Northern Ontario School of Medicine
École de médecine du Nord de l'Ontario

Laurentian University
Université Laurentienne

Researching the Effects of the Presence and Absence of Ionizing Radiation (REPAIR): a deep-underground biology experiment investigating the role of naturally occurring background radiation

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Laurentian University, Northern Ontario School of Medicine

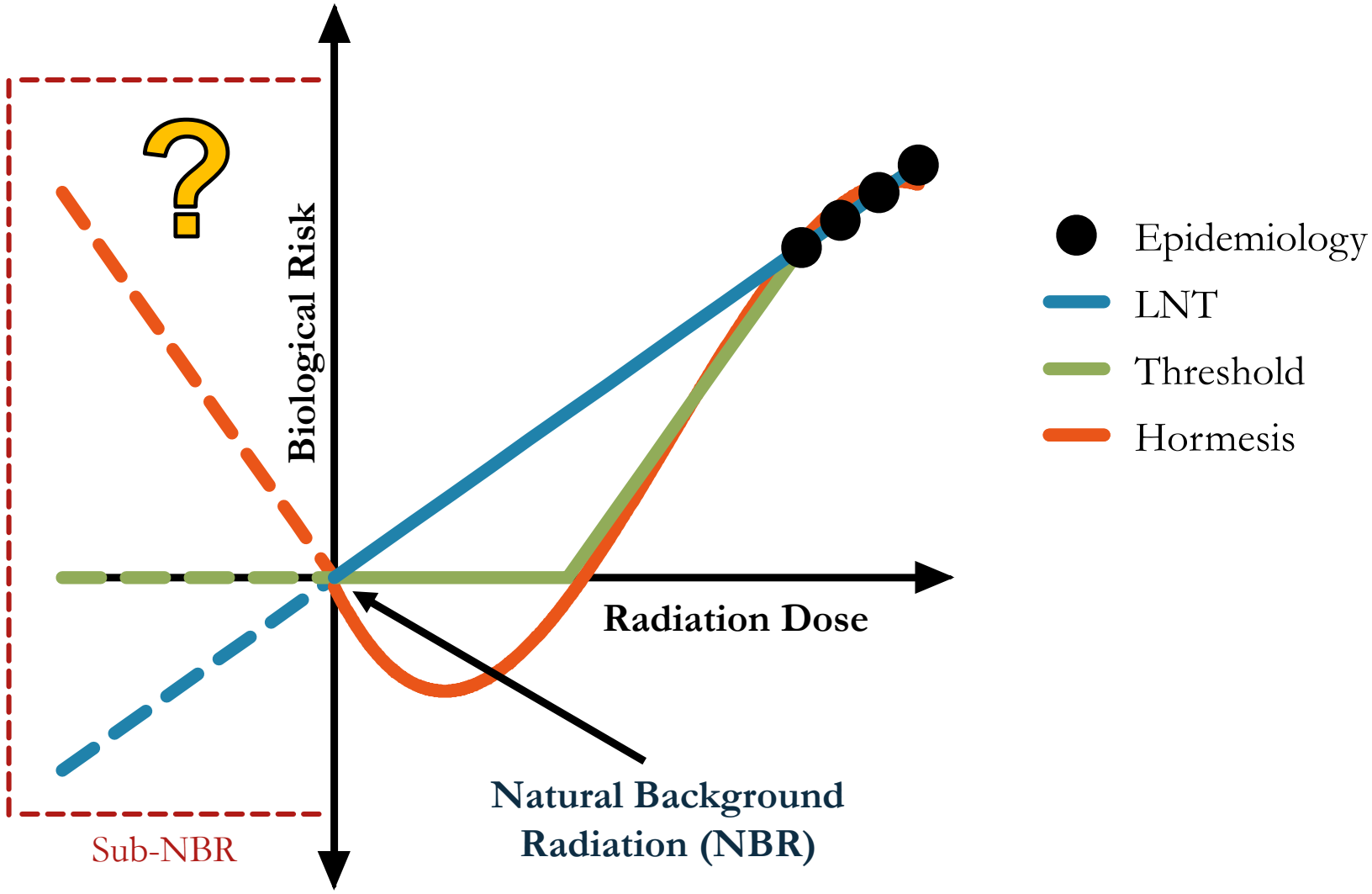
2021 Northern Health Research Conference
Theme 3 – Environment and Health

Disclosure of Affiliations, Financial and In-Kind Support:

I am an Mitacs funded employee of Laurentian University

The REPAIR Project is supported through Natural Sciences and Engineering Research Council of Canada (NSERC) Collaborative Research and Development (CRD) and MITACS grants in partnership with Bruce Power Inc and the Nuclear Innovation Institute.

Radiobiological models of risk



Where can we empirically investigate below background radiobiology?

An ideal radiologically "quiet" environment for sub-NBR radiobiology experimentation

Deep-underground research laboratory
(Inherent shielding from rock overburden)

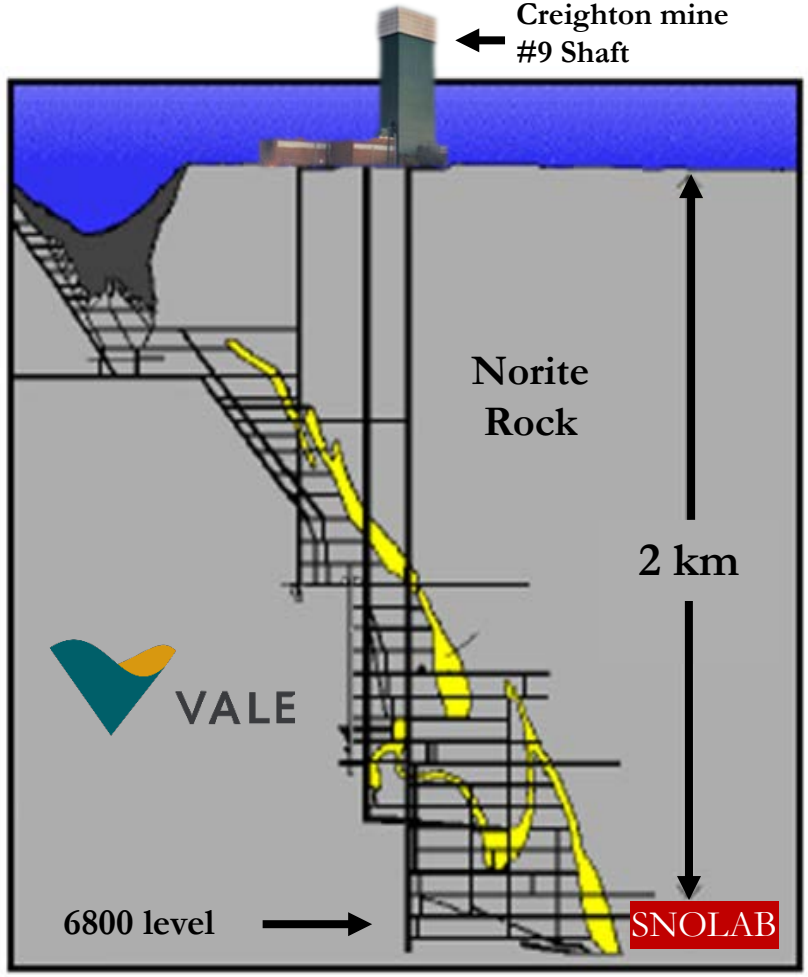
2 km (6,800 ft) underground
(6 km water equivalent)

~5,000 m^2 (53,000 ft^2) laboratory space

Class 2000 clean room
(less than $2x10^3$ particles >0.5µm per ft^3)

$5x10^7$ reduction cosmic radiation
(shielded by rock overburden)

HEPA filtration of 50 m^3 s^{-1}
(10 full lab air exchanges per hour)



Getting there . . .

Est. 2015, “bio-logistical” pilot project

Can we run a biology experiment with the technical and logistical limitations of a laboratory environment located 2km below the surface of the planet within an active mining operation?

Yes!

Especially when you explain where the experiment is located to a prospective graduate student

Lake whitefish (*Coregonus clupeaformis*) embryonic development

	Surface	Surface	Underground	Underground
Temperature	5°C	3°C	5°C	3°C
Dishes (50 embryos per dish)	39	38	43	42
Embryos	1,950	1,911	2,150	2,100

Embryos fixed at 40, 60, 80% development

- Hatch rate
- Survival
- Morphometrics:
  - Body length
  - Body weight
  - Yolk Conversion Efficiency
  - Yolk area
  - Yolk weight

“Bio-logistical” pilot project

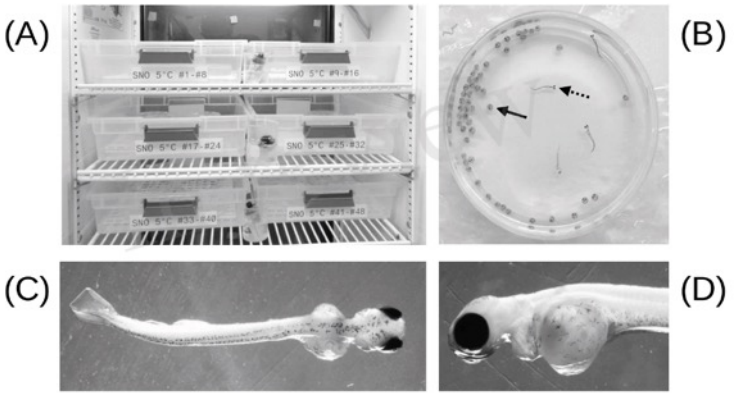
Frontiers In Earth Science - Special Edition:
The Biogeochemistry, Biophysics, Radiobiology, and Technical Challenges of Deep Subsurface Research

frontiers

A research environment 2 km deep-underground impacts embryonic development in lake whitefish (Coregonus clupeaformis)

Jake Pirkkanen[1], Andrew M. Zarnke[2], Taylor Laframboise[1], Simon J. Lees[3, 4], T.C. Tai[1, 2, 5], Douglas R. Boreham[1, 2, 5, 6], Christopher Thome[1, 2, 5*]

[1] Department of Biology, Laurentian University, Sudbury, ON, Canada, [2] Biomolecular Sciences Program, Laurentian University, Sudbury, ON, Canada, [3] Department of Biology, Lakehead University, Thunder Bay, ON, Canada, [4] Medical Sciences Division, Northern Ontario School of Medicine, Thunder Bay, ON, Canada, [5] Medical Sciences Division, Northern Ontario School of Medicine, Sudbury, ON, Canada, [6] Bruce Power, Tiverton, ON, Canada

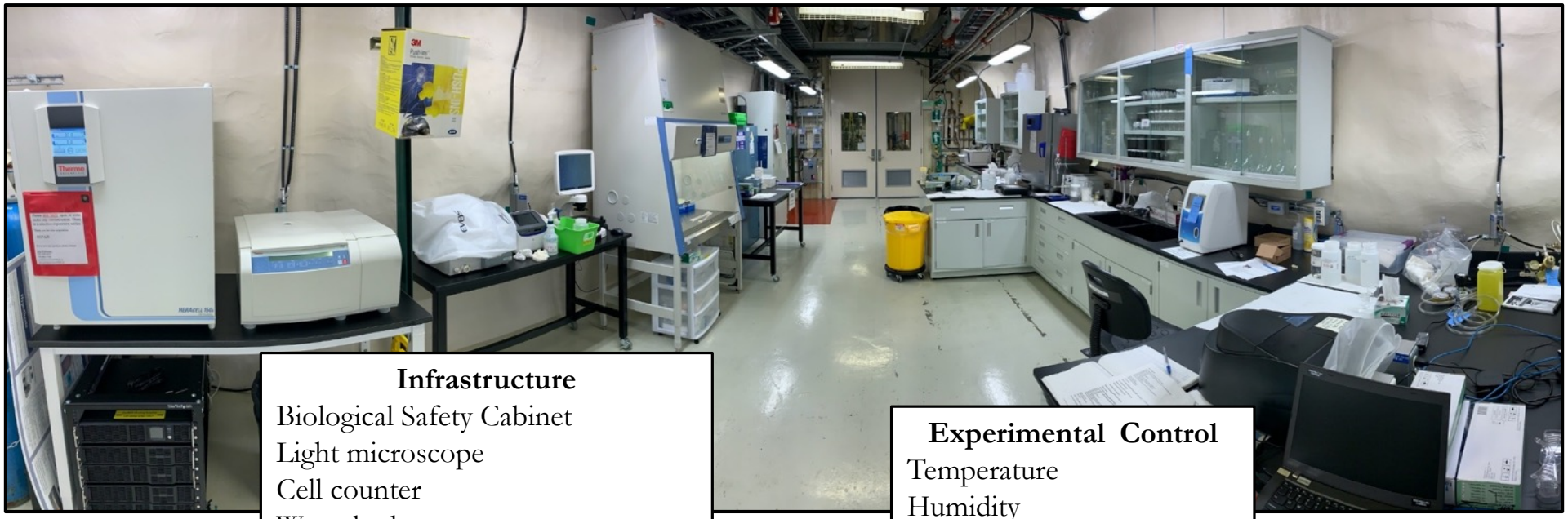


From the pilot project’s inception, our goal was to establish the ability to perform modern molecular and cellular biology endpoints, and assay these in a variety of model systems

REPAIR Est. 2016

Researching the **E**ffects of the **P**resence and **A**bsence of **I**onizing **R**adiation

A deep-underground sub-NBR life sciences radiobiology research project



Infrastructure

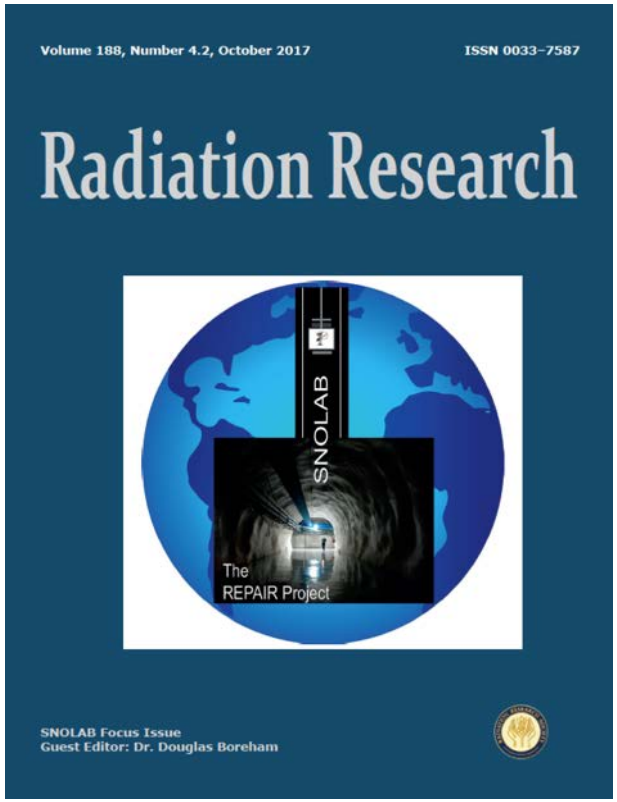
Biological Safety Cabinet
Light microscope
Cell counter
Water bath
Centrifuge
Tissue culture incubator (control)
Uninterrupted Power Systems *

Experimental Control

Temperature
Humidity
Gas/atmosphere levels
Access to samples
NBR constituents

*** Diesel backup completed September 2021**

Radiation Research - REPAIR Focus Issue



RADIATION RESEARCH **188**, 470–474 (2017)
0033-7587/17 $15.00

DOI: 10.1667/RR14654.1

COMMENTARY

The REPAIR Project: Examining the Biological Impacts of Sub-Background Radiation Exposure within SNOLAB, a Deep Underground Laboratory

Christopher Thome,[a,b,1] Sujeenthar Tharmalingam,[a,b,1] Jake Pirkkanen,[b,1] Andrew Zarnke,[b,1] Taylor Laframboise[a] and Douglas R. Boreham[a,b,c,2]

[a] *Division of Medical Sciences, Northern Ontario School of Medicine and* [b] *Department of Biology, Laurentian University, Sudbury, Canada, P3E 2C6; and* [c] *Bruce Power, Tiverton, Canada, N0G 2T0*

RADIATION RESEARCH **188**, 512–524 (2017)
0033-7587/17 $15.00

DOI: 10.1667/RR14911.1

REVIEW

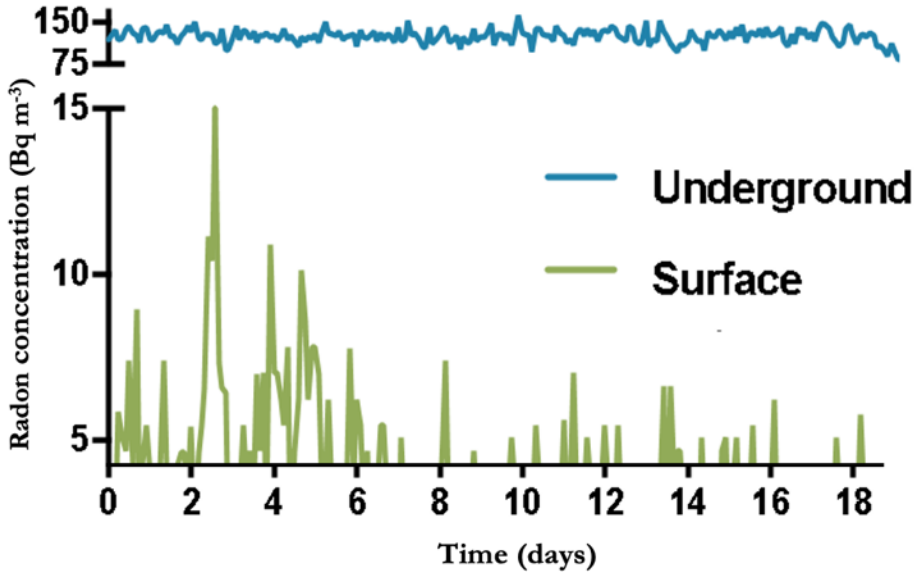
The CGL1 (HeLa × Normal Skin Fibroblast) Human Hybrid Cell Line: A History of Ionizing Radiation Induced Effects on Neoplastic Transformation and Novel Future Directions in SNOLAB

Jake S. Pirkkanen,[a,1] Douglas R. Boreham[a,b,c] and Marc S. Mendonca[d,2]

[a] *Department of Biology, Laurentian University, Sudbury, Ontario, Canada, P3E 2C6;* [b] *Northern Ontario School of Medicine, Sudbury, Ontario, Canada, P3E 2C6;* [c] *Bruce Power, Tiverton, Ontario, Canada, N0G 2T0; and* [d] *Department of Radiation Oncology, Radiation and Cancer Biology Laboratories, and Department of Medical & Molecular Genetics, Indiana University School of Medicine, Indianapolis, Indiana 46202*

The radon hurdle

Radon ($t_{1/2}$ 3.8d) levels are significantly elevated deep underground compared to the surface, and represent a significant experimental contaminant for sub-NBR studies

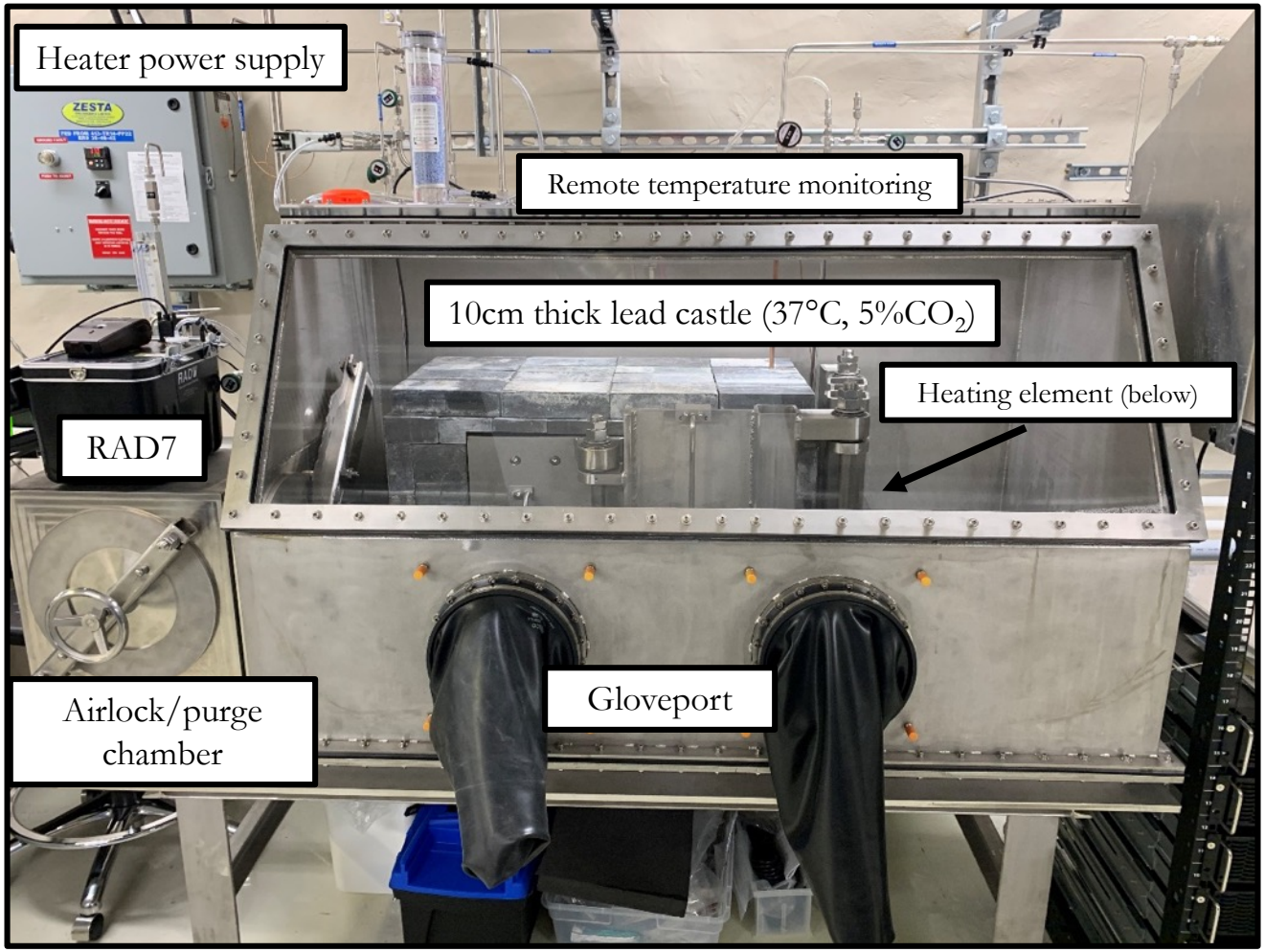


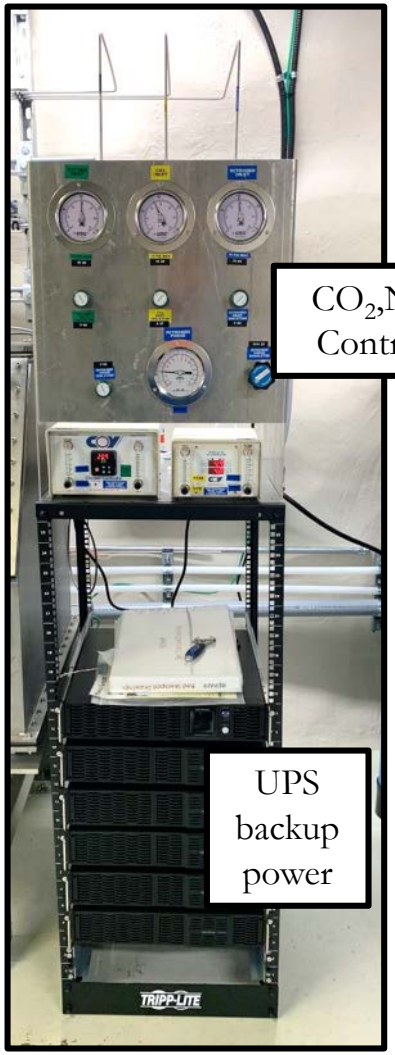
Gas cylinders (CO_2, N_2, O_2) are aged underground for a minimum of one month

(N_2 source upgraded to LN_2 dewar in 2019)

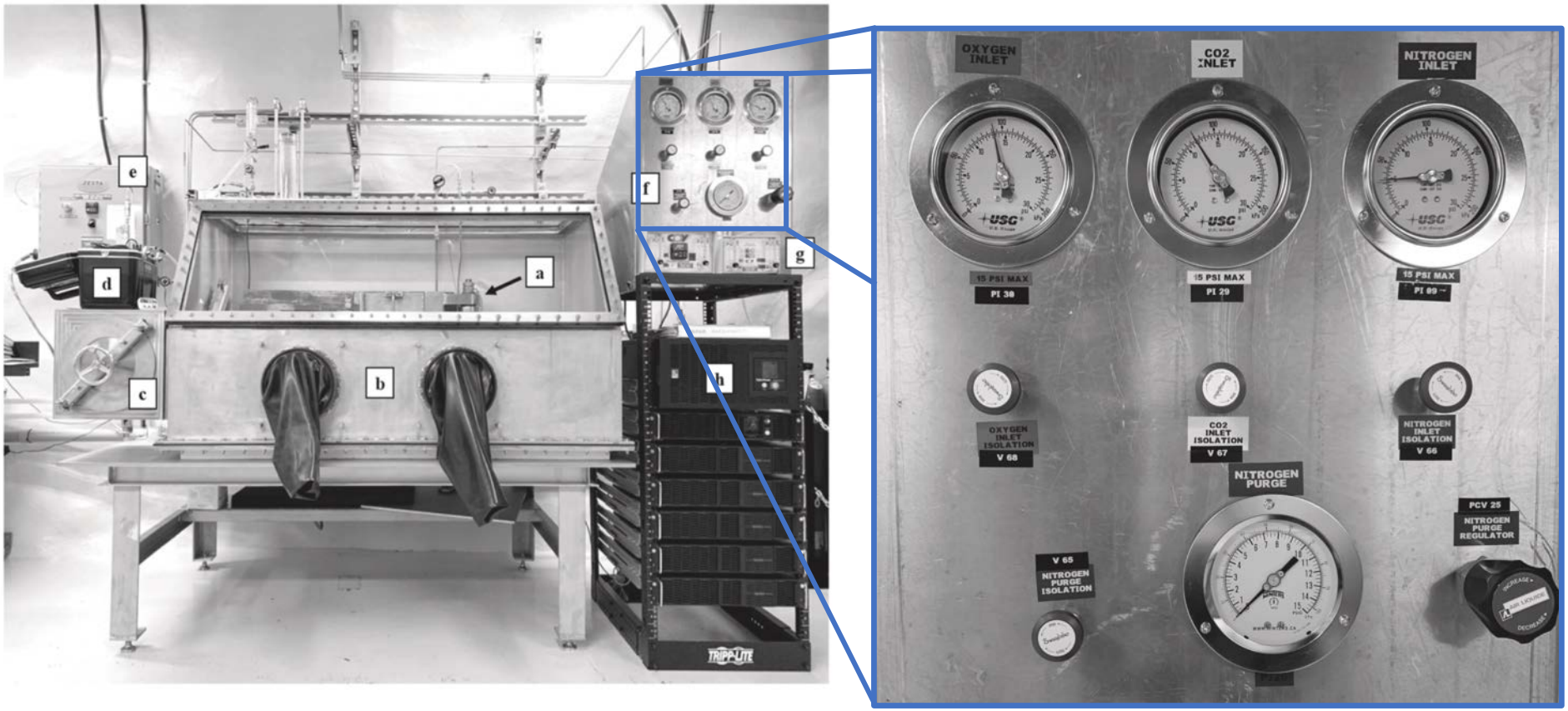
It was necessary to engineer and construct an instrument capable of maintaining our biological samples as well as reducing additional components of NBR (notably ^{222}Rn)

Sub-Natural Background Radiation
Specialized Tissue Culture Incubator (STCI)





Sub-Natural Background Radiation
Specialized Tissue Culture Incubator (STCI)

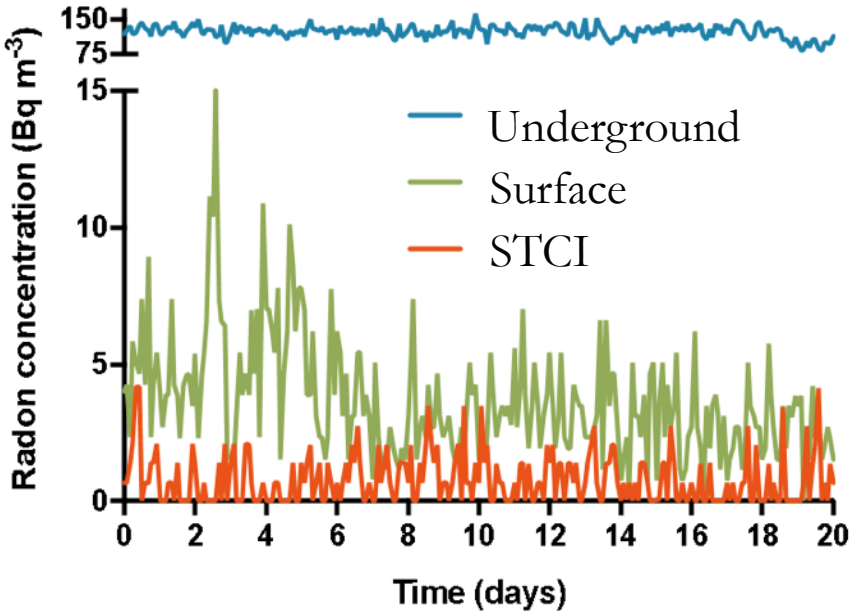


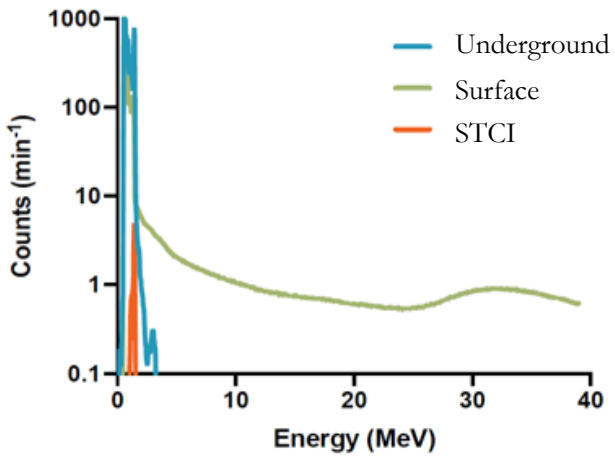
Sub-Natural Background Radiation
Specialized Tissue Culture Incubator (STCI)

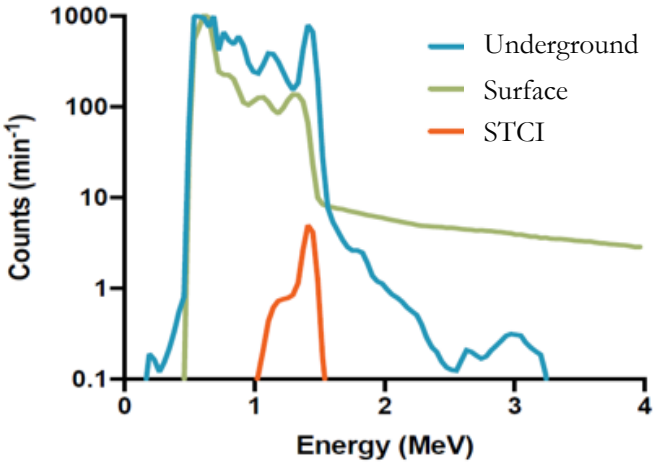
Over-pressurization bubbler system (UPW + oil)

In-line gas mixture humidification system

Sub-Natural Background Radiation Specialized Tissue Culture Incubator (STCI)







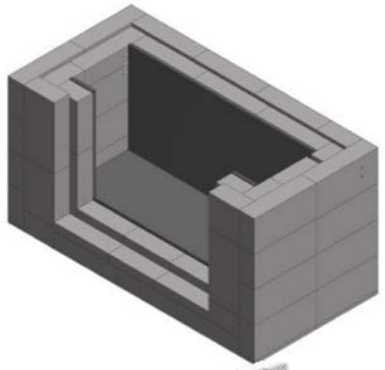
The STCI is a novel instrument which is successful at reducing levels of NBR components below what is ambiently found at the surface, making investigations into the biological significance of their absence possible

Sub-Natural Background Radiation Specialized Tissue Culture Incubator (STCI)

Contents lists available at ScienceDirect

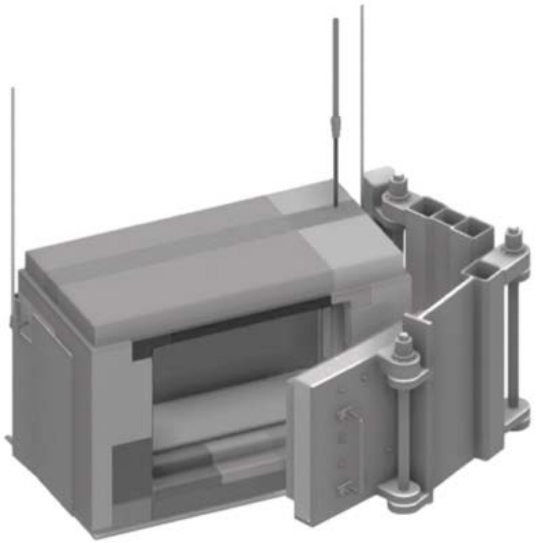
Journal of Environmental Radioactivity

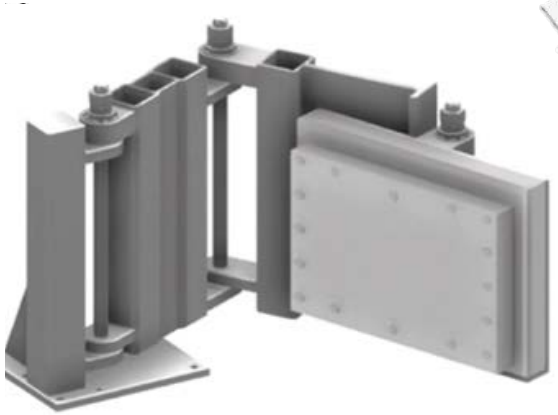
journal homepage: http://www.elsevier.com/locate/jenvrad

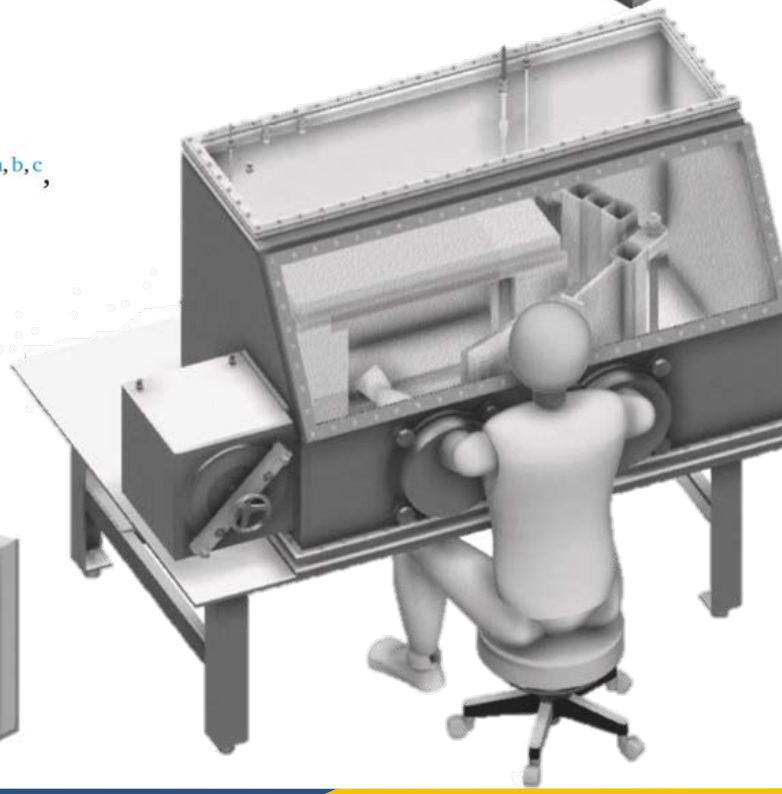


A novel specialized tissue culture incubator designed and engineered for radiobiology experiments in a sub-natural background radiation research environment

Jake Pirkkanen [a,b,c], Taylor Laframboise [a], Peter Liimatainen [d], Tom Sonley [d], Stephen Stankiewicz [d], Mike Hood [d], Mehwish Obaid [d], Andrew Zarnke [b,c], T.C. Tai [a,b,c], Simon J. Lees [e,f], Douglas R. Boreham [a,b,c,g,h], Christopher Thome [a,b,c,h,*]

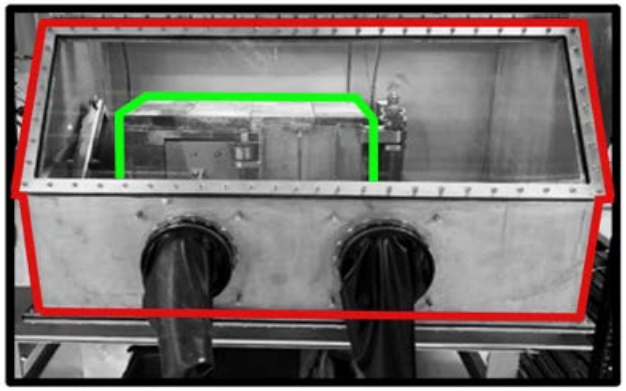


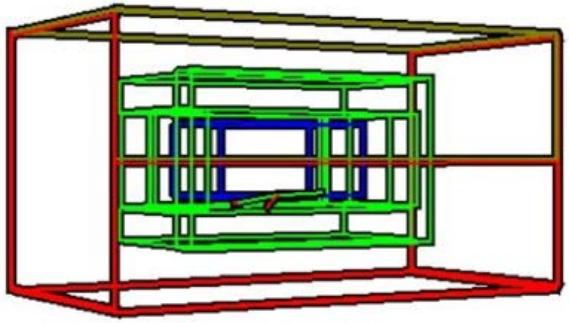


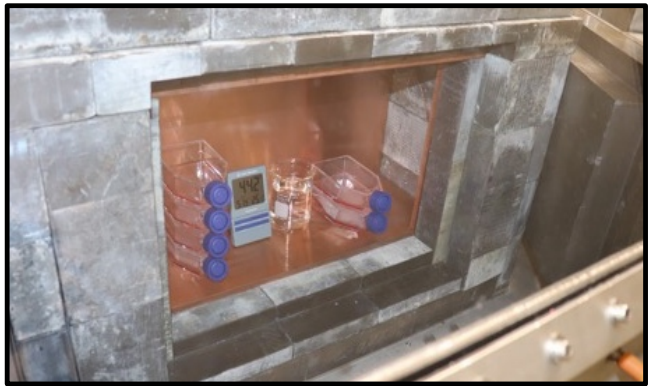


Natural Background Radiation Characterisation

GEANT4 Monte Carlo simulation-based modeling of each experimental environment. The model considers calculated or measured alpha, gamma, neutron and muon components as well as the ^{40}K and ^{14}C constituents of tissue culture nutrient media







Cells incubating underground within the STCI's lead castle

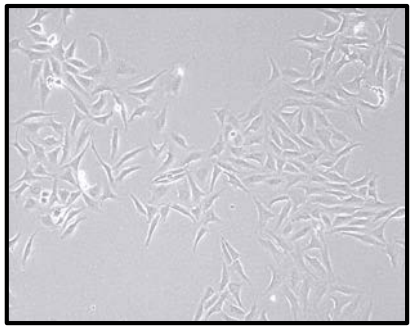
Table 2. Absorbed dose rate calculations for the three radiation environments. Dose rates were calculated in a water scoring volume.

Particle type	Surface control (nGy hr^{-1})	Underground control (nGy hr^{-1})	Sub-background (nGy hr^{-1})
Gamma	5.78 ± 0.03	7.67 ± 0.01	0.0427 ± 0.0013
Neutron	4.52 ± 0.04	0.0045 ± 0.0002	0.00169 ± 0.00002
Muon	55.27 ± 0.40	Negligible	Negligible
^{222}Rn	0.044 ± 0.014	1.45 ± 0.17	0.009 ± 0.011
^{40}K	2.41 ± 0.19	2.41 ± 0.19	2.41 ± 0.19
^{14}C	0.0175 ± 0.0001	0.0175 ± 0.0001	0.0175 ± 0.0001
Low LET[a]	63.48 ± 0.62	10.10 ± 0.20	2.47 ± 0.19
High LET[b]	4.56 ± 0.05	1.45 ± 0.17	0.01 ± 0.01
Total	68.04 ± 0.67	11.55 ± 0.37	2.48 ± 0.20

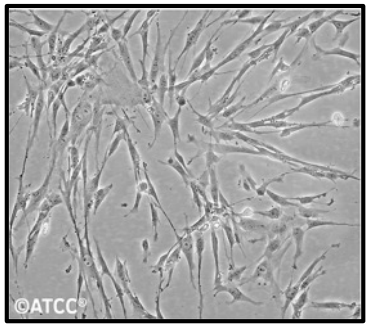
[a]Low LET = Gamma, Muon, ^{40}K, ^{14}C.
[b]High LET = Neutron, ^{222}Rn.

Experimental Plan

The goal of the REPAIR Project is to investigate the biological effects of the absence of NBR in a variety of complex multicellular model systems

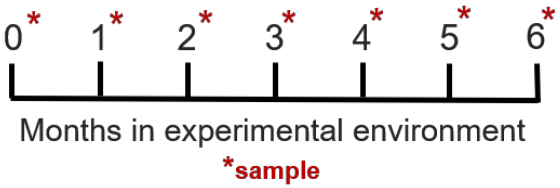


Mammalian cells



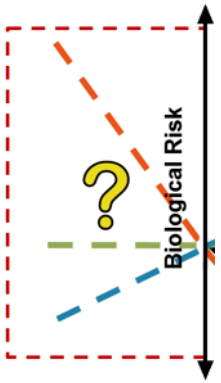
Yeast

Nematode worms



- Baseline response
- Radiation challenge

- Gene expression
- Cell growth
- Survival
- DNA damage
- Mutation
- Transformation



Active REPAIR experiments:

- **The Effects of a Sub-Natural Background Radiation Environment 2km Underground on Biological Systems**

HeLa (D98/AH2) x Normal Fibroblast (GM0077)
↓
CGL1

- **The Role of Natural Background Radiation on Neurological Development and Processes**

- **The Role of Anhydrobiosis on Yeast in a Sub-Natural Background Radiation Environment**

FLIES IN A MINE

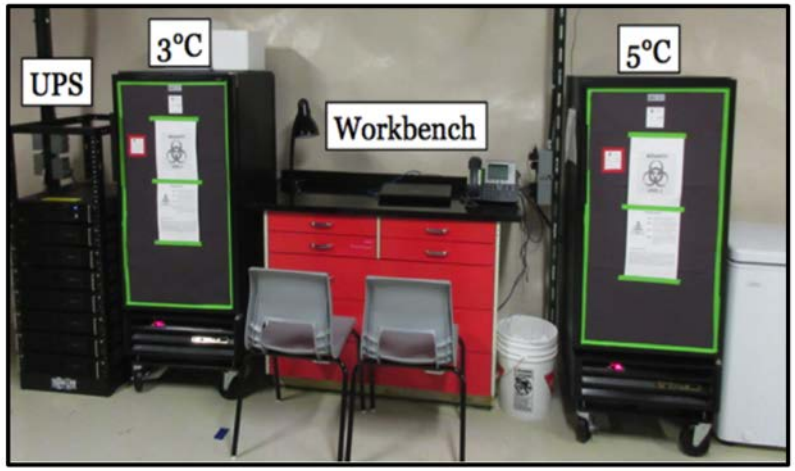
Transport eggs underground
*Fall 2021

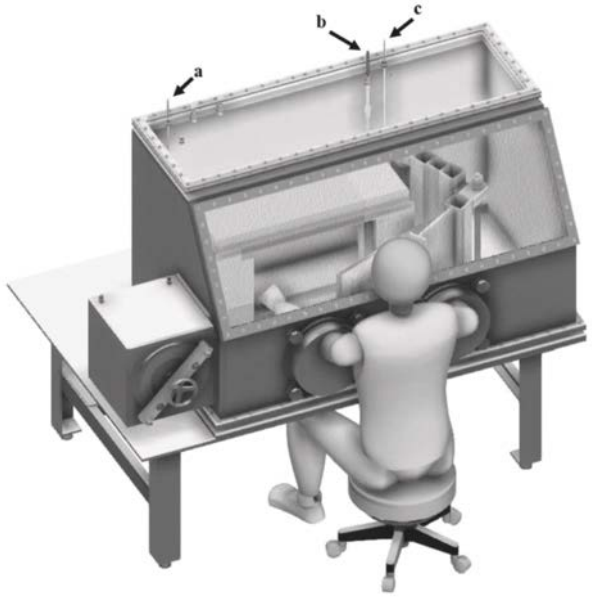
→

10 days

→

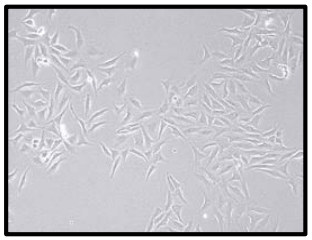
- Weight
- Triglycerides
- Total lipids





REPAIR has grown in the last 5 years from a small pilot project to a multi-institution collaboration supporting almost two dozen people, including several PIs, post-doctoral fellows, doctoral students, master's students, undergraduate students, and research technicians.

REPAIR is incredibly excited to have expanded our experimental capabilities/infrastructure and completed our first underground protracted experiments in a sub-NBR environment. We look forward to continuing these studies with new biological model systems!



REPAIR

Thank you for your time!

SNOLAB

NUCLEAR INNOVATION INSTITUTE

NSERC CRSNG

Bruce Power™

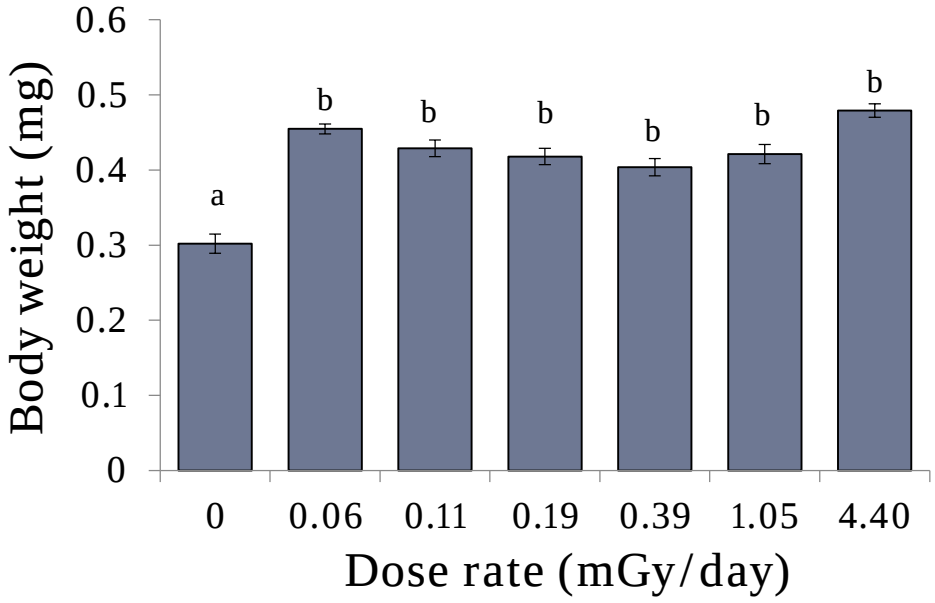
MITACS

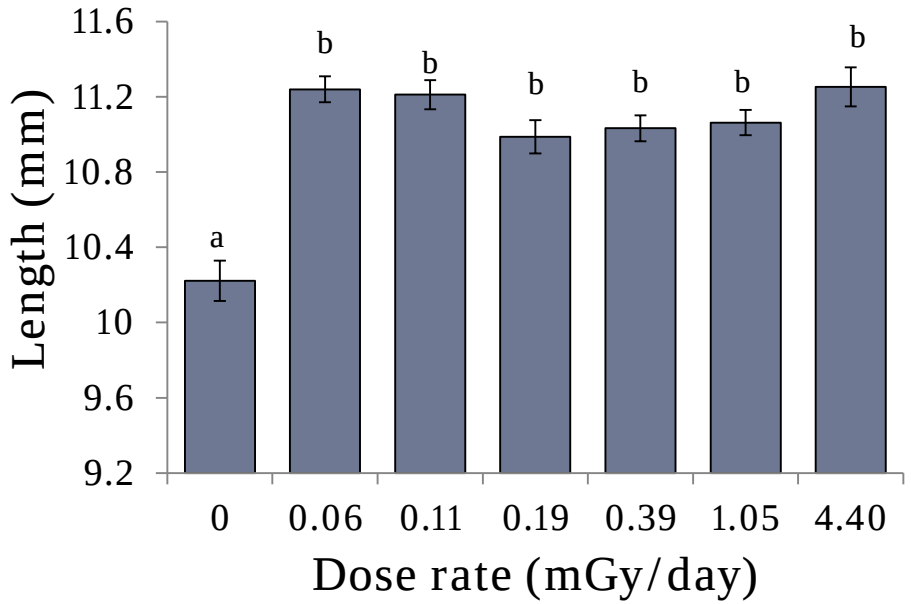
Supplemental material

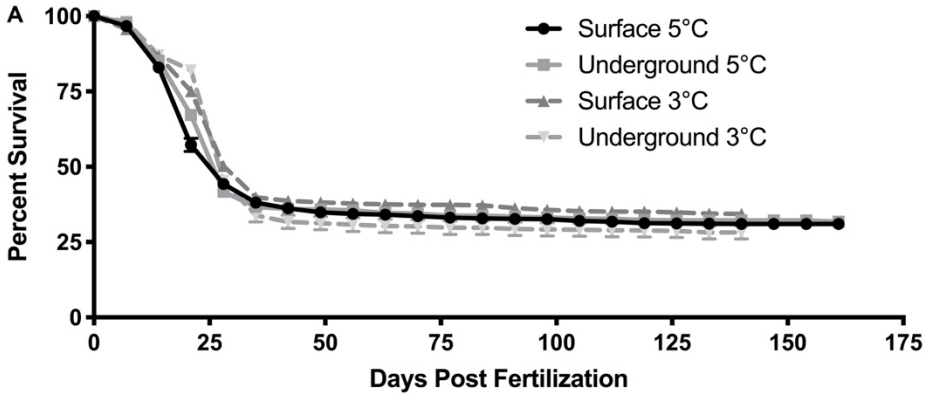
> Radiat Res. 2017 Oct;188(4.2):475-485. doi: 10.1667/RR14574.1. Epub 2017 Jul 24.

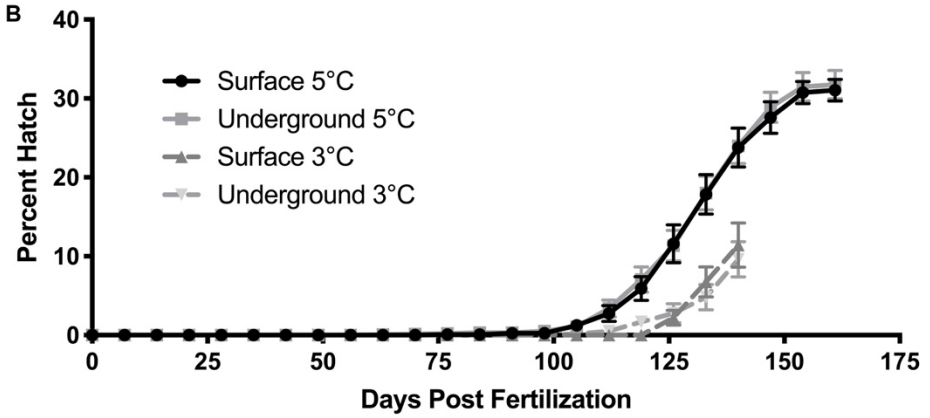
Initial Characterization of the Growth Stimulation and Heat-Shock-Induced Adaptive Response in Developing Lake Whitefish Embryos after Ionizing Radiation Exposure

Christopher Thome [1], Charles Mitz [1], Emily N Hulley [1], Christopher M Somers [2], Richard G Manzon [2], Joanna Y Wilson [3], Douglas R Boreham [1 4]

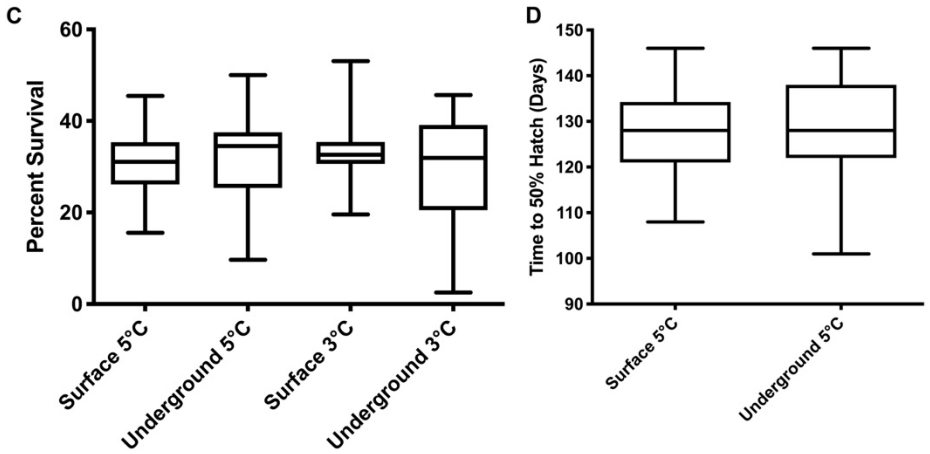


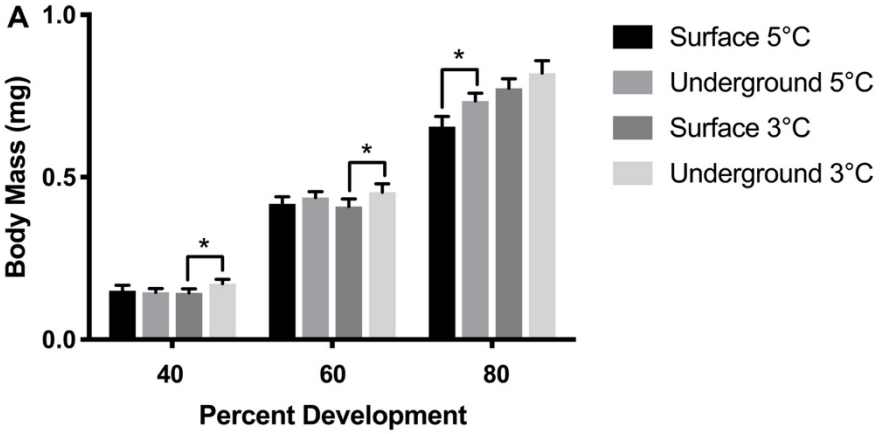


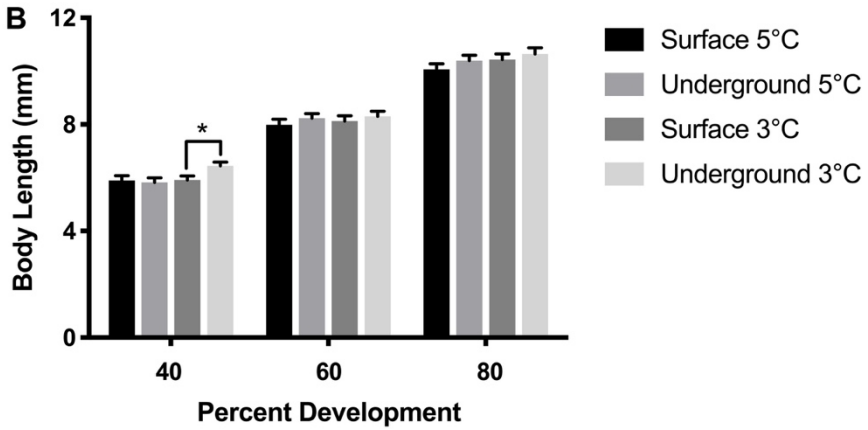


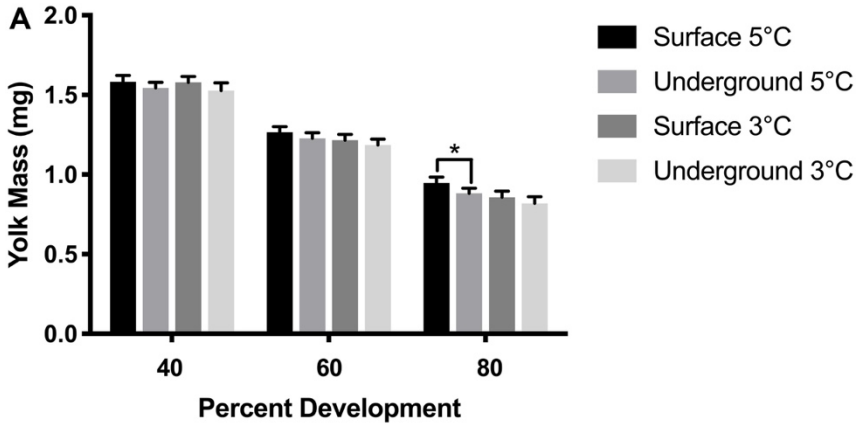


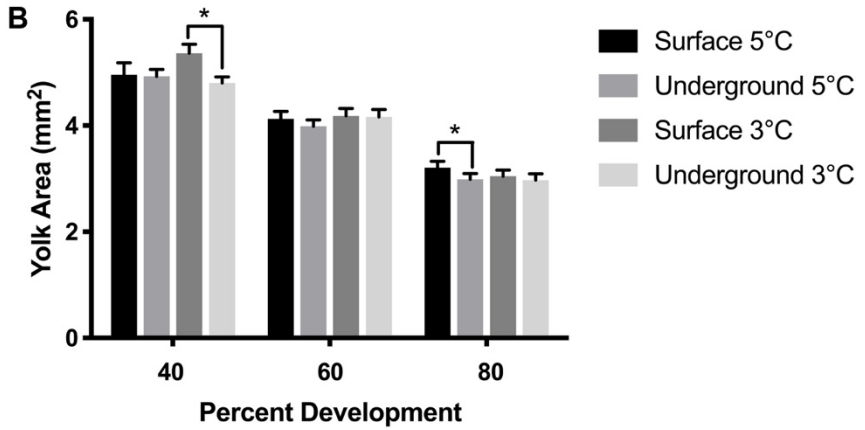
$$\mathrm{YCE}\ (\%) = \frac{\mathrm{yolk\ \ free\ \ body\ \ dry\ \ mass}}{(1\ \mathrm{dpf\ \ yolk\ \ dry\ \ mass} - \mathrm{yolk\ \ dry\ \ mass})} \times 100$$



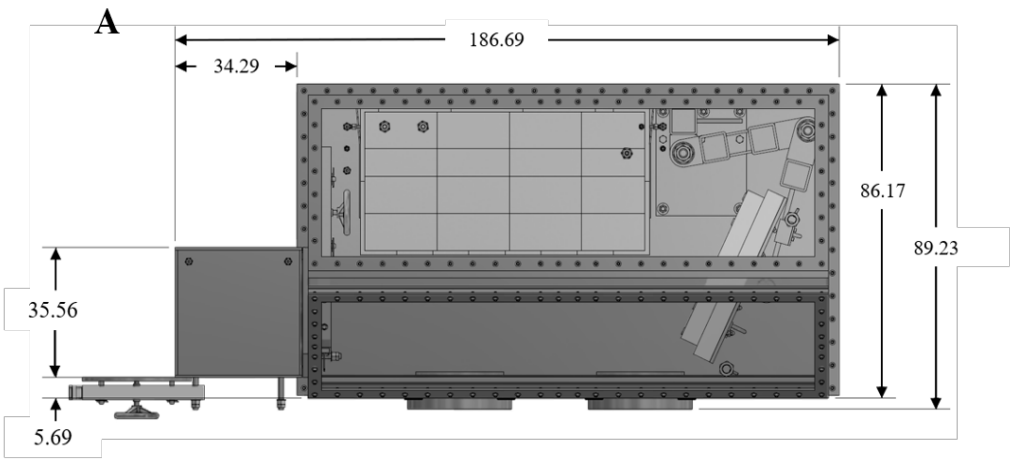

A
1.0
0.5
0.0
Body Mass (mg)
*
*
*
40
60
80
Percent Development
Surface 5°C
Underground 5°C
Surface 3°C
Underground 3°C

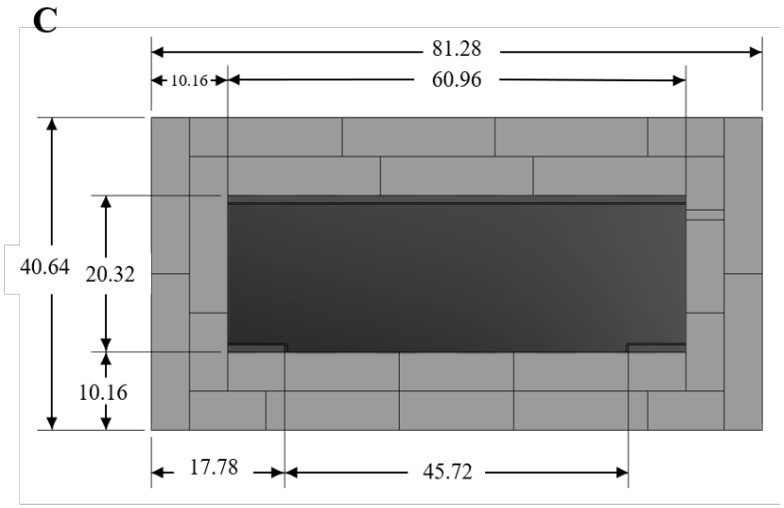

B
12
8
4
0
Body Length (mm)
*
40
60
80
Percent Development
Surface 5°C
Underground 5°C
Surface 3°C
Underground 3°C

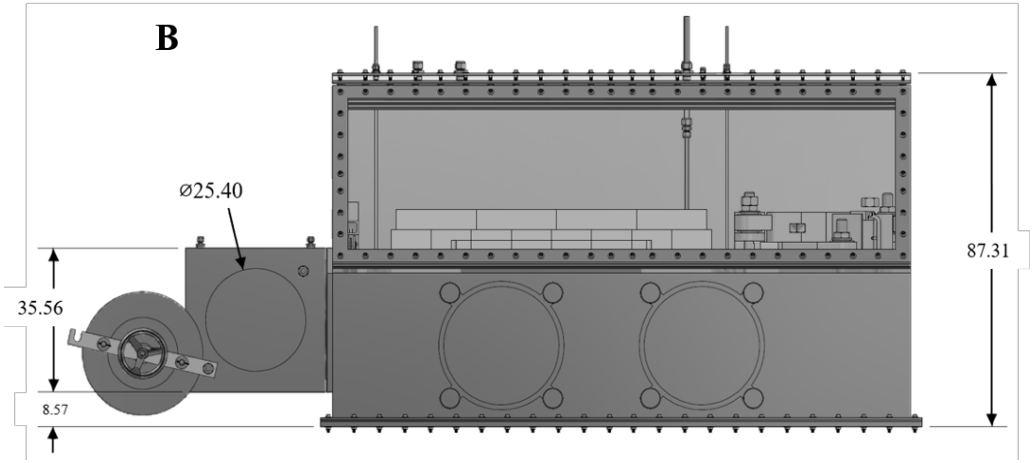

A
2.0
1.5
1.0
0.5
0.0
Yolk Mass (mg)
*
40
60
80
Percent Development
Surface 5°C
Underground 5°C
Surface 3°C
Underground 3°C

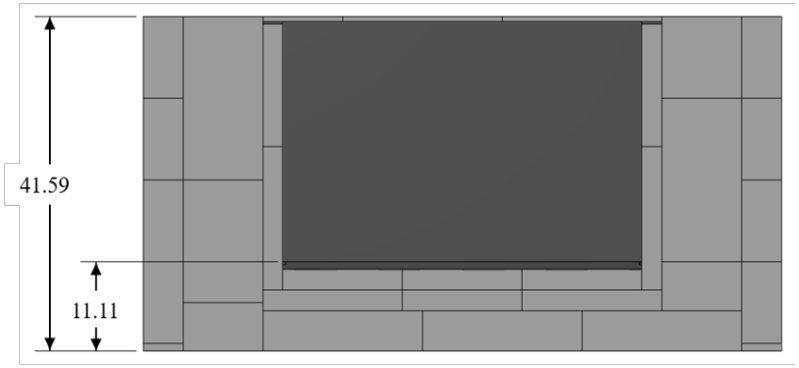

B
6
4
2
0
Yolk Area (mm²)
*
*
40
60
80
Percent Development
Surface 5°C
Underground 5°C
Surface 3°C
Underground 3°C

Sub-Natural Background Radiation
Specialized Tissue Culture Incubator (STCI)









Radiation Protection Dosimetry (2021), pp. 1–10 https://doi.org/10.1093/rpd/ncab120

DOSIMETRIC CHARACTERISATION OF A SUB-NATURAL BACKGROUND RADIATION ENVIRONMENT FOR RADIOBIOLOGY INVESTIGATIONS

Konnor J. Kennedy[1], Alexandre LeBlanc[1], Jake Pirkkanen[2,3,4], Christopher Thome[1,2,3,4,5], T.C. Tai[2,3,4], Robert LeClair[1,3] and Douglas R. Boreham[2,3,4,5,*]

Table 2. Absorbed dose rate calculations for the three radiation environments. Dose rates were calculated in a water scoring volume.

Particle type	Surface control (nGy hr^{-1})	Underground control (nGy hr^{-1})	Sub-background (nGy hr^{-1})
Gamma	5.78 ± 0.03	7.67 ± 0.01	0.0427 ± 0.0013
Neutron	4.52 ± 0.04	0.0045 ± 0.0002	0.00169 ± 0.00002
Muon	55.27 ± 0.40	Negligible	Negligible
^{222}Rn	0.044 ± 0.014	1.45 ± 0.17	0.009 ± 0.011
^{40}K	2.41 ± 0.19	2.41 ± 0.19	2.41 ± 0.19
^{14}C	0.0175 ± 0.0001	0.0175 ± 0.0001	0.0175 ± 0.0001
Low LET[a]	63.48 ± 0.62	10.10 ± 0.20	2.47 ± 0.19
High LET[b]	4.56 ± 0.05	1.45 ± 0.17	0.01 ± 0.01
Total	68.04 ± 0.67	11.55 ± 0.37	2.48 ± 0.20

[a]Low LET = Gamma, Muon, ^{40}K, ^{14}C.
[b]High LET = Neutron, ^{222}Rn.

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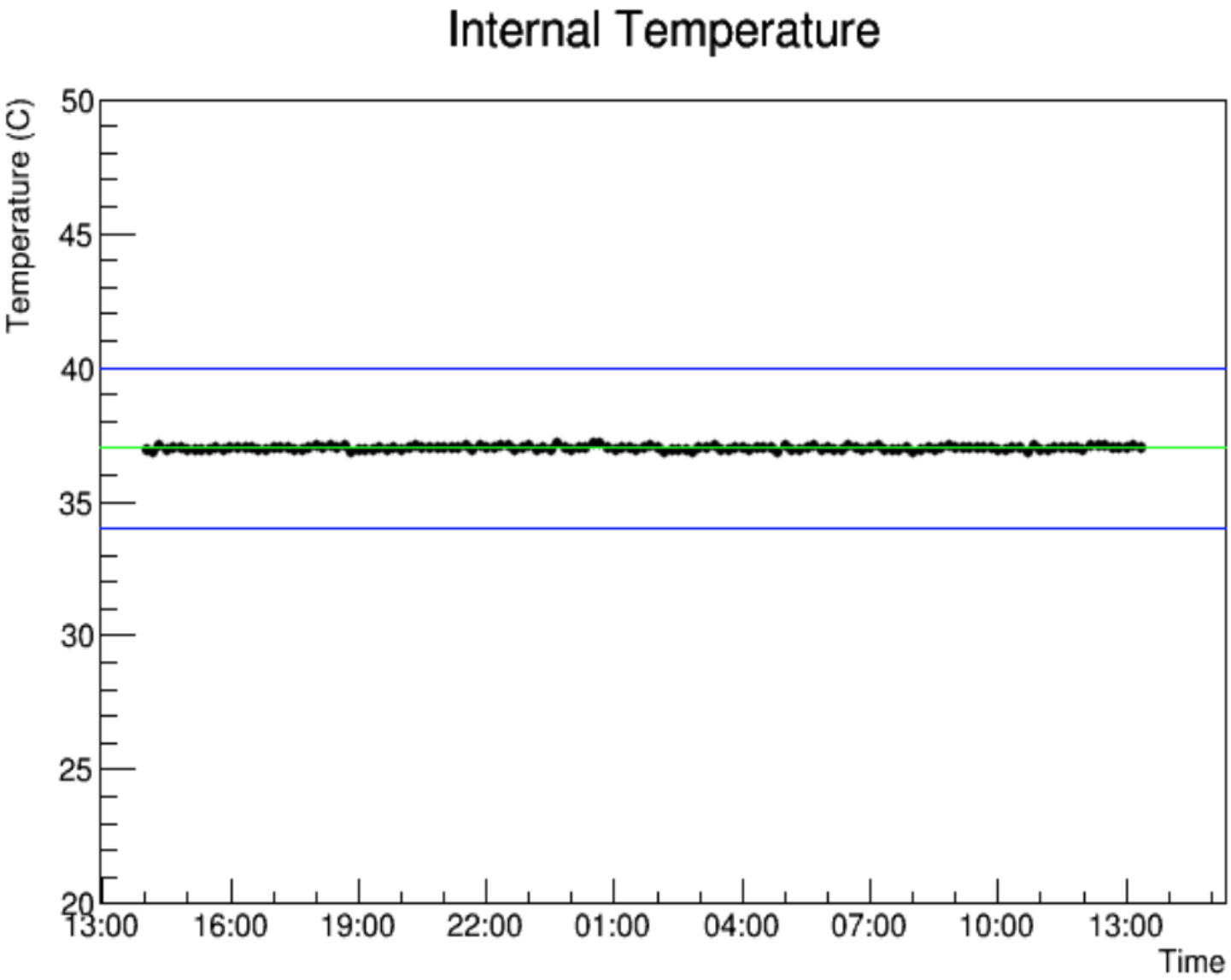
The ^{40}K activity was measured using a high purity germanium detector (HPGe) (Canberra 2011, Canberra Coaxial Detector) at SNOLAB as previously described (23). The sample was left in the detector for 6.819 days, and the count rate was established at the end of the counting cycle.

The ^{14}C measurements were performed at the André E. Lalonde Accelerator Mass Spectrometry Laboratory at the University of Ottawa as previously described (24). Briefly, the sample was combusted **using a Thermo Flash 1112 elemental analyzer**, and the CO_2 trapped within a Pyrex seal. The combusted liquid sample was then analyzed by a Mega Volt tandem accelerator mass spectrometer as outlined in Kieser et al (25).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Temperature (C)
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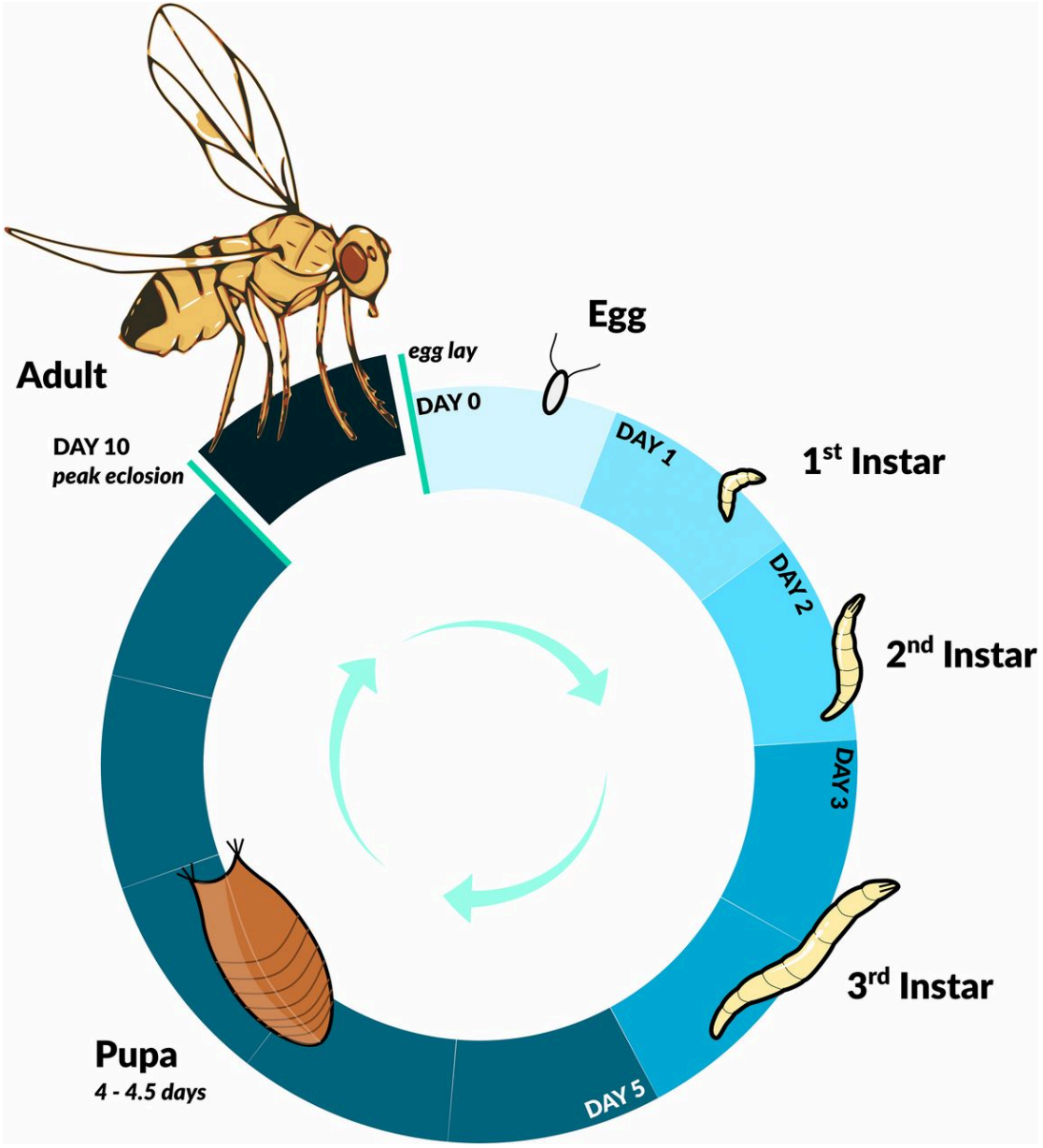
45
40
35
30
25
20
13:00
16:00
19:00
22:00
01:00
04:00
07:00
10:00
13:00
Time


Adult
egg lay
DAY 0
Egg
DAY 1
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DAY 2
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DAY 3
3rd Instar
DAY 5
Pupa
4 - 4.5 days
DAY 10
peak eclosion