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 Biological Investigation Deep Underground

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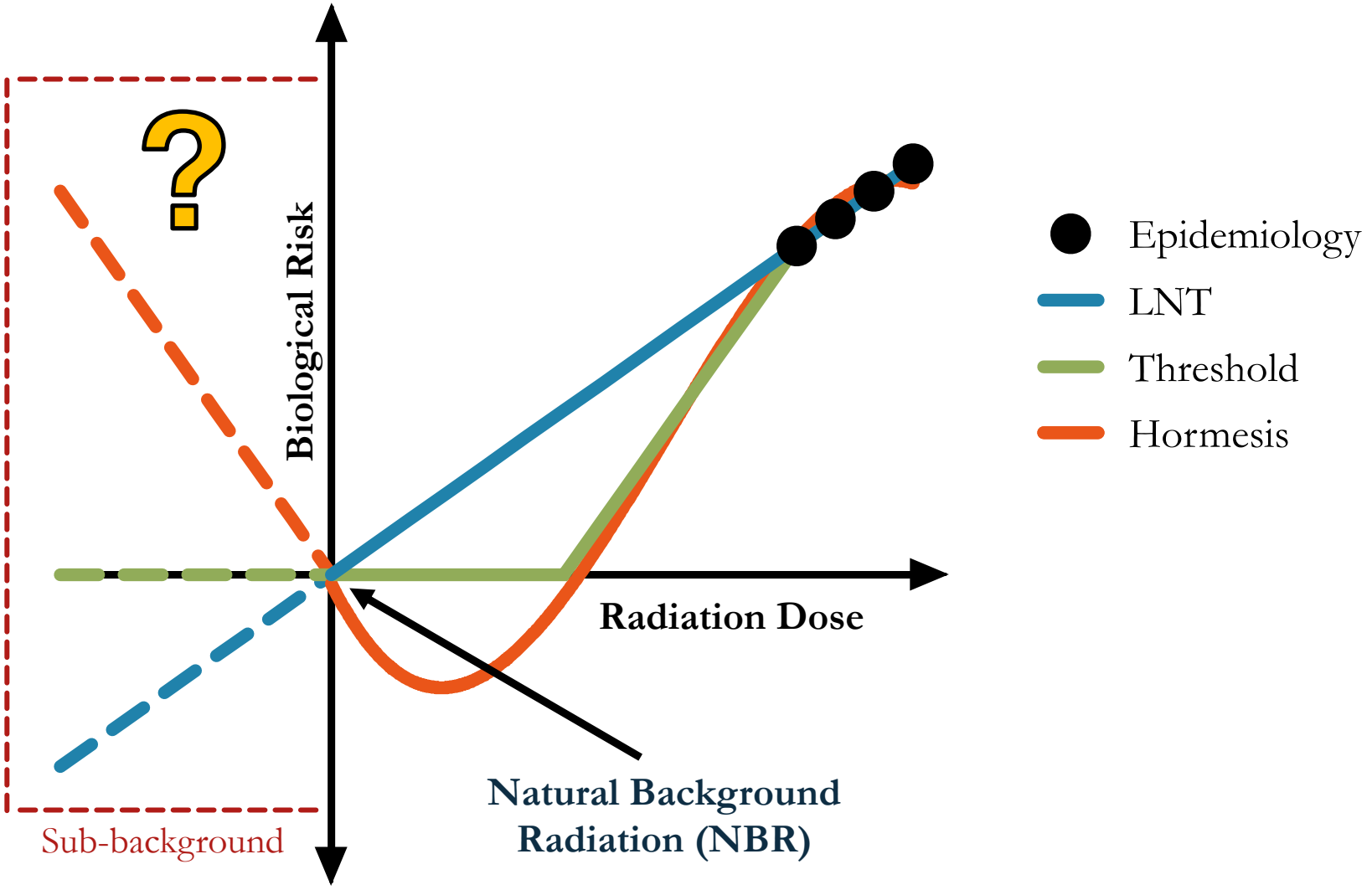
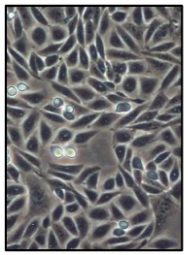
Laurentian University, Northern Ontario School of Medicine

SNOLAB Future Projects Workshop
May 11th, 2021

Radiobiological models of risk



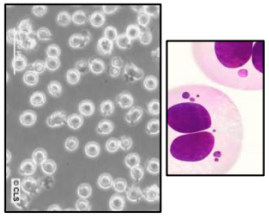
Previous sub-NBR work



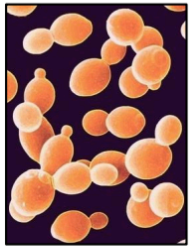
Removal of NBR impairs growth. This effect is ameliorated when NBR is artificially re-introduced.

- Paramecium shielded with lead (Planel *et al* 1976)

- Blue-green algae (*Synechococcus lividus)* shielded with lead (Conter *et al* 1983)
- Yeast (*Saccharomyces cerevisiae*) shielded with lead/cadmium (Gajendiran and Jeevanram 2002)
- Bacteria (*Deinococcus radiodurans)* grown in WIPP (Smith *et al* 2011)

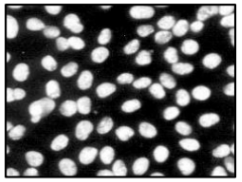


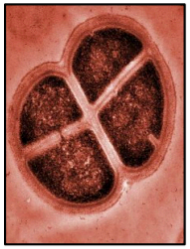
- Mouse lymphoma L5178Y cells shielded with lead or iron (Taizawa *et al* 1992, Kawanishi *et al* 2012)



Removal of NBR impairs repair capacity of induced damage.

- Survival fraction in yeast (*Saccharomyces cerevisiae*) shielded with lead/cadmium (Gajendiran and Jeevanram 2002)





- Background and induced mutation rate in Chinese hamster V79 cells grown in Gran Sasso Underground Laboratory (LNGS) (Satta *et al* 2002)
- Micronuclei formation and ROS scavenging in human lymphoblastoid TK6 cells grown in LNGS (Carbone et al 2010)

Hypothesis

Natural background radiation has an essential biological role and helps to maintain genomic stability

Prolonged exposure to a sub-natural background radiation environment will be detrimental to living biological systems

Where can this question be empirically investigated?

The ideal radiologically "quiet" environment for sub-NBR radiobiology studies

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

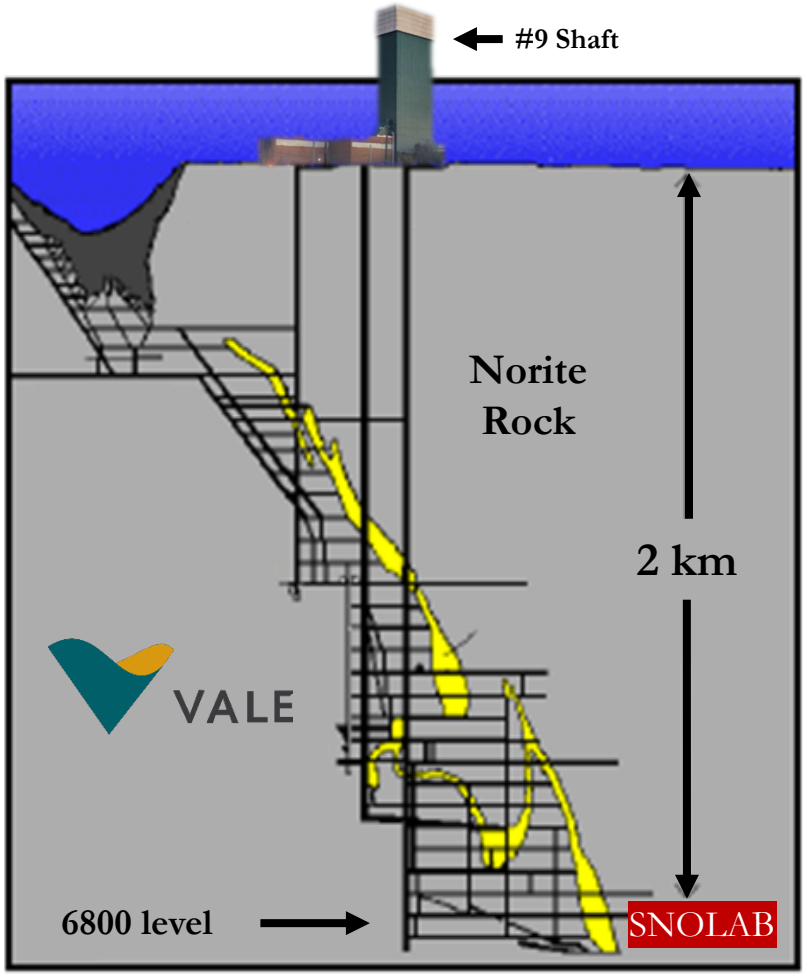
2 km (6,800 ft) underground
(6 km water equivalent)

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

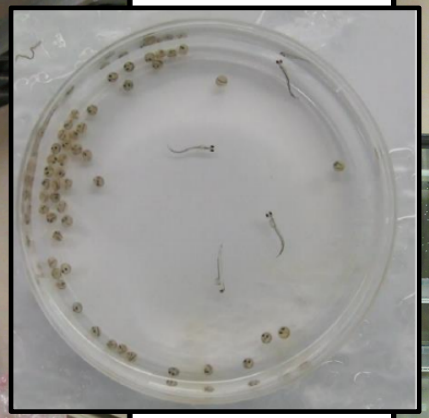
Class 2000 clean room
(less than $2x10^3$ particles >0.5µm per ft^3)

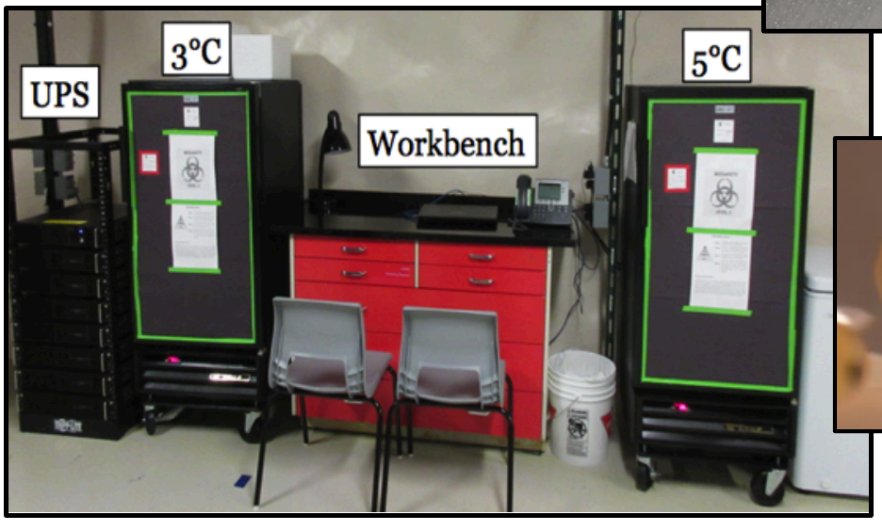
$5x10^7$ reduction cosmic radiation
(shielded by rock overburden)

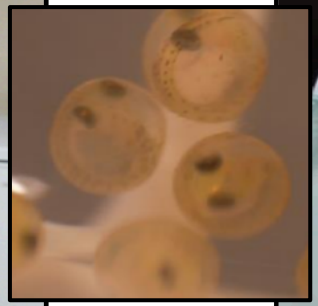
HEPA filtration of 50 m^3 s^{-1}
(10 full lab air exchanges per hour)



REPAIR Est. 2015







Initial "bio-logistical" pilot project

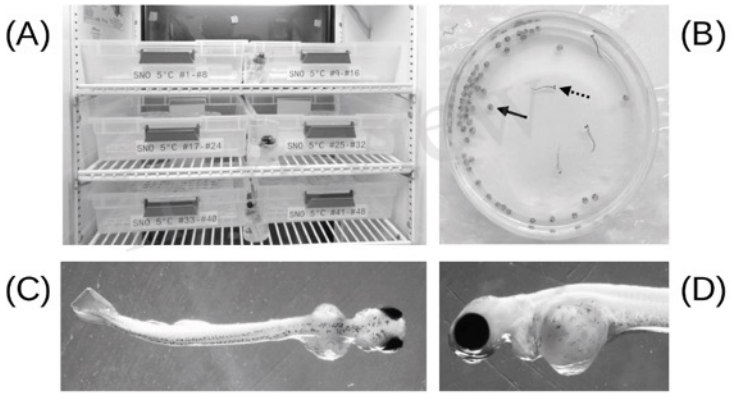
Frontiers In Earth Science - Special Edition:
The Biogeochemistry, Biophysics, Radiobiology, and Technical Challenges of Deep Subsurface Research

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*]

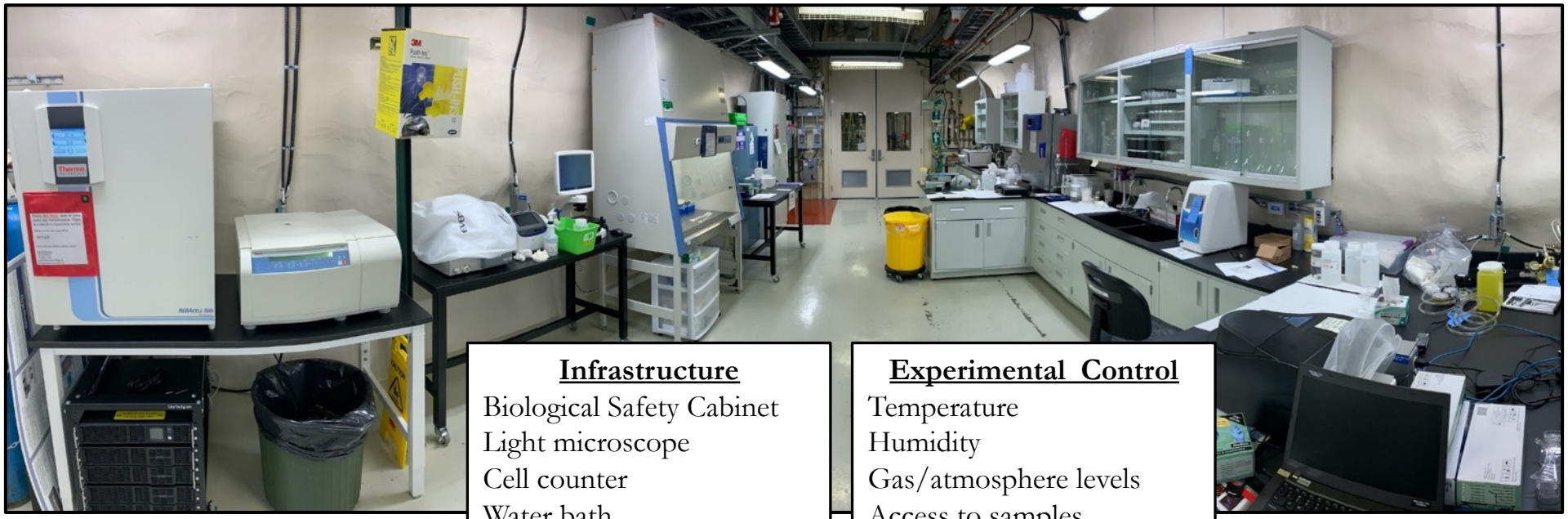
[1] Department of Biology, Laurentian University, Sudbury, ON, Canada, [2] Biomolecular Sciences Program, Laurentian University, Sudbury, ON, Canada, [3] Department of Biology, Lakehead University, Thunder Bay, ON, Canada, [4] Medical Sciences Division, Northern Ontario School of Medicine, Thunder Bay,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



From REPAIR'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

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

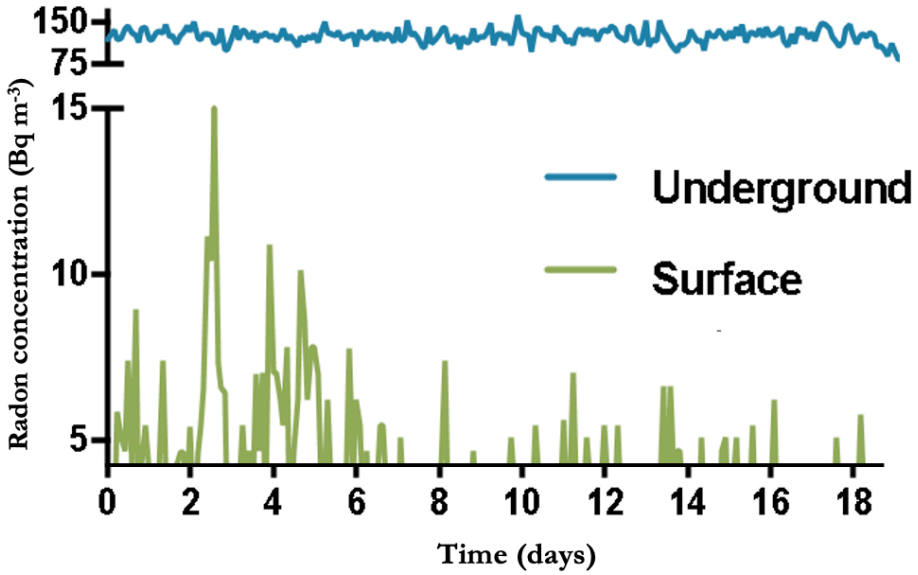
Biological Safety Cabinet
Light microscope
Cell counter
Water bath
Centrifuge
Tissue culture incubator
UPS

Experimental Control

Temperature
Humidity
Gas/atmosphere levels
Access to samples
NBR constituents

The radon "hurdle"

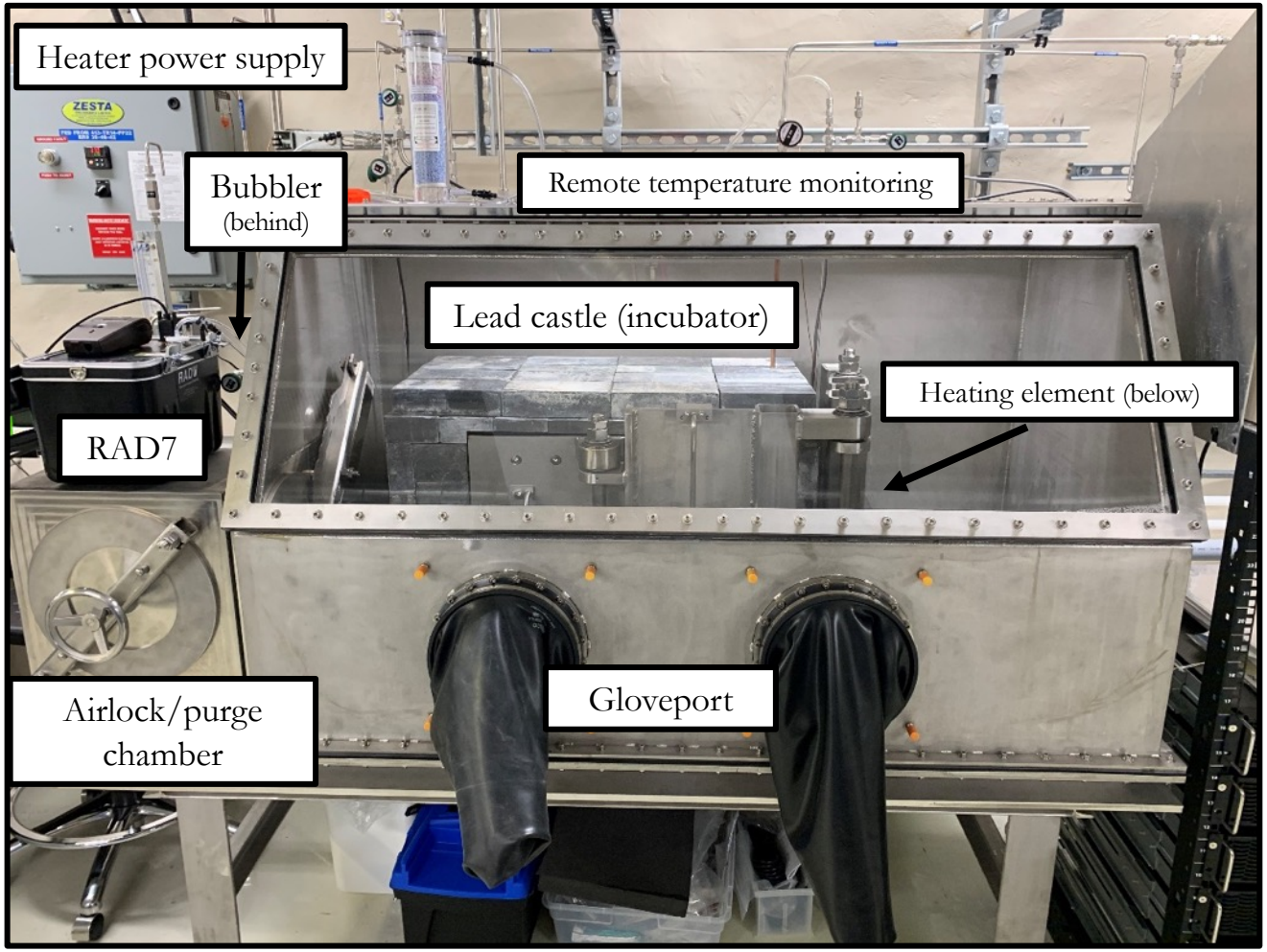
Radon ($t_{1/2}$ 3.8d) levels are significantly elevated deep underground compared to the surface, and represent a significant experimental contaminant for sub-NBR studies

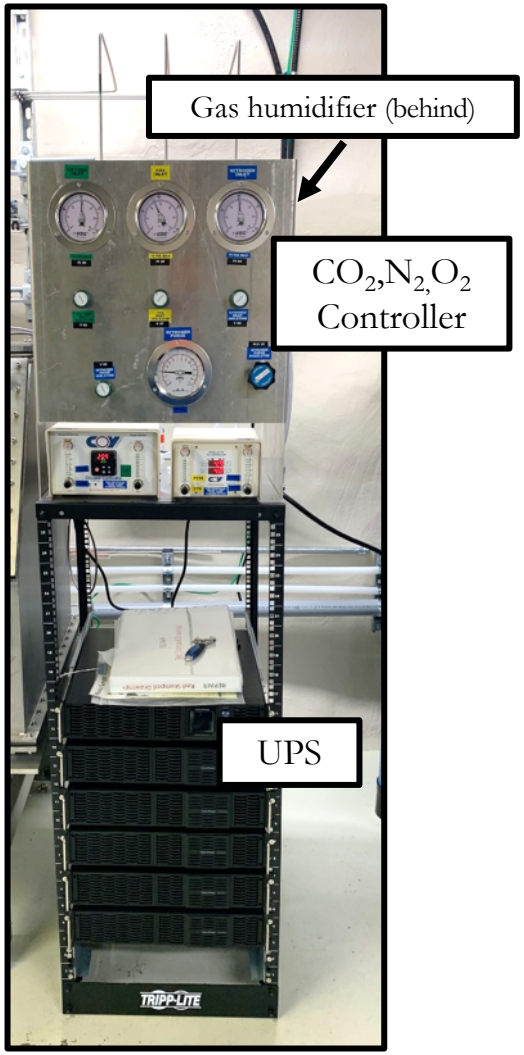


Gas cylinders (CO_2, N_2, O_2) used in biological sample maintenance are aged underground for a minimum of one month

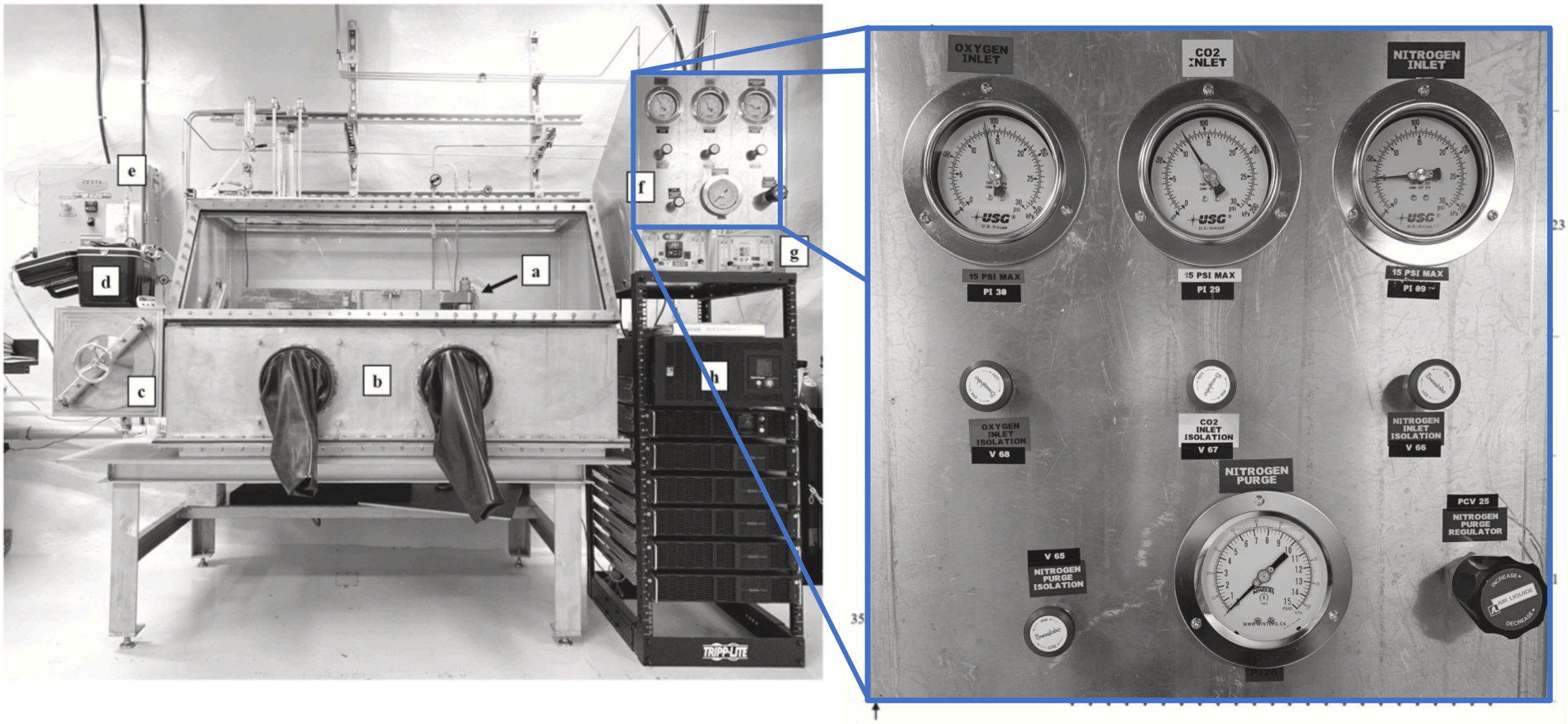
It was necessary to engineer and construct an instrument capable of maintaining our biological samples as well as reducing additional components of NBR (notably ^{222}Rn)

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





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



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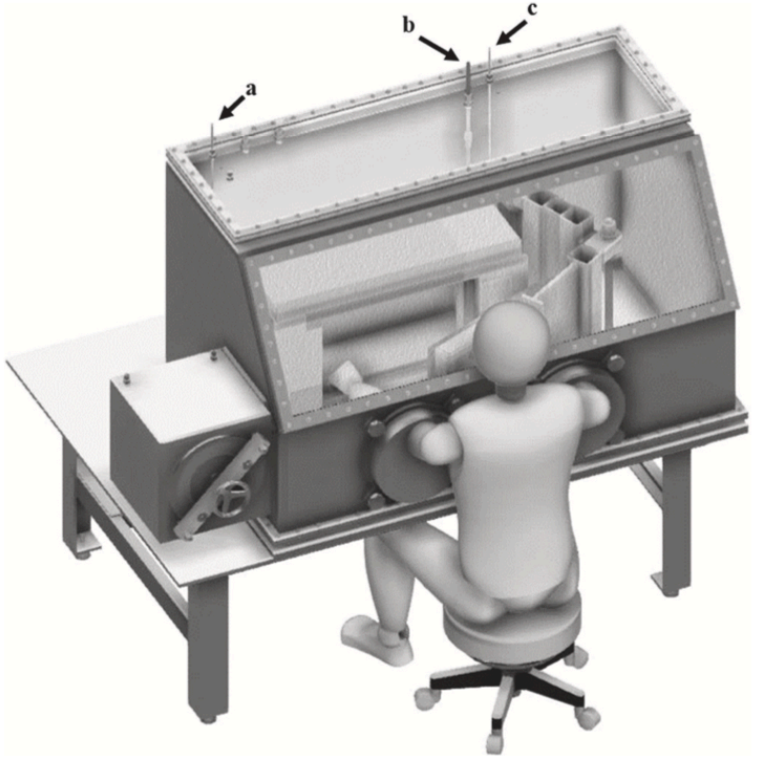
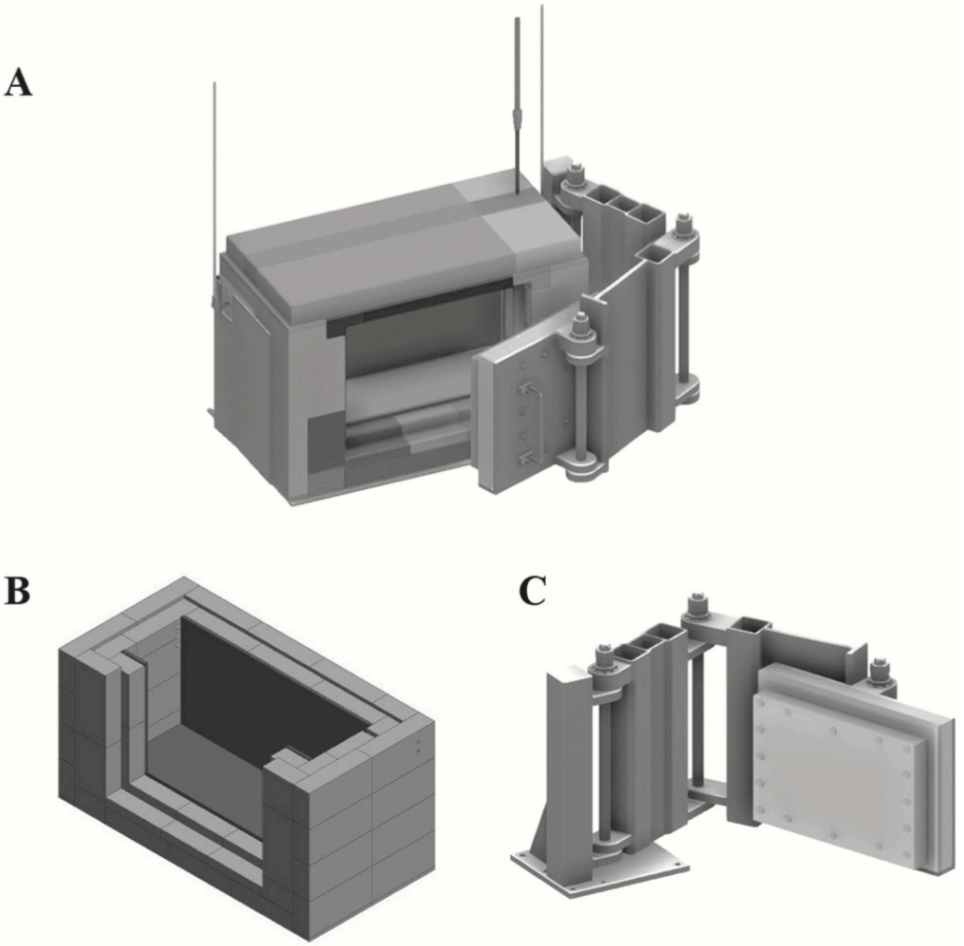
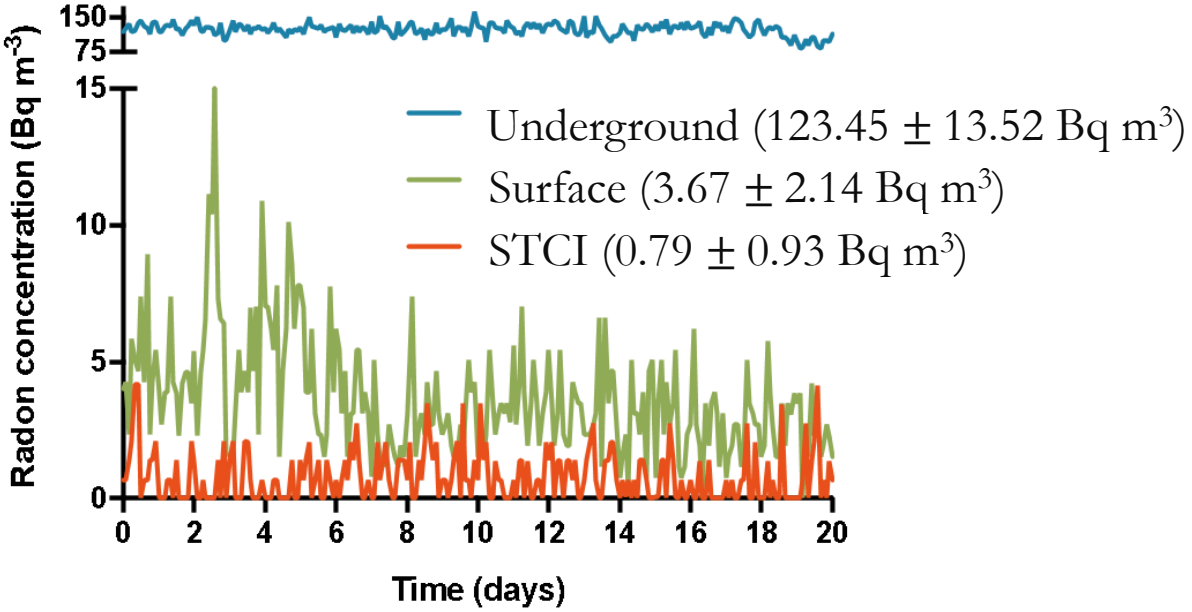
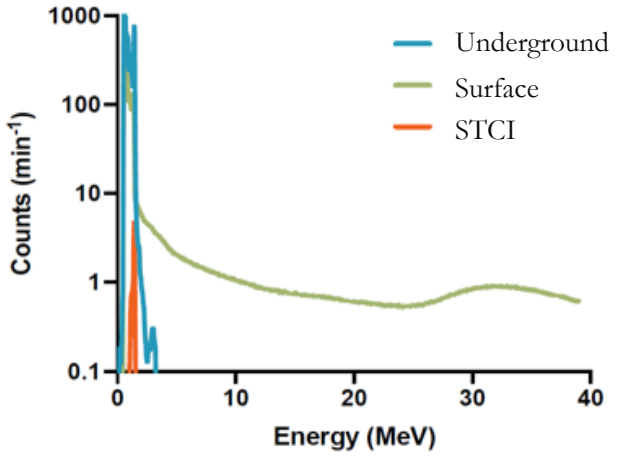
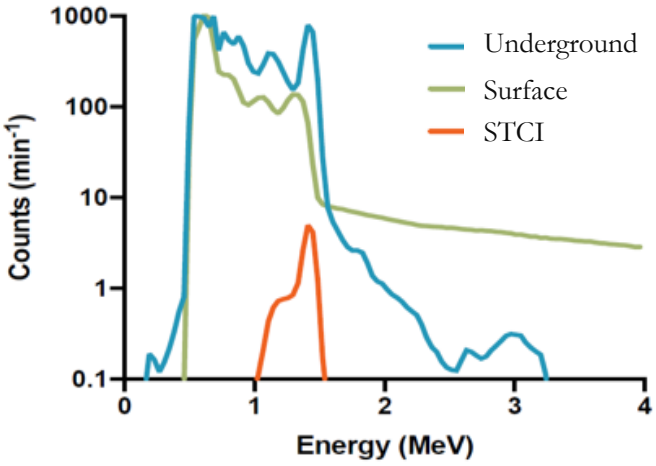




Sub-Natural Background Radiation Specialized Tissue Culture Incubator (STCI)







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)

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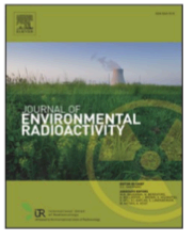
journal homepage: http://www.elsevier.com/locate/jenvrad

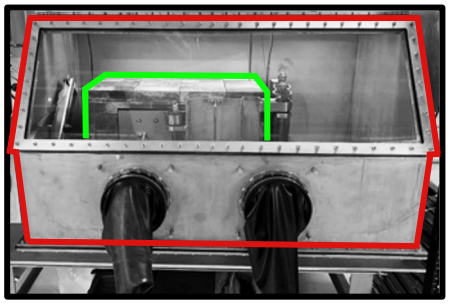


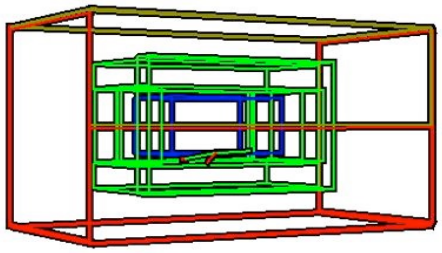
A novel specialized tissue culture incubator designed and engineered for radiobiology experiments in a sub-natural background radiation research environment

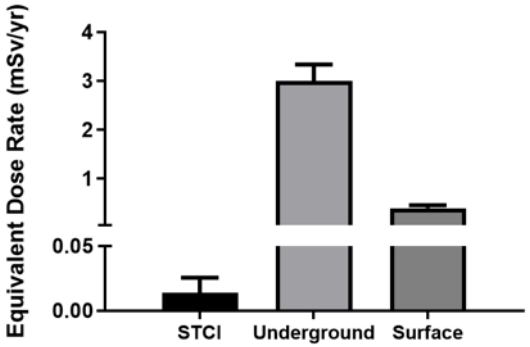
Jake Pirkkanen [a,b,c], Taylor Laframboise [a], Peter Liimatainen [d], Tom Sonley [d], Stephen Stankiewicz [d], Mike Hood [d], Mehwish Obaid [d], Andrew Zarnke [b,c], T.C. Tai [a,b,c], Simon J. Lees [e,f], Douglas R. Boreham [a,b,c,g,h], Christopher Thome [a,b,c,h,*]

Natural Background Radiation Characterization

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



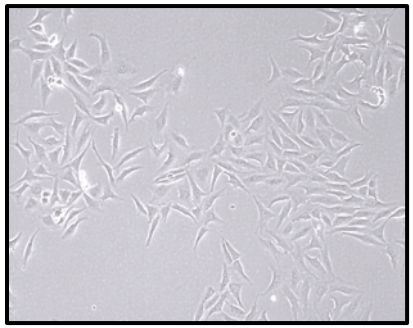




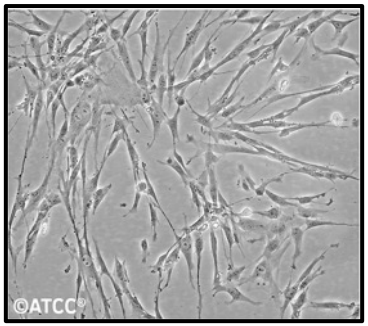
"Dosemetric characterization of a sub-natural background radiation environment for radiobiology investigations" Currently in review: Radiation Protection Dosimetry

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

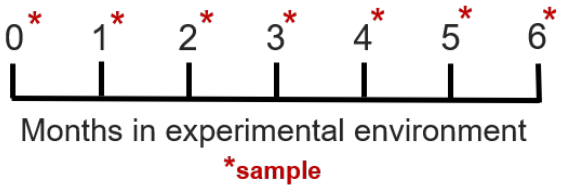


Mammalian cells



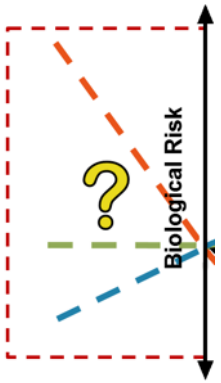
Yeast

Nematode worms

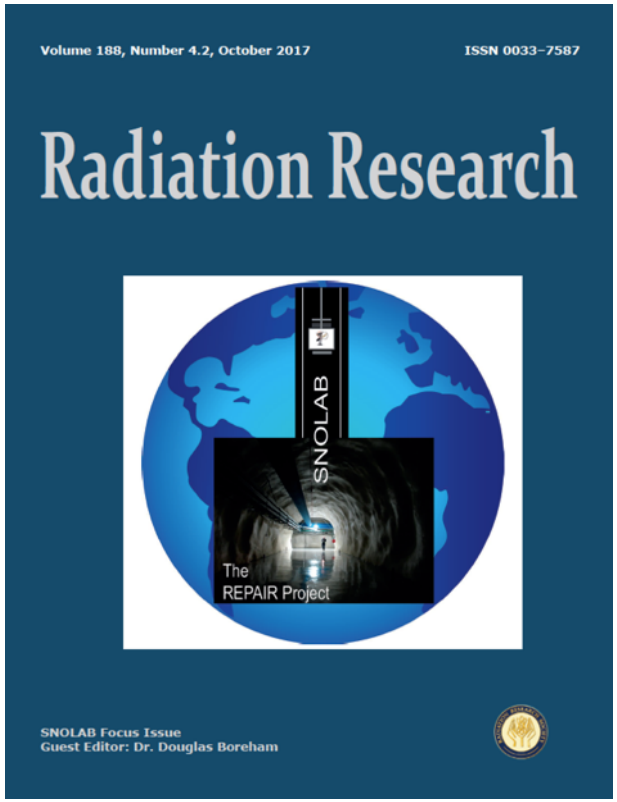


- Baseline response
- Radiation challenge

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



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

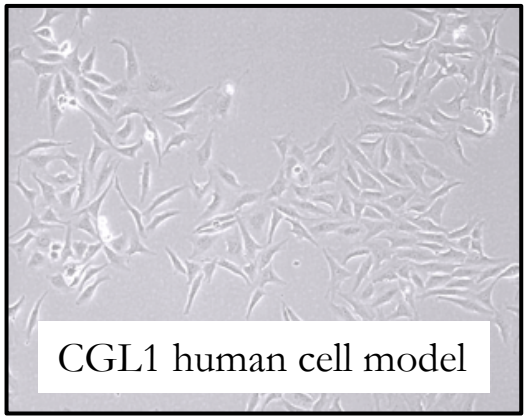
Jake S. Pirkkanen,[a,1] Douglas R. Boreham[a,b,c] and Marc S. Mendonca[d,2]

[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*

Current experimental progress

Data are currently being analyzed from our first 4-month protracted exposure experiment. Human cells were cultured at the surface and underground, and every month assayed for:

- Growth rate
- Radiation challenge survival
- Enzymatic activity
- Gene expression
- Invasion
- Migration
- Adhesion



CGL1 human cell model

Cells incubating underground within the STCI's lead castle

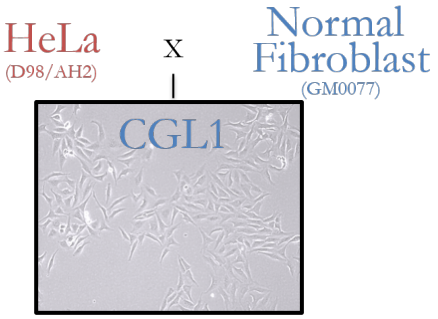
Current experimental progress

The REPAIR Project currently has two funded 3-year Mitacs Accelerate Industrial Post-Doctoral Fellowships

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

  Supervisor: Dr. Christopher Thome (NOSM)

  Status: 4-month experiment completed, preparing to replicate



GW-3 Operations Approval for nematode worm and *C. elegans* experiments: March 2021

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

  Supervisor: Dr. Sujeenthar Tharmalingam (NOSM)

  Status: C. elegans officially underground as of May 7th, 2021!

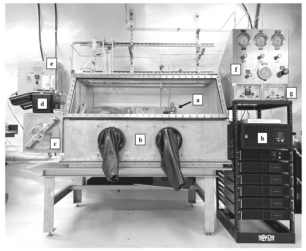
- The Role of anhydrobiosis on yeast in a sub-NBR environment

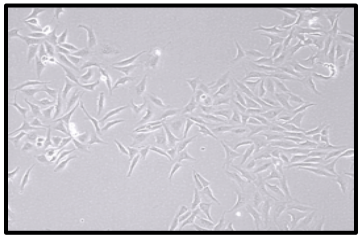
  Status: Preparing samples at surface for relocation underground

Current experimental progress

Category	Metric	Reporting Year (2019-2020)
Canadian Institutions	# of Canadian Academic Institutions	1
	# of Canadian Industrial/Private Institutions	1
International Institutions	# of International Academic Institutions	2
	# of International Industrial/Private Institutions	
Canadian Collaborators	# of faculty/scientists	5
	# of technicians, engineers, technologists	2
	# of Post-Doctoral Fellows/Associates	2
	# of Doctoral students	2
	# of Masters students	2
	# of Undergraduate students	
International Collaborators	# of faculty/scientists	3

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





REPAIR

Thank you for your time!

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