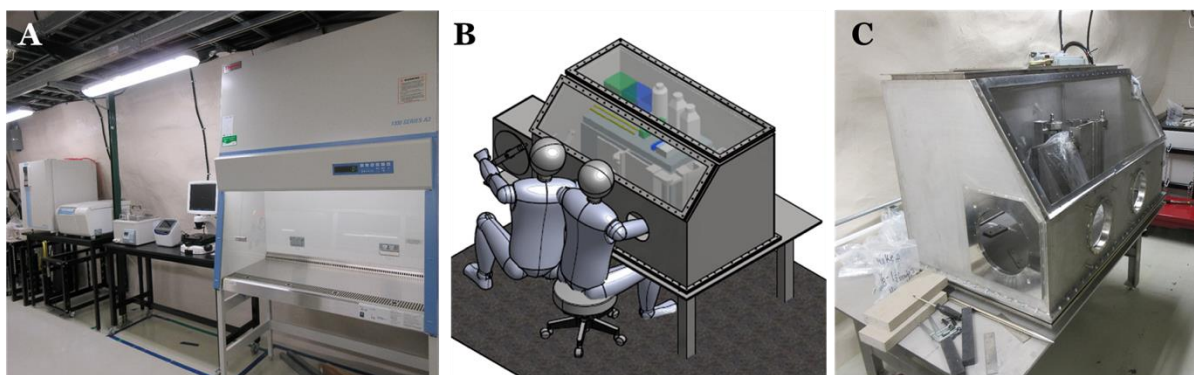


Figure 1. SNOLAB project cell culture systems. A) Cell culture systems underground in SNOLAB. B) Schematic of low-radon specialized tissue culture incubator underground in the SNOLAB C) Low-radon specialized tissue culture incubator in-situ in the SNOLAB.

Characterization of the radiation environment within the glovebox has been conducted and commissioning reveals excellent reduction of natural background radiation components. This represents a significant achievement in the field of low-dose radiation research and has novel implications for our future sub-natural background radiation



experiments. A manuscript detailing the engineering, construction, and commissioning of the STCI (Specialized Tissue Culture Incubator) has been published (Kennedy 2021).

A Monte Carlo simulation was conducted on the REPAIR project framework underground and above ground at NOSM (Northern Ontario School of Medicine), which includes the STCI geometry. Work since has been focused on getting the resulting manuscript from this work ready for publication. There have been several changes that have gone into refining and improving the simulation. One such change was improving the efficiency of the GPS (General Particle Source) within the simulation. As a result, the simulation has become much more accurate and representative of what is occurring within the different radiation environments.

This simulation and measurement of the radiation environment both underground and above ground is one of the most comprehensive and detailed of any of the other labs who are actively conducting sub-natural background radiation research. The resultant dose rates from the simulation show substantial reductions in cosmic and terrestrial radiation from the STCI configuration (as well as the result of having the chemical sciences laboratory deep underground in SNOLAB). This specialized facility enables the research team to explore the effects of radiation, or lack thereof, on living organisms. The following sections outline that research using the facility.

### Cell Culture System

Work has been performed in the NOSM laboratory with the cell culture systems and endpoints to be used underground. This included the FADU (Fluorescence Analysis DNA Unwinding), comet and transformation genetic assays. Transformation assays have been run using the CGL-1 and WI-38 cell lines to study cancer induction. The CGL-1 cell line is a hybridization between the tumorigenic (*i.e.*, tumor causing) human HeLa cell and a normal human cell and its extensive use for radiation exposure research has established specific cellular and genetic endpoints. These endpoints include the ability to identify specific genetic changes that occur after radiation exposure and that differ between tumorigenic and non-tumorigenic cells. The WI-38 cell line consists of normal human cells and can serve as a control when compared to other cell lines.

A modern molecular biology endpoint has been optimized that will allow the selective removal of genes of interest from the cell culture model systems. The removal of specific genes will allow researchers to explore the mechanisms driving any radiation-induced effects observed in the SNOLAB.



## Irradiator

Installation of the X-RAD 320 X-ray cabinet irradiator was completed at the NOSM West Campus in 2020. This is the same model of irradiator that is currently being used at the East campus, allowing for parallel research to be performed at the West Campus and serving as a second surface lab site to perform radiation studies, which is a critical control for confirming results.

## Research Activities and Results

Please refer to the 2021 and 2022 Annual Public Health and Environment Research Report for an outline of previous research activities and results from this research program including:

- A series of experiments performed on the CGL-1 human hybrid cell line and the WI-38 fibroblasts under various low-dose radiation conditions. The overall goal of this work was to understand the effects of low dose radiation on well-studied cell lines commonly used for radiobiology experiments.
- The experiments above expanded to include MDA-MB-231 cells in 2021, which were performed in order to study the molecular mechanisms for breast cancer induction.

A new line of research was started in 2021 in collaboration with NASA to use a yeast as a model organism for radiation research.

## Adaptive Response in Yeast

The adaptive response is a process where cells previously exposed to low doses of a stressor obtain a level of resistance as a form of protection against subsequent higher doses of the same or different environmental stressor. Several different stressors have been shown to induce the adaptive response, particularly ionizing radiation and thermal stress.

To better understand the adaptive response and the molecular mechanisms underlying the biological effects of low dose stresses, three different strains of yeast (*Saccharomyces cerevisiae*) were used as test organisms and subjected to thermal stress. The three strains of yeast include the BY4743 wild type strain which is the parental strain used to create collections of gene deletion mutants and two genetically engineered strains provided by the BioSentinel Group at NASA Ames Research Center. The BioSentinel strains consist of a wild type strain and a radiosensitive rad51 deletion mutant strain. A collaboration between NOSM/NII and NASA will study how these yeast strains respond to high dose space radiation (while travelling in deep space) compared to ultra-low shielded cosmic radiation environment in the SNOLAB.





Heat shock response in the three yeast strains was assessed to determine a) the degree of cell killing and b) the optimal timing for the delivery of a priming heat shock to initiate an adaptive response prior to a higher heat shock. After determining the optimal dose of thermal stress to induce an adaptive response, whole transcriptomic analysis was performed to identify all the molecular pathways involved in initiating and maintaining this adaptive response. Evaluation of the transcriptomic data revealed that all three strains of *Saccharomyces cerevisiae* showed a distinct molecular response to the thermal stress when compared to the untreated controls. The data suggests that genes associated with protein folding, nutrient metabolism, and reproduction are significantly altered during the thermal adaptive response in this study.

In 2022, desiccated yeast samples were stored in the sub-NBR radiation environment at SNOLAB to assess the impact of radiation damage accumulation over time. Data was collected for up to 48 weeks of continuous incubation in SNOLAB and in the control laboratory at NOSM. Two different yeast strains were used; a wild-type strain and a strain with a knockout for the RAD51 gene which makes them more susceptible to DNA double strand breaks. A subset of yeast from each NBR location was rehydrated at 4-week intervals and assayed for survival, growth and metabolic activity. Overall, yeast which were incubated in SNOLAB had a reduced survival compared to control samples (Figure 2). There was also a reduction in growth rates and metabolic activity in SNOLAB. These data suggest that prolonged exposure to a sub-NBR environment is detrimental to desiccated yeast.

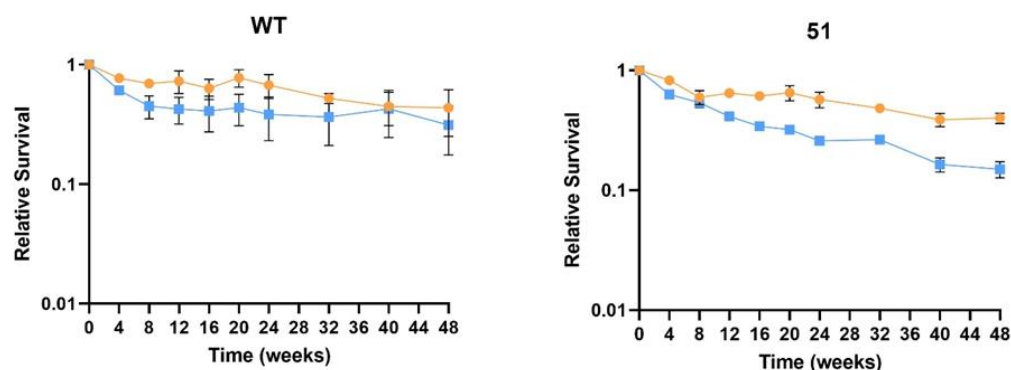


Figure 2: Survival of desiccated yeast samples incubated in SNOLAB (blue line) and the surface control laboratory at NOSM (orange line) for 48 weeks. The wild-type (WT) yeast is shown on the left, while the RAD51 knockout yeast is shown on the right. Samples were rehydrated at 4-week intervals for survival analysis. Data represent the average of three independent replicates  $\pm$  SEM and were compared using an ANCOVA.



A second experiment with desiccated yeast in SNOLAB was initiated in 2022. The main addition to this new experiment is the removal of naturally occurring  $^{40}\text{K}$  from the yeast samples. A detailed dosimetry in the sub-NBR environment in SNOLAB was performed and determined that approximately 97% of the residual radiation exposure in SNOLAB comes from exogenous  $^{40}\text{K}$  (Figure 3). To reduce this, researchers have purchased refined potassium that has a lower level of  $^{40}\text{K}$ . Removal of radioactive  $^{40}\text{K}$  from the yeast samples will provide a much more significant reduction in NBR.

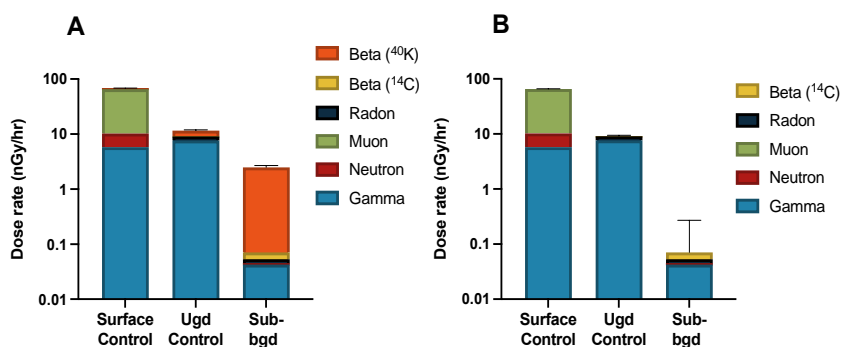


Figure 3. Background radiation dose rate calculations in sub-background (STCI) and two control environments using Monte Carlo simulations. Dose rates are shown with  $^{40}\text{K}$  (A) and without  $^{40}\text{K}$  (B).

Prior to preparing samples for SNOLAB, the concentration of potassium required for yeast to survive the desiccation protocol needed to be determined. To test this, yeast were cultured and desiccated using four different potassium concentrations; 0.1, 1, 3 and 10 mM. Shortly after desiccation, samples were rehydrated and survival was determined. The three higher concentrations (1, 3, and 10 mM) produced optimal levels of post-desiccation survival whereas the 0.1 mM concentration had reduced survival. Based on these results, all future experiments will be run at 1 mM—the lowest concentration that produced sufficient desiccation survival—in order to reduce experimental costs.

Next, to confirm that there was a significant reduction in background radiation dose rates, the activity of  $^{40}\text{K}$  was measured in normal and refined media. Samples were counted multiple times using a HPGe (high purity germanium) detector. The results confirmed that the refined media had approximately 1/10<sup>th</sup> the radiation dose rate as normal potassium. Using this data, the background radiation dose rate in each of our experimental locations from all sources of radiation were calculated. It was determined that the radiation dose rate in SNOLAB was 0.31 nGy/hr compared to 68.0 nGy/hr on surface, representing a 219-fold reduction in natural background radiation.

With the dosimetry completed and the optimal potassium concentration selected, yeast samples were prepared for long-term incubation in SNOLAB. Briefly, yeast were grown in the normal or refined media for 7 days. Samples were then desiccated in 96-well plates and were transported to SNOLAB. Control samples were kept at the NOSM laboratory. Desiccated yeast samples were incubated in SNOLAB and at NOSM for 6



months. Every 4 weeks, a subset of samples are being rehydrated and assayed for survival, growth rates and metabolic activity. Preliminary analysis of the results suggest that the yeast incubated in the sub-NBR environment have lower survival and growth rates.

### Novel methods in biodosimetry

The primary aim of this project is to identify novel biodosimetry biomarkers for low dose radiation exposures. In particular, the team is focused on identifying small non-coding RNA molecules known as microRNA (miRNA) as radiation biomarkers. miRNAs have emerged as a potential class of biomolecules for diagnostic biomarker applications. miRNAs are small non-coding RNA molecules produced and released by cells in response to various stimuli. miRNAs demonstrate remarkable stability in a wide range of biological fluids, in extreme pH fluctuations, and after multiple freeze–thaw cycles. Given these advantages, identification of miRNA-based biomarkers for radiation exposures can contribute to the development of reliable biological dosimetry methods, especially for low-dose radiation (LDR) exposures.

Large-scale miRNA profiling has been challenging since miRNA molecules are very short in length (18–22 base-pairs). In 2021, the research team overcame this challenge by developing a next-generation sequencing technology that can identify and profile millions of miRNA molecules in biological fluids.

## **2023 Results**

### Adaptive Response in Yeast

During 2023, the team focused on experiments using the yeast model systems. Two separate experiments were completed using desiccated yeast. Both experiments utilized two different strains of yeast; a normal wild type and an isogenic recombinational repair deficient rad51 knockout strain (rad51Δ). The knockout makes the yeast deficient in their ability to repair DNA double strand breaks.

In the first experiments, desiccated yeast samples were stored in the normal background surface control laboratory (68.0 nGy/hr) and in the sub-background environment within SNOLAB (10.1 nGy/hr) for up to 48 weeks. Post-rehydration survival, growth rate, and metabolic activity were assessed at multiple time points. Survival in the sub-background environment was significantly reduced by a factor of 1.39 and 2.67 in the wild type and rad51Δ strains, respectively. Post-rehydration metabolic activity measured via alamarBlue reduction remained unchanged in the wild type strain but was 26% lower in the sub-background rad51Δ strain. These results demonstrate that removing natural background radiation negatively impacts the survival and metabolism



of desiccated yeast, highlighting the potential importance of natural radiation exposure in maintaining homeostasis of living organisms.

In the second yeast experiment, a further background dose rate reduction was accomplished by incorporating 10 cm of lead shielding and a tailored yeast nutrient broth containing a low concentration of the radioactive isotope potassium-40 (40K). Radon was removed via the use of the radon-free gas-fed glovebox. These radiation mitigating techniques have resulted in an experimental background dose rate of  $0.08 \pm 0.01$  nGy/hr, roughly 835x lower than the control dose rate from NBR ( $66.8 \pm 0.4$  nGy/hr). The yeasts were stored in their respective environments for 32 weeks. Samples were collected and assayed every four weeks. Assays utilized were survival, measured via colony forming units; growth, quantified via optical density; metabolic activity, measured via alamarBlue fluorescence doubling time; and petite phenotype incidence, measured via comparison of colony formation on agar dishes containing fermentable and nonfermentable carbon sources.

Survival was not significantly impacted by radiation background in either strain. Post-rehydration metabolic activity, however, was found to be significantly impacted by background dose rate in both strains with a 30.8% decrease in the ultra-low background-exposed Wild Type strain and 111.5% decrease in the *rad51Δ* strain. These results further support the notion that there is a metabolic impact resulting from ultra-low sub-background radiation exposure in desiccated yeast. This is a further indication of the role of natural radiation exposure in the maintenance of normal biological function.

### Novel methods in biodosimetry

Large-scale miRNA profiling has been challenging since miRNA molecules are very short in length (18–22 base-pairs). Moreover, extraction of high-quality miRNA from blood samples requires optimization of current extraction techniques.

In 2023, the team established a robust pipeline for profiling total miRNA fractions from blood using next-generation sequencing technology. This approach enabled them to characterize the blood miRNA profile of four-month-old mice exposed to radiation doses of 100 mGy or 2 Gy, with analysis conducted 6 hours post-exposure. Compared to sham, exposure to 2 Gy led to significant upregulation of 12 miRNAs and downregulation of 6 miRNAs at the 6-hour mark post-irradiation. Moreover, 7 miRNAs showed significant upregulation following 100 mGy exposure (miR-126a-5p, miR-133a-3p, miR-125a-5p, miR-125b-5p, miR-99a-5p, miR-1a-3p, miR-126a-3p). Notably, miR-126a-5p and miR-133a-3p were exclusive to the 100 mGy group, while the remaining 5 miRNAs were upregulated in both 100 mGy and 2 Gy groups. RT-qPCR validation supported the reliability of these miRNAome findings.



In conclusion, the team identified two low-dose radiation (LDR) miRNA serum biomarkers (miR-126a-5p and miR-133a-3p) and several high-dose miRNA biomarkers with potential applications in biological dosimetry.

## Outcomes

<b>Table 1: 2023 scientific outputs<sup>1</sup></b>	
<b>Source</b>	<b>Type</b>
Pirkkanen, J., Laframboise, T., Peterson, J., Lapointe, M., Mendonca, M.S., Tai, T.C., Lees, S.J., Tharmalingam, S., Boreham, D.R., Thome, C. August 2023. The role of natural background radiation in maintaining normal cellular function in the CGL1 human hybrid model system. Poster presentation. International Congress on Radiation Research. Montreal, Quebec.	Presentation
Lapointe, M., Laframboise, T., Couture, L.E., Boreham, D.R., Thome, C. August 2023. Protracted exposure to a sub-background radiation environment negatively impacts the anhydrobiotic recovery of yeast. Poster presentation. International Congress on Radiation Research. Montreal, Quebec.	Presentation
Lapointe, M., Boreham, D.R., Thome, C. October 2023. The REPAIR project; Investigating the role of natural background ionizing radiation. Oral presentation. Radiation Research Society Fall Workshop. Bozeman, Montana.	Presentation
Tharmalingam, S. October 2023. microRNA as a biomarker for low dose radiation exposure. Oral Presentation. Radiation Research Society Fall Workshop. Bozeman, Montana.	Presentation

## 2024 Research Plan

### Adaptive Response in Yeast

Experimentally, work will continue on the 40K depleted yeast experiment. The team is in the process of optimizing additional experimental endpoints that can be incorporated into the SNOLAB experiments. This includes a novel metric for quantifying DNA double strand breaks in desiccated yeast. They plan to redeploy desiccated yeast within SNOLAB (and the surface control laboratory) in May 2024 for a 12 month continuous experiment.

<sup>1</sup> Please see previous Annual Reports for a comprehensive listing of previous publications.



The team has also initiated the next set of experiments using the CGL1 human cell culture system. Experiments commenced in February 2024 and will run for 4 consecutive months. The goal of this experiment is to examine the transcriptional response in human cells exposed to sub-background radiation. Similar to previous experiments, samples will be collected once a month for 4 months. Full global transcriptomic analysis will be performed to identify genes that may be regulation the biological response to natural background radiation exposure. Based on the interesting results identified in yeast with respect to metabolism, the team will also be tracking the metabolic rate of human cells across the 4-month sub-NBR exposure.

In 2023, the team had one PhD student (Michel Lapointe) successfully defend his PhD thesis. He will be continuing to work on the SNOLAB project as a post-doctoral fellow.

### Novel methods in biodosimetry

The team's objective is to discover blood miRNA biomarkers induced by long-term radiation exposure (spanning days to months). Furthermore, they aim to investigate potential sex differences in the blood miRNA profile following radiation exposure. Finally, they seek to identify acute and chronic miRNA biomarkers associated with radon exposures.

### Key Researchers

Dr. Simon J. Lees, Northern Ontario School of Medicine

Dr. Sujeenthara Tharmalingam, NII Research Chair at the Northern Ontario School of Medicine

Dr. Chris Thome, NII Research Chair at the Northern Ontario School of Medicine



## Biological effects of radon gas exposure (2023)

The primary aim of this project is to investigate the biological effects of radon gas exposure. The economic burden for radon gas detection and mitigation strategies is substantial for Canadian industries and residential home dwellers. However, the mechanisms of radon interactions with living systems and the level at which detrimental cellular effects can occur is still largely unknown.

A custom radon chamber is being constructed for this project in Northern Ontario, matching the chamber that was previously built in Adelaide, Australia. Using this unique piece of infrastructure, the team will expose mice to varying concentrations of radon gas for different time intervals. Lung tissue, along with other organ systems, will be analyzed for molecular and genomic markers of carcinogenesis.

The expected outcomes of this research will benefit Canada through improved public policy regarding safe levels of radon exposure. The data from this project will help in assigning dose thresholds for radon exposure to ensure the safety of workers and the general public. The data will also help to ensure that Canadian citizens and industries are not spending unnecessary time and resources monitoring and mitigating safe levels of radon in enclosed dwellings.

### Research Activities and Results

Funding for this project was received in Summer 2023 through an NSERC Alliance Grant. During the first two years of the grant, the new radon chamber will be constructed and commissioned in Adelaide. The chamber will then be shipped to Canada for installation at NOSM University. While the new chamber is being constructed, radon experiments will be conducted in Adelaide using the existing radon chamber.

Experiments have commenced on the first cohort of mice in Adelaide after receiving ethical approval from Laurentian University. This cohort uses a strain of mice that has a specific gene (p53) knocked out in lung tissue, making them more susceptible to lung cancer. Mice are being exposed to 1000 Bq/m<sup>3</sup> of radon for up to 6 months. At several timepoints, a subset of mice will be euthanized for analysis.

The first set of lung tissue has been collected. These samples are being prepared for shipment to Sudbury for processing. The remaining samples will be collected by August 2024. The team will specifically be looking at genomic and transcriptional changes in lung, spleen, blood, liver, kidney, heart and brain. They will also be quantifying changes in the gut microbiome that may occur due to radon exposure.



## 2024 Research Plan

During 2024, construction will continue on the NOSM University radon chamber. It is anticipated that construction will be close to complete by the end of 2024. Commissioning of the chamber will then commence in 2025. The first set of animal exposures will be complete by August 2024. Analysis of the tissue samples from these mice will be complete by the end of 2024. It is anticipated that these data will result in 2 peer reviewed publications. Starting in September 2024, the second cohort of radon experiments will commence. The goal of these experiments will be to evaluate the effects of short vs long term radon exposure, as well as to compare the biological response across different radon concentrations (100-4000 Bq/m<sup>3</sup>).

### Key Researchers

Dr. Simon J. Lees, Northern Ontario School of Medicine

Dr. Sujeenthara Tharmalingam, NII Research Chair at the Northern Ontario School of Medicine

Dr. Chris Thome, NII Research Chair at the Northern Ontario School of Medicine





## NEUDOSE (2019-2022)

Started in 2015, **NEU**tron **DOS**imetry & **E**xploration (NEUDOSE, pronounced “new dose”) was a satellite mission that was designed and built by researchers at McMaster University, Bruce Power, and the NII to study the effects of neutron radiation on human health in space. The website for the NEUDOSE satellite project can be accessed at <https://mcmasterneudose.ca/>.

The scientific objective of NEUDOSE satellite was to increase our understanding of the risks associated with prolonged exposure to space radiation by investigating the contribution of charged particles and neutrons to the total ambient dose equivalent in low-Earth orbit (LEO). NEUDOSE featured a novel dosimeter combining a tissue-equivalent proportional counter (TEPC) and a plastic anti-coincidence detector, allowing it to accurately measure neutron radiation and differentiate it from other types of space radiation, such as protons and heavy ions. This technology is crucial for understanding astronaut radiation exposure and developing effective protection strategies.

### Research Activities and Results

NEUDOSE was launched to the International Space Station (ISS) aboard SpaceX CRS-27 on March 15, 2023, at 00:30 UTC, and was deployed from the ISS on April 24, 2023, at 12:15 UTC. The first signals from NEUDOSE were received by amateur radio operators over the south of Australia 36 minutes after deployment.

During the first month, student operators at McMaster University worked to establish two-way communication and commission the systems. However, a software anomaly caused NEUDOSE to enter a perpetual boot loop, preventing successful contact and commissioning of the instrument. Despite these challenges, the CubeSat’s other systems, including its custom-designed radio, were confirmed to be operational, providing valuable insights for future missions.

In June 2023, the Canadian Space Agency (CSA) selected McMaster’s Pitch Resolving Spectroscopy for Electron Transport (PRESET) project for funding through the Canadian CubeSat Program (CCP). Building on the success of NEUDOSE, the PRESET project will advance technologies in space radiation detection, magnetometry, digital signal processing, satellite communications, and attitude determination and control. Additionally, NEUDOSE team members Hanu, Byun, and Johnston joined the NASA Botany Proposal, which aims to grow plants at the Lunar South Pole, with potential development of NEUDOSE-2 to monitor radiation there.



The financial support from Bruce Power, the Nuclear Innovation Institute (NII), and the CSA's Canadian CubeSat Program (CCP) was crucial for establishing McMaster's satellite research infrastructure. The technology developed for NEUDOSE will serve as a foundation for astronaut radiation safety in future lunar and Mars missions. The mission also provided valuable training for over 10 graduate students and approximately 150 undergraduates, many of whom have successfully entered the space industry, marking NEUDOSE as a significant milestone in McMaster's space research and technology development.

The CSA has funded a proposal to develop a CNP-TEPC instrument, which is the same one developed for the NEUDOSE mission, to be deployed on the outside of the ISS for a 16-week period in 2027. The project will officially commence in September 2024.

### 2024 Research Plan

The funding for this research project ended in 2022. The financial support of this project was to design and fabricate the novel radiation detector, which was successful during the 4-year funding window. This project is now concluded.

### Key Researchers

Dr. Andrei Hanu, Bruce Power  
Dr. Soo Hyun Byun, McMaster University  
Dr. Fiona McNeill, McMaster University  
Dr. Eric Johnston



## Neutron Spectroscopy at Bruce Power (2022-2023)

Accurate neutron dosimetry is a challenging task due to their interaction methods and low interaction probability at certain energies. The problem of dosimetry is made more difficult due to the non-uniform radiation weighting factor, resulting in an estimate of the input neutron spectrum to determine dose. This implies that in order to perform neutron dosimetry, the neutron spectrum must first be determined, which can be a difficult measurement requiring very specialized equipment and long data acquisition times. As a result, most field dosimeters will try to use approximate spectra, in conjunction with tailored instrument response functions, to produce a neutron dose with reasonable dose uncertainties. As a result, most neutron field dosimeters operate with a -20% to +50% tolerance, which is considered standard for such instruments.

When performing practical dosimetry in a mixed-field environment, the neutron dose is often a small contributor to the overall dose received, and this tolerance is typically accepted since it tends to produce higher doses reported on average. However, as the neutron dose starts to become a significant contributor to overall dose, this overestimate can result in inflated (and non-received) dose that is recorded. At Bruce Power there are cohorts of workers who have neutron doses that make a significant portion of their total dose, suggesting the need for more accurate neutron dose measurements.

This project was developed to explore the neutron spectra in various locations at CANDU reactors and characterize the under/overestimation of doses caused by various field dosimeters. These measurements will be used to inform methods of dose reduction and future experiments.

### Research Activities and Results

The work on this project began in May 2022, with the researchers starting by examining potential neutron spectrometers, measurement locations and logistics. In June 2022, measurements were taking place at Bruce Power using a Nested Neutron Spectrometer (NNS), a SNOOPY dosimeter, and a handful of personal dosimeters on human phantoms. Measurements were taken for two weeks at various locations where it was expected that workers would receive significant neutron doses. After a successful data collection campaign, the research group began unfolding the NNS data and producing more accurate neutron spectra and doses.

The initial findings of the first measurement campaign proved the assumptions, the SNOOPY and personal dosimeters will overestimate dose in soft (i.e., lower energy than



calibrated against) neutron fields. A manuscript documenting these findings and outlining the procedures is being developed and will be submitted in 2023.

In addition to the initial measurement campaign, there was an obvious need to do more work characterizing neutron doses at CANDU reactors. OPG purchased their own NNS, with Bruce Power purchasing a ROSPEC, the NIST standard neutron spectrometer. A longer measurement campaign took place in 2023 to perform a cross-comparison of these two spectrometers with the original NNS used in 2022, as well as several other field dosimeters.

In 2023, the first paper showing the results of NNS measurements was submitted to Applied Radiation and Isotopes Journal, summarizing the findings. Given the soft neutron fields on the reactivity deck, the SNOOPY overestimated dose by approximately 50% where workers harvest <sup>177</sup>Lu at the isotope production system (IPS). This publication lays the groundwork for correcting the dose measurements at the IPS, as well as using the same technique at other places on-site where neutron spectra are much softer than the standard calibration sources.

### Outcomes

<b>Table 2: 2023 scientific outputs for Neutron Spectroscopy</b>	
<b>Source</b>	<b>Type</b>
Hanu, A.R., Atanackovic, J., Boyd, C., Johnston, E.M. 2024. Characterization and mapping of the neutron fields around Bruce Power’s <sup>177</sup> Lu isotope production system. Applied Radiation and Isotopes. Volume 208, 111284.	Publication

### 2024 Research Plan

The majority of the project and experimental work was completed in 3 testing campaigns during 2023. The other test campaigns included additional measurements at Bruce Power, some measurements at OPG, and a test using additional neutron spectrometers and dosimeters.

In 2024, the research team will prepare manuscripts for each of these and pursue publications, further outlining the impact of accurate spectrometry on neutron dose calculations.



## Key Researchers

Dr. Anthony Waker, Ontario Tech

Dr. Andrei Hanu, Bruce Power

Dr. Jovica Atanackovic, Ontario Power Generation

Dr. Eric Johnston



## Computer Vision for Occupancy Mapping (2022-2023)

As outlined in the Neutron Spectroscopy section of this report, accurate neutron dosimetry is difficult; practically, it is more reasonable to provide conservative overestimates to the neutron dose. Instead of using inaccurate personal neutron dosimeters, a more accurate (see Neutron Spectroscopy, pg. 18) field dosimeter will be used to provide a dose estimate for workers in an area with significant neutron fields.

To do so, workers are required to carry a large, heavy and cumbersome neutron survey meter known as the SNOOPY. Generally, and by procedure, workers will place the SNOOPY instrument in a location near to where they are working and where the neutron dose rates are the highest – even if the workers are not exposed to the highest dose rate measured by the SNOOPY instrument. Once the work is complete, all the workers are assigned the integrated dose measured by the SNOOPY instrument for the duration of the job. As a result of this practice, workers are assigned doses that are conservative but overestimated when compared to the effective dose that was received by each individual. This dosimetry practice places upper limits on the amount of dose that each operator can receive.

Alternatively, neutron dosimetry may be performed indirectly using the product of stay-time (i.e., the time spent by each worker in the radiation field) and the most conservative dose rate that the worker may have been exposed to. By procedure, indirect neutron dosimetry may be performed up to an exposure of 20 mrem per worker per job. However, it currently faces the same limitations that the stay-time must be multiplied by the most conservative (i.e., highest) neutron dose rate that could be experienced in that location. To correct this overestimation, it would be necessary to track the worker's presence more accurately in the radiation field. Knowing the worker's location more accurately would enable the use of more accurate dose rates, resulting in a lower (yet still upper bound) on the dose received.

### Research Activities and Results

At the onset of the project, it was decided that the research team would keep as many parameters of the solution as generic as possible, resulting in a minimal time to adapt the solution to changes in hardware and deployment location. With that in mind, there were many tasks throughout this project that were designed to ensure the system was highly configurable.

Following obtaining and setting up the required instrumentation, backend code was developed for real time ingestion of camera signals utilizing hardware acceleration for both decoding and encoding of camera signals, as well as multiprocessing in Python. With the completion of the backend for communicating with the cameras, efforts were



made to correct for the distortions present in all imaging systems. Correcting for these distortions involves imaging a known, flat, calibration pattern. This was found to be most easily, and successfully, performed by displaying the pattern on a flat monitor. Having a corrected image is necessary for maintaining a linear correlation between position in image and position in the real world of the object being imaged.

With the cameras corrected, the next step of the process was to establish an algorithmic determination of the camera's position in the world relative to a point that all cameras have in view. This process involves another well-known pattern that remains visible in all camera views. The relative distortion of the pattern in the image produced by each camera can be used to determine the position and rotation of the camera (referred to as pose) relative to the calibration object. See Figure 4 for an example of the calibration mat showing a pose estimate.



Figure 4: An example of the three axes being drawn at the origin of the calibration mat after pose estimation. The correct orientation and position of the three drawn axes ensure that there was a successful pose estimation. This also ensures that the position of the camera and rotation relative to the origin is accurate.

In 2022, the camera pose was improperly overestimating the distances of the cameras to the pose estimation mat, which was believed to be caused by an insufficient number of calibration points inside the pattern.

By the end of the first phase of the project, all the challenges from 2022 were overcome, and a working demo and video documenting the approach and results were produced for Bruce Power. The calibration routine still produced inaccurate results, but these were determined to be the result of poor image quality of the mat under certain lighting and angle conditions, which need to be addressed to improve the accuracy of the auto calibration routine.



In order to determine a worker's location, an AI is used to draw a bounding box around each worker. Using the centre of the bounding box as the location of the worker, a straight-line trajectory was produced in 3D-space from the camera to the worker, and this vector was used in the triangulation procedure. Once the list of vectors for all candidates in each camera was produced, a least squares routine looked at quadruplets of vectors to determine the worker position. While exhaustive, this routine produced results within the required timeframe (less than 1/30<sup>th</sup> of a second), and was deemed sufficient for the demonstration.

## 2024 Research Plan

In late 2023, this project was renewed for a second phase after a 9-month hiatus. The main goal of the second phase would be to improve the calibration routine/mat, and look at non-exhaustive approaches to perform the triangulation. The first challenge related to accuracy of the camera position calculation using the calibration mat, resulting in workers being placed incorrectly by up to 1 foot. The general approach would be to create a larger calibration mat with larger squares, and to use a matte finish to reduce glare in daylight conditions.

The second issue was algorithmic in nature, related to the rapid growth of possible solutions as the number of workers increased in each camera. The 2022 version of the program was able to compute worker location for 3 workers using 4 cameras in real time (i.e. within 1/30 of a second since the cameras are 30 frames per second), however the introduction of a fourth worker took the calculation to 1 second per frame. While this may be reasonable for the experiment in mind, there will be obvious issues when a fifth or more workers are added.

A new approach to the trajectory calculation was formulated, using transformations to put all four cameras into the same virtual 2d space, collapsing the triangulation in 3-space problem into a problem of overlapping bounding boxes. This approach is expected to produce significant improvements as the number of workers in frame grows.

## Key Researchers

Dr. Richard Garnett, Ontario Power Generation (formerly at Nuclear Innovation Institute)  
Dr. Eric Johnston  
Virat Tripathi, Nuclear Innovation Institute  
Dr. Andrei Hanu, Bruce Power





## Environment Research Programs

### Aquatic Biota (2018-2023)

This research program investigates the research gaps identified in the course of the previous Whitefish research program.

A Mitacs grant application was successfully submitted in 2017 seeking support for four post-doctoral fellows for an additional five years of research. These post-doctoral fellows are completing research on the following areas:

1. Determine the genetic population structure of Lake Whitefish, Round Whitefish, and Yellow Perch using advanced DNA analysis techniques.
2. Examine potential mechanisms causing mortality during embryogenesis and reduced fitness later in life by comparing transcriptomes of fish using advanced DNA analysis techniques.
3. Determine the survival and fitness of Lake and Round Whitefish subjected to thermal discharges during embryonic development.
4. Assess the survival of embryos from a model species (Yellow Perch) reared in varying temperatures reflective of thermal discharges.

An NSERC application was received in 2018 in support of research addressing three remaining key questions:

1. Thermal effects in Lake and Round Whitefish hatchlings and juveniles: What are the effects of variable and increased incubation temperatures on Lake and Round Whitefish hatchlings and juveniles?
2. Thermal effects in spring spawning fish: Using Yellow Perch as a model species, what are the effects of variable and increased incubation temperatures on the embryonic, hatchling and juvenile stages of spring spawning fish?
3. Population structure of Lake and Round Whitefish and Yellow Perch: What are the underlying population structures and habitat use of Lake and Round Whitefish and Yellow Perch in Lake Huron and near Bruce Power? What are the boundaries of the populations near Bruce Power?

### Research Activities and Results

#### Whitefish

Please see previous Annual Reports on the Environment@NII webpage for an in-depth discussion of previous results.

The team has completed analyses on Lake and Round Whitefish reared with fall cooling and spring warming compared to constant incubation. The goals were to assess the



impact of seasonality on whitefish development. Mortality was higher, hatching earlier, body weight and length decreased, yolk weight increased and jaw gape decreased with constant warming temperatures in both Lake and Round Whitefish.

When a fall cooling period was included in the temperature regimes, these effects were ameliorated for Round Whitefish (body weight, length, yolk weight) and to a lesser degree Lake Whitefish (body length only). Jaw gape was impacted by incubation temperature in both species and fall cooling minimized the effect of temperature. Interestingly, the fall cooling period had a marked effect on Lake Whitefish eye development and greatly reduced eye size. This well supports prior findings that the fall cooling period is a critical window for Whitefish development.

Due to the large effect on the thyroid gland, the team included treatments in 2023 that manipulated this system with either supplemental thyroid hormone or a goitrogen (compound that interferes with thyroid function). This experiment will provide clear data on whether the effects in the post hatching phase is related to delayed thyroid development with warmer incubation.

### Yellow Perch

Perch were reared at three constant temperatures (12, 15 or 18°C). At hatch, 12°C incubated fish exhibit greater swimming behaviour compared to fish incubated at either 15 or 18°C. At hatch, 18°C incubated fish consumed less oxygen relative to their colder conspecifics (15°C), and possibly experienced mitochondrial dysfunction. Cardiac development was more advanced at hatch in 18°C fish. Warmer incubated fish had diminished movement and increased oxygen consumption at 20 days post-hatch (DPH), demonstrating long-term disruptions to larval yellow perch.

Previous work showed increased growth rates at 20 DPH in 18°C incubated fish, a possible link to elevated metabolism. This study suggests that elevations in embryonic incubation temperatures may cause metabolic dysfunction and diminished movement in perch. The team has previously shown that eye diameter is larger in perch reared at higher temperature, and so they tested the ability of young perch to detect light using an electroretinogram.

Fish were acclimated to darkness to maximize sensitivity to light and then flashed with light of increasing brightness. Fish reared at higher temperatures detected lower light levels, gave a larger response to light, and saturated at lower light levels. Yellow perch reared at higher temperatures can detect light at a younger age. Yet, despite advantages in the eye development, these fish do not behaviourally discern changes in light.



Finally, the team continued to investigate the effects of rearing temperature on the thyroid system; fish reared at the warmest temperature have delayed thyroid development compared to those reared at cooler temperatures. They examined the expression of key genes involved in thyroid development (3 genes) and function (2 genes) in the post-hatching phase and found altered gene expression in all genes examined. This continues to support the team’s findings that thyroid development and function is altered with higher rearing temperature and may explain many of the effects they have noted in the post-hatching phase.

Population Structure of Lake and Round Whitefish and Yellow Perch

The team completed compound-specific stable isotopes analysis (CSIA) of 154 Lake and Round Whitefish from areas adjacent to Bruce Power, as well as several sites for comparison in the main basin of Lake Huron. The CSIA shows a wide diversity of food web origins for spawning phase adult fish present in the area around Bruce Power and confirms previous findings that there is no evidence for a local population. The team is currently generating a manuscript for publication based on the CSIA. They recently completed lab work for CSIA of 90 yellow perch from 6 different areas of Lake Huron for similar assessment of ecological population subdivision. In addition, these same 90 perch are being genotyped at several thousand single nucleotide polymorphisms for genetic comparisons.

Outcomes

<b>Table 3: 2023 scientific outputs for Aquatic Biota<sup>2</sup></b>	
<b>Source</b>	<b>Type</b>
Harman, A., Mahoney, H., Thompson, W.A., Fuzzen, M.L.M., Aggarwal, B., Laframboise, L., Boreham, D.R., Manzon, R.G., Somers, C.M., Wilson, J.Y. (2023) Effect of elevated embryonic incubation temperature on the temperature preference of juvenile lake (Coregonus clupeaformis) and round whitefish (Prosopium cylindraceum). Conservation Physiology, Volume 11, Issue 1, coad067	Publication
Rutko et al. Spatial and temporal variation in the life history traits of yellow perch (Perca flavescens) in the Canadian waters of Lake Huron. Submitted to Plos ONE.	Publication
Thompson, WA*, Masood, N., Easwaramoorthy, M., Hartenstein, P., Laframboise, L, Chow, E., Choh, V., McCulloch, D., Manzon, R., Somers, C., and Wilson JY. Rearing temperature	Presentation

<sup>2</sup> Please see previous Annual Reports for a comprehensive listing of previous publications.



influences the development and function of the eye and behavioural performance of yellow perch larvae. Canadian Ecotoxicity Workshop Ottawa, Ontario Oct 2-5, 2023.	
Easwaramoorthy, M. *, Thompson, W.A., Fraz, S., Laframboise, L., Hartenstein, P., D., Manzon, R., Somers, C., Wilson, J.Y. The effect of rearing temperature on the cardiometabolic development of yellow perch ( <i>Perca flavescens</i> ). Canadian Ecotoxicity Workshop Ottawa, Ontario Oct 2-5, 2023. Best student presentation award	Presentation

### 2024 Research Plan

This project was slated to finish in 2022 but was extended to complete experiments and publish the results. Final results are still pending. In 2024, the team will complete analyses from 2023 yellow perch samples: data analysis is ongoing. Key data will be collected in 2024 to finish experimental needs for behavioural effects in the post hatching phase and to assess eye function in fish reared at higher temperatures. Finally, the team will complete the Whitefish analyses and submit for publication 2 papers on the effects of seasonality in Lake and Round Whitefish

### Key Researchers

Dr. Joanna Wilson, McMaster University  
 Dr. Chris Somers, University of Regina  
 Dr. Richard Manzon, University of Regina



## Environmental DNA (eDNA) (2021-2023)

Water use in power generation, both directly through dams and indirectly in once-through cooling systems used by nuclear power plants, results in the entrainment of early-life stage fishes, collectively referred to as ichthyoplankton. Identifying species in ichthyoplankton samples is challenging due to the lack of unique morphological characters differentiating early-life stage fish. Unlike morphological classification, DNA sequence-based identification is not hampered in early-life stage fish, making it particularly useful for ichthyoplankton monitoring. Next-generation sequencing approaches, such as DNA metabarcoding, offer a promising high-throughput alternative to conventional DNA barcoding and make it possible to sequence samples containing DNA from many species and thousands of individuals. For ichthyoplankton, DNA metabarcoding increases the number of individuals that can be processed without significantly increasing costs and effort associated with sorting, extracting, and sequencing each individual specimen separately. DNA metabarcoding also makes it possible to identify species from environmental DNA (known as eDNA) samples to detect entrained species from DNA shed into the water-cooling systems of power generation facilities.

eDNA samples are often more easily collected than larval fishes, and in applications where accessing the sampling location can be difficult, such as nuclear power plants, can be collected by autonomous water samplers. The abundance of each species in bulk ichthyoplankton samples and eDNA samples are often determined by the proportional abundance of each species in the many thousands of sequencing reads generated. However, the accuracy of these estimates are debated, and to be useful for quantifying larval fish entrainment, need to be evaluated empirically.

### Research Activities and Results

#### DNA Metabarcoding Primers on Genomic DNA

Numerous approaches for DNA metabarcoding have been developed for fish. These range from protocols designed to maximize detection and resolution for local fish diversity or for the global diversity of bony fish and have been implemented in large-scale monitoring programs. These approaches, while often highly effective, need to be evaluated for the taxa inhabiting the systems that they are being used to monitor.

Prior to 2023, a set of primers and protocol were shown to work well on the species typically entrained at Bruce Power (see previous Annual Reports for further details). Some additional development of these primers to adapt them to the Ion Torrent instrument used to sequence samples at GenFish was completed in 2023.



## Comparison of species detections from metabarcoding of bulk tissue samples and eDNA

This subobjective could not be tested using data collected from Bruce Power because delays in the installation of the pump system prevented larval fish and eDNA collection. However, preliminary results from the eDNA autosampler (deployed in 2022) and contemporaneous net-based fish collection data, as well as collaborations with GenFish (see <https://gen-fish.ca/> for more information) on similar projects, indicate that species detections are often similar across methodologies.

In addition, the success of the more automated approach to eDNA sample collection (eDNA autosampler) shows that eDNA-based surveys can be much less labour intensive than conventional sampling, ideal for implementations like Bruce Power where physical sampling can be very challenging.

The team also looked at evaluation of forensic approaches to determine the number of individuals in bulk tissue and eDNA samples. While simulations suggest that the species-specific forensic-based method for enumerating larval fish abundance shows promise, they were unable to test it further due to the lack of eDNA or larval samples collected from Bruce Power.

## Quantitative measure of biomass from bulk tissue samples and eDNA

To estimate the potential bias in the relative abundance of species calculated from ichthyoplankton DNA metabarcoding data the anterior halves of larval fishes were extracted and used for species identification through single-individual barcoding, while the posterior halves were combined into bulk samples and sequenced using DNA metabarcoding to compare abundance estimation methodologies (Figures 5 and 6).

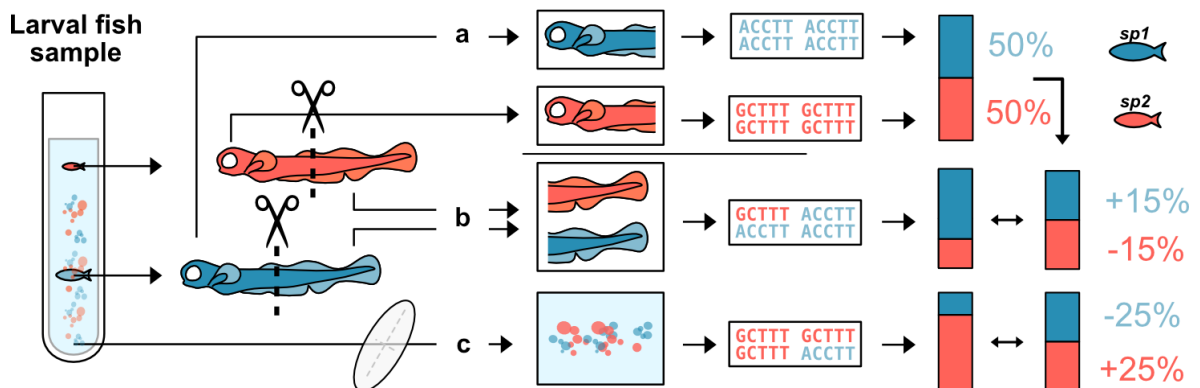


Figure 5. Schematic of DNA extraction and sequencing methodology. Larval fishes were each cut in half with the anterior half sequenced separately. These sequencing results were used to determine the true relative abundance of each sample. The homogenized posterior halves of each fish and the filtered storage ethanol were extracted separately. Relative abundance was estimated by the

proportion of sequencing reads for each species. Relative abundance estimates were compared to true abundance for accuracy.

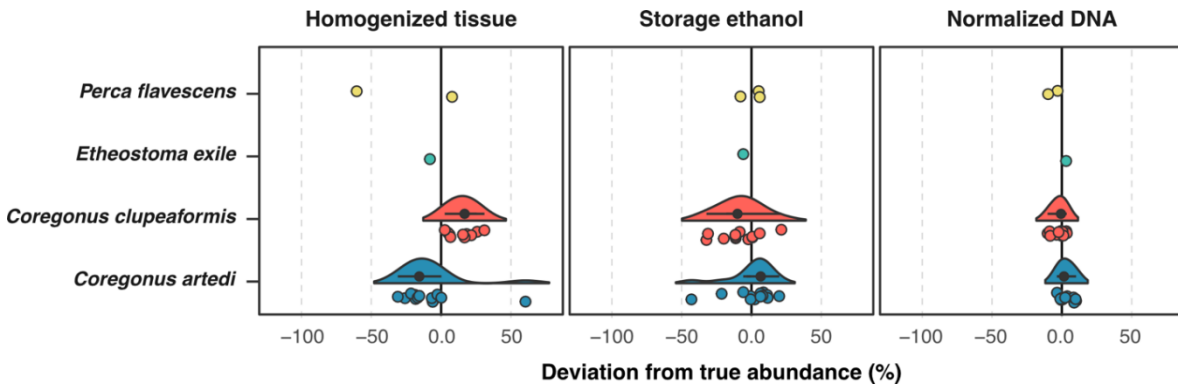


Figure 6. Sample accuracy of bulk DNA metabarcoding abundance estimates. Accuracy of the relative abundance estimates for each species in each sample. Black circles indicate the median value for each species and horizontal lines indicate the interquartile range. Data only from samples that contain more than one species.

In addition, the aforementioned abundance estimates were compared to ethanol-derived DNA (the filtrate of the ethanol used to store larval fish specimens) as a proxy for eDNA and simulations were conducted using past entrainment monitoring data from Bruce Power and other published datasets to evaluate the impacts of bias in abundance estimates in larger scale studies (Figure 7).



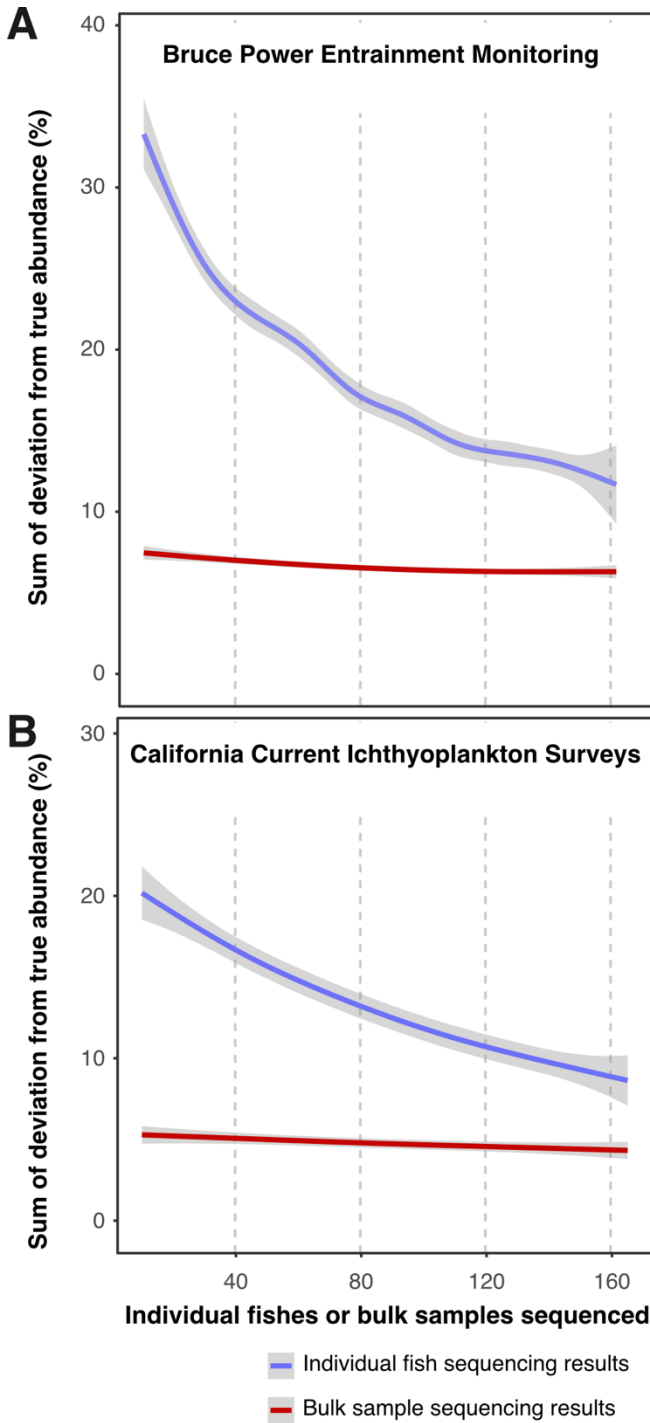


Figure 7. Simulation-based comparison of the accuracy of single fish and bulk sample sequencing approaches. Curves show the sum of the deviation from the true abundance across subsamples when abundance estimates were based on randomly subsampled individuals (blue curve) or simulated bulk sequencing results from an equal number of randomly sampled larval tows (red) at Bruce Power or the California Current.





The team found that despite the biases associated with larval fish size variation, homogenized tissue samples and storage ethanol approaches produce more accurate estimates of abundance than conventional single-fish sequencing methods when the number of individuals/samples sequenced was kept consistent. This suggests that DNA metabarcoding can be used to estimate abundance from bulk ichthyoplankton samples, especially when storage ethanol is included as template DNA, and should be incorporated into large-scale surveys to help eliminate biases associated with inadequate sampling.

#### Long-term monitoring of changes in species entrained.

Because of delays associated with the implementation of the entrainment survey at Bruce Power, the team was unable to compare the species entrained with other samples collected more broadly across Lake Huron. However, the samples used to measure biomass from bulk tissue samples, provided by the Saugeen Ojibway Nation's Coastal Waters Monitoring Program, did provide some insight into the feasibility of long-term monitoring using these approaches. The team has discussed how the DNA metabarcoding sequencing approach developed as part of this project could be integrated into the Coastal Waters Monitoring Program and other long-term monitoring programs. Comparative analyses of these results from 2021 with results from the same region a decade earlier (2011) has also begun.

#### 2024 Research Plan

The 2024 research plan for this project is currently pending the installation of sampling equipment at Bruce Power and will be revisited at that time. Postdoctoral researcher Alexander Van Nynatten, formerly at the University of Toronto, has taken a new postdoctoral position at the University of Victoria. Analysis of the eDNA autosampler data will be conducted by Markell Morphet, a PhD Candidate in Nicholas Mandrak's lab. Professors Nicholas Mandrak and Nathan Lovejoy will lead future iterations of this project.



## Outcomes

<b>Table 4: 2023 scientific outputs for Environmental DNA</b>	
<b>Source</b>	<b>Type</b>
Electric Power Research Institute Great Lakes Interest Group Annual Meeting. Virtual meeting. December 6, 2022.	Presentation
Pathway to Increase Standards and Competency of eDNA Surveys Annual Meeting. Guelph, ON. June 18-23, 2023.	Presentation

## Key Researchers

Alexander Van Nynatten, University of Toronto

Dr. Nicholas Mandrak NE, University of Toronto



## Fairy Lake (2021-2023)

The goals of this project were to characterize the current condition of Fairy Lake, both in terms of physicochemical conditions and the structure of the biological community, and then to use that knowledge to develop recommendations regarding the potential for restoration or other targeted remediation actions.

This project commenced in May 2021 and progressed through two summers of field work. The 2.074 km<sup>2</sup> drainage area for Fairy Lake was mapped, and the planned water, sediment, amphibian, and vegetation sampling was completed (Figure 8). After the data analysis was completed, the team submitted a final project report on May 1, 2024. The report included recommendations for potential restoration and remediation of the lake.

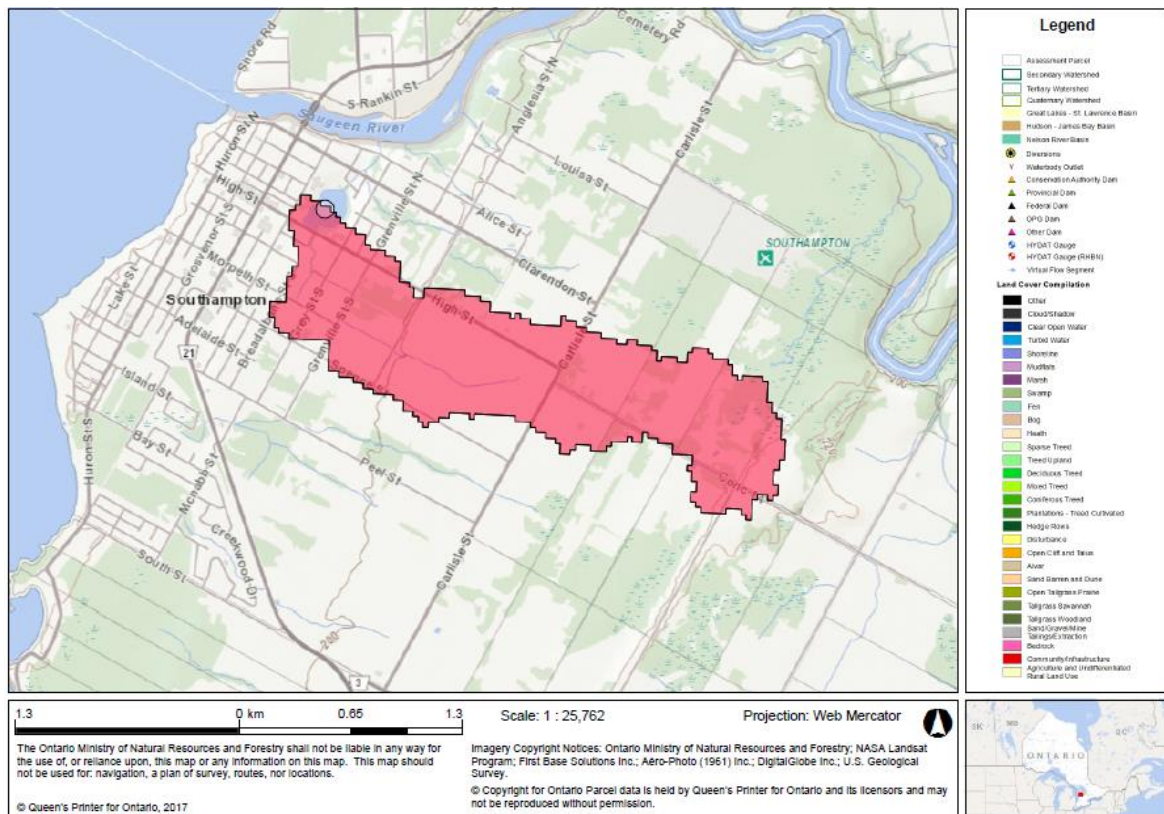


Figure 8: Drainage area of Fairy Lake, measuring 2.074 km<sup>2</sup>, containing 0.030 km<sup>2</sup> of wetland area and 0.022 km<sup>2</sup> of lake area. The mean elevation of the drainage area is 203.895 m above sea level and the mean slope of the drainage area is 1.623%. The mean annual temperature is 7.2 deg C and the total annual precipitation measures 1145 mm.

## Research Activities and Results



Fairy Lake has experienced water quality issues including cloudy water, low oxygen, and overabundant plant and algae growth. Several factors may contribute to the water quality problems, including external nutrient and sediment inputs from the agriculturally dominated watershed, internal phosphorus loading due to the invasive bottom-feeding Common Carp, or mid-summer oxygen depletion as invasive Curly-leaf Pondweed plants rot in the lake.

Identifying what actions might effectively improve the water quality in Fairy Lake requires understanding these contributing factors. To achieve this understanding, a team of researchers from the University of Waterloo undertook a pre-feasibility study in 2021-2022.

The team analysed water and sediment samples, surveyed frogs, fish and invertebrate indicators of lake health, and monitored the growth pattern of Curly-leaf Pondweed. The results indicate that Fairy Lake is eutrophic with high levels of the nutrients phosphorus and nitrogen. It also has high levels of total coliforms, which frequently exceeded the limit of 200 E. coli cfu/100 mL of water, set by the Guidelines for Canadian Recreational Water Quality.

They also determined that the suspended solids causing water clarity issues in mid to late summer is not mineral but likely algae, based on the organic content of suspended solids and the high chlorophyll-a concentrations that were observed.

Curly-leaf Pondweed, though not a desirable native plant, is playing an important role in Fairy Lake by helping maintain water clarity and oxygen levels in the spring and early summer (see Figures 9-11). The turbid conditions in Fairy Lake establish following the natural midsummer senescence of this plant.



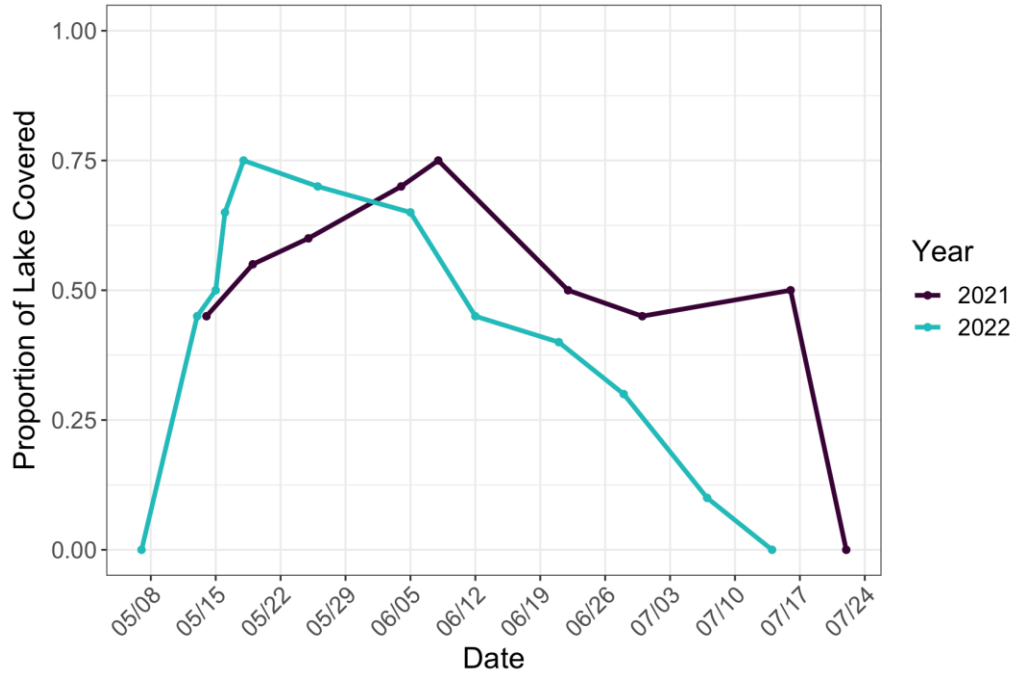


Figure 9: Extent of Curly-leaf Pondweed coverage of Fairy Lake, from data collected in 2021 and 2022. The highest proportion of Curly-leaf Pondweed cover occurred in early June in 2021 and mid-May in 2022.

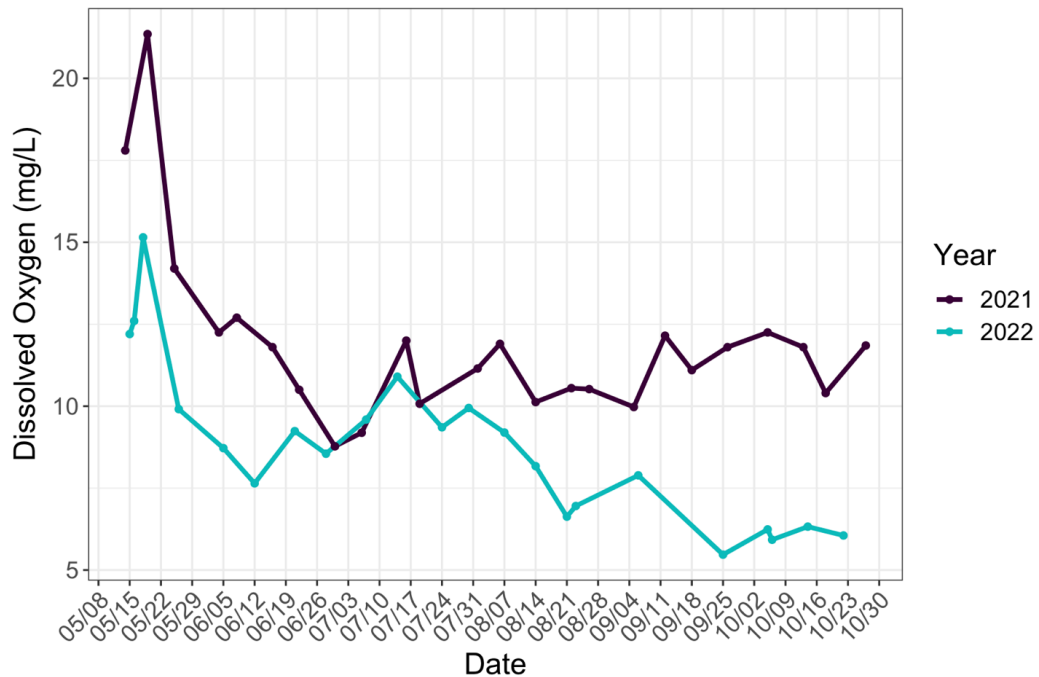


Figure 10: Dissolved oxygen concentrations in Fairy Lake, measured from water samples collected in 2021 and 2022.



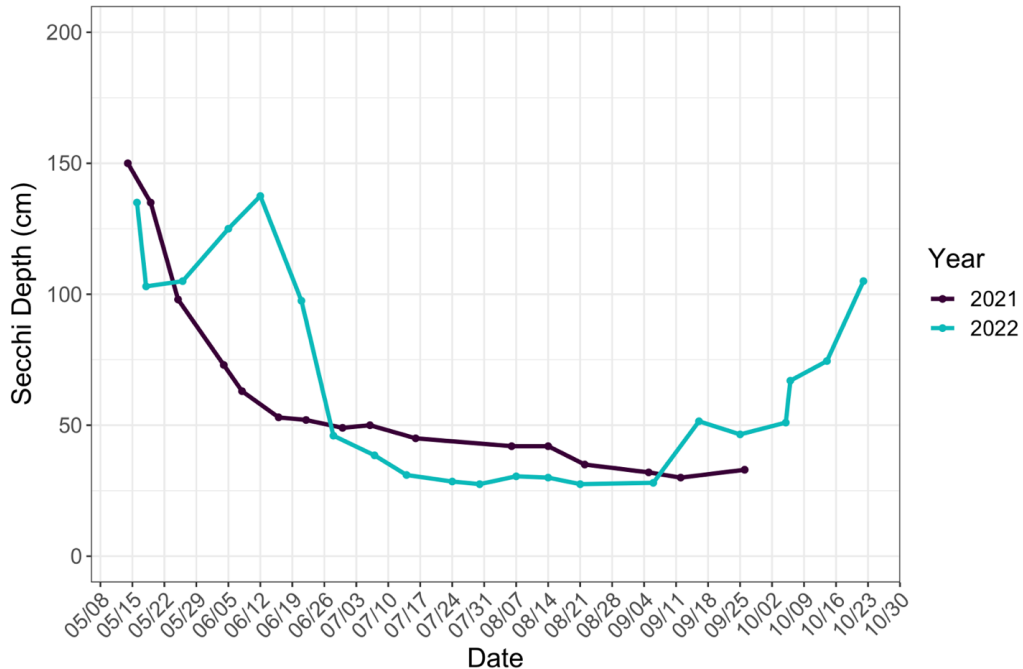


Figure 11: Secchi depth measurements from Fairy Lake in 2021 and 2022. In terms of water clarity, the Secchi depth measurements illustrate the sharp declines in water clarity that follow the senescence of Curly-leaf Pondweed in the lake.

The team also confirmed that Common Carp are present in Fairy Lake, but they are challenging to catch and fishing them out is not likely a viable management option. Instead, a biocide would likely be necessary to ensure the complete removal of Common Carp from Fairy Lake. It is likely that the Common Carp are contributing to turbid conditions by resuspending sediment and recirculating phosphorus after the Curly-leaf Pondweed has senesces for the summer.

However, with agricultural run-off from the watershed, external sources of nutrients to Fairy Lake indicate that water quality issues would persist even if Common Carp were eradicated. Using herbicide to remove the Curly-leaf Pondweed without controlling Common Carp might worsen the water quality issues. Due to the external nutrient inputs, the team recommends that interventions to enhance the water quality in Fairy Lake focus on reducing nutrient inputs, rather than on eradicating invasive plants or fish. Recommendations include:

1. Soften the hardened shoreline near High Street
2. Post signage to raise awareness about invasive species spread & water quality (Figures 12-14)
3. Prevent feeding geese and carp
4. Construct water treatment wetland to mitigate water quality from High Street

Additionally, an annual harvest of Curly-leaf Pondweed plant matter from the lake in late May or early June (before it senesces) could improve late summer water quality by



taking the nutrients bound in the plant matter out of circulation. However, this would require safe disposal of the plant matter to prevent spreading the invasive plant. It would not eliminate Curly-leaf Pondweed from the lake, but could help mitigate the effects of external nutrient inputs.

**DO NOT FEED THE BIRDS & FISH**  
**HELP IMPROVE WATER QUALITY**

**RISKS OF FEEDING THE WILDLIFE**

Any addition of food to the ecosystem adds nutrients to the Lake, and encourages birds – primarily **Canada Geese** – to loiter and defecate in the water. It also encourages the growth of invasive species such as **Common Carp**.

**WHY IS WATER QUALITY IMPORTANT?**

Simply put, better water quality results in a healthier Lake. Loitering and defecating birds can lead to high concentrations of *E. coli*, which is harmful not only to plants and animals in the Lake, but to humans as well. Additionally, the increase in nutrients caused by bird and fish food can lead to Lake eutrophication and algal blooms.

**HELP IMPROVE WATER QUALITY**

Thoroughly clean and dry boats and equipment after use.

Do not feed the wildlife in Fairy Lake.

A - Canada Goose (*Branta canadensis*)  
 B - Common Carp (*Cyprinus carpio*)  
 Image courtesy of George Chernilevsky under CC BY-SA 3.0

Logos: Sauguenay Shores, SAUGUENAY RIVER, WATERBODIES LABORATORY, WATKINS UNIVERSITY, IPCC

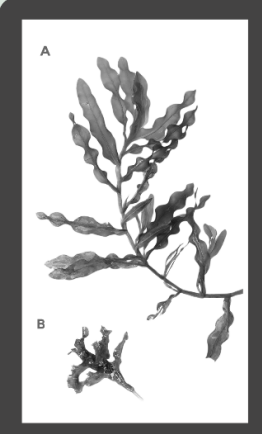
Figure 12: Example informational signage that could be erected at Fairy Lake.



# HELP STOP THE SPREAD OF INVASIVE SPECIES IN FAIRY LAKE

### CURLY-LEAF PONDWEED


Curly-leaf Pondweed (*Potamogeton crispus*) is native to Eurasia, Africa and Australia, but is invasive in North America. It produces seeds and buds that look like small brown pinecones (Fig. B) in early summer. It is able to begin growing very early in the spring, even under the ice.



**A - Mature Curly-Leaf Pondweed**  
Image courtesy of Stefan Leffner under CC BY-SA 4.0


**B - Curly-Leaf Pondweed bud**  
Image courtesy of Leslie J. Mehrhoff under CC BY 3.0

### HELP PREVENT ITS SPREAD



Thoroughly clean and dry boats and equipment after use.

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Do not feed the wildlife in Fairy Lake.




Figure 13: Example informational signage that could be erected at Fairy Lake.





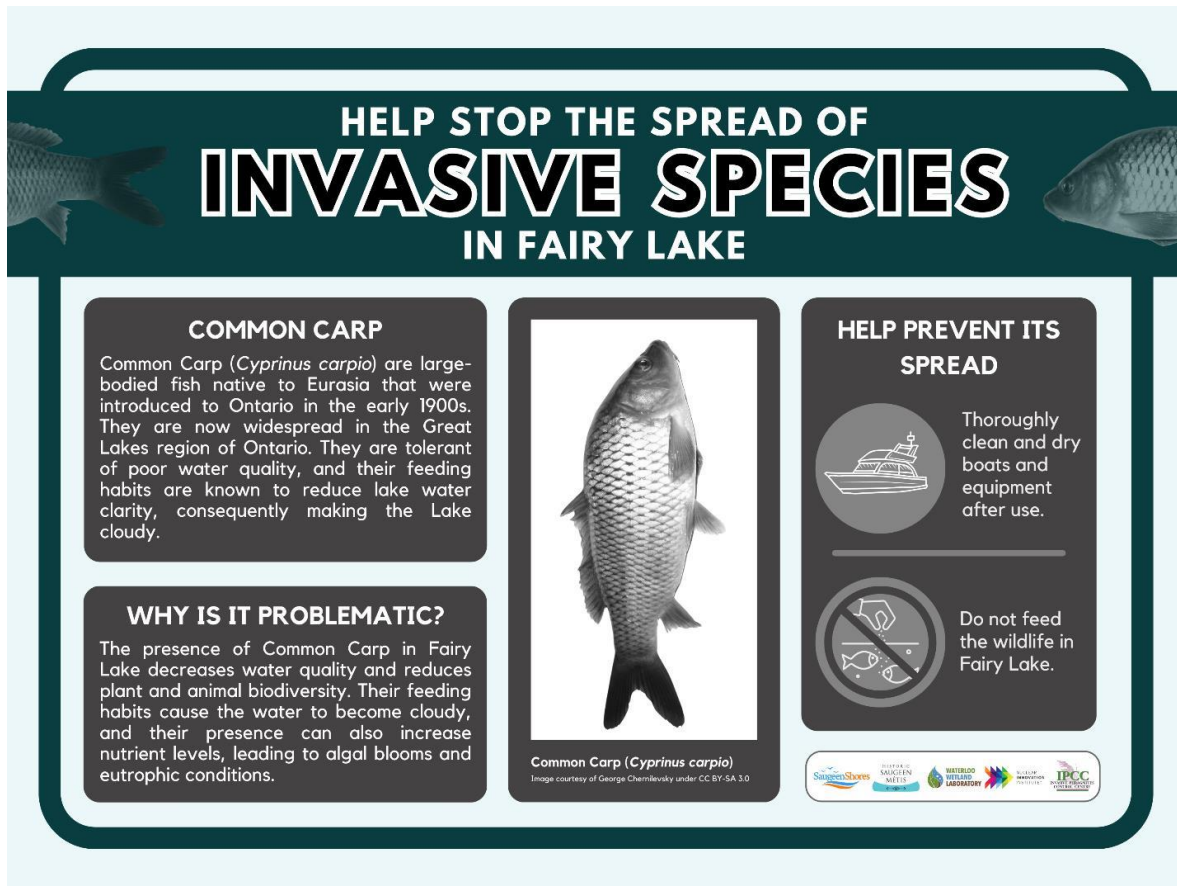


Figure 14: Example informational signage that could be erected at Fairy Lake.

## Public Engagement and Outreach

In 2022, three presentations were hosted for the public. The first was a summary of the 2021 field results to the project core team; this meeting was held on March 28, 2022. The researchers also presented to the Town of Saugeen Shores council, sharing the same results on the July 25, 2022. A lakeside in-person outreach event was held on October 4, 2022 at Fairy Lake, hosted by the research team and NII. This event was attended by 63 people from the community, including students from the local elementary school.

An initial project goal included the development of a project webpage as an outreach component. In 2023, after consulting with the Town of Saugeen Shores, the project team decided not to move forward with the website and instead to prioritize other forms of outreach.

In 2023, research assistant Adrienne Mason gave a presentation on Fairy Lake at the Huron Fringe Birding Festival on May 29, and another at MacGregor Point Provincial Park. Mason also hosted an Earth Day garbage picking event at Fairy Lake on April 23, 2024, which was attended by 20 people.



Fairy Lake participated in the Living Lakes project in 2022 and 2023. In 2024, the volunteer kit for Living Lakes was passed on to the science teacher at G.C. Elementary School who plans to continue the volunteer monitoring with her class.

Finally, the research team presented their final results to the Town of Saugeen Shores and NII at a meeting held on June 13, 2024.

#### Key Researchers

Dr. Rebecca Rooney, University of Waterloo

Dr. Heidi Swanson, University of Waterloo

Adrienne Mason, University of Waterloo



## The Climate Project (2023)

The world is saturated in news and information about the perils of climate change. From a stream of scientific studies to daily climate change-related news, this global scale can make the issue less tangible—and less urgent.

There is a pressing need to localize news and information about climate change, to provide actionable intelligence that helps people better understand what a changing global climate could mean for where they live.

The Climate Project was created to be a living, trusted and accessible digital hub with scientific research findings from qualified sources in academia, municipal, provincial and federal governments, conservation authorities, NGOs, industry partners and sources of local Indigenous knowledge.

Its purpose is to share the body of localized research and scientific knowledge on climate change pertaining to people in this region—those in Bruce, Grey, and Huron counties and local Indigenous communities—all located within the Saugeen Ojibway Nation Territory.

The Climate Project is intended to be a living site that will continue to grow as new research is added and in response to questions that audiences pose. There is also a significant outreach program component to the project, which is currently under development.

### Project Activities

In May 2023, NII hired a science communicator, Dr. Stephanie Keating, to research and write the content for the Climate Project website. Written content was completed by December 2023. The website was designed in Wix by NII Graphic & Web Designer Summer Goodeve.

Content on the Climate Project website includes highlights of Environment@NII research, such as the Aquatic Biota and Environmental DNA projects, as well as other Bruce Power environmental research and spotlights on local community organizations dedicated to climate change and sustainable practices. Stakeholders who contributed to the project include the Saugeen Ojibway Nation Environment Office, the Historic Saugeen Métis, ALUS, the Lake Huron Coastal Centre, Maitland Valley Conservation Authority, and the Ontario Federation of Agriculture.



Content is presented in accessible, engaging language, and is formatted as a story told in three chapters: Air, Water and Land. The content of the chapters is as follows:

#### Air

- Weather and air quality, including air temperature, precipitation patterns, extreme weather, tornadoes, forest fires and air quality

#### Water

- Physical characteristics, including temperature, water levels, ice cover and water quality
- Shoreline processes, including shorelines, bluffs, erosion, trees and plants, and dunes
- Ecosystem changes, including fish, the Lake Huron food web, the Aquatic Biota program, the environmental DNA program, Indigenous-led research such as Together with Giigoonyag, the ciscoes of Lake Huron, the Saugeen Ojibway Nation Coastal Waters Monitoring Program, invasive species, and Fairy Lake.

#### Land

- Agriculture and livestock, including growing season, livestock, diseases and pests
- Land management, including carbon sequestration, climate and soil health, soil best practices, and a spotlight on ALUS
- Forests, including a history of forests in Saugeen-Bruce, forests and climate change, forest management, effects of water, invasive species, and species at risk
- Wetlands and watersheds, including wetlands as a carbon sink, disappearing wetlands, rivers and streams, conservation authorities, and watershed report cards

In February 2024, Dr. Stephanie Keating was brought on as Director, Environment@NII, with a primary component of the role dedicated to managing the Climate Project. A communications plan was developed that includes schedules for blog posts and social media, as well as press releases and media communications.

### Public Engagement and Outreach

On January 23, 2024, NII presented a preview and summary of the Climate Project to the Women's Probus Club of Saugeen Shores.



## 2024 Activity Plan

The 2024 activity plan includes the formal launch of the Climate Project website and development of the outreach and communications strategy for 2024 and 2025. Specific goals include identifying key areas of interest and concern for stakeholders and key audiences, establishing baselines for website and social media engagement, engaging with community and NGO groups concerned about climate change and nuclear power, and bringing a local perspective on climate change to students and teachers.

## Key Personnel

Dr. Stephanie Keating, Nuclear Innovation Institute

Dana Van Allen, Nuclear Innovation Institute

Summer Goodeve, Nuclear Innovation Institute



## Conclusion

Environment@NII is proud to play a role in facilitating this research and in sharing the stories and outcomes of these projects with audiences in nuclear and far beyond.

NII looks forward to continuing on-going research projects and developing new avenues of research in 2024. Priority areas include research into the health of Lake Huron and its ecosystems, understanding the effects that everyday radiation has on our lives, and providing actionable intelligence to improve worker health and safety. NII is grateful to Bruce Power for funding these leading-edge projects and supporting scientific rigor and independent academic research in these critical areas.

