

# eureka

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Undergraduate Science Journal

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**FEATURING**

**Dr. Shereen Hamza**

*Teaching through a  
new lens*

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**Eureka Journal**

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*Eureka* is a student-founded and student-run initiative.

This journal offers undergraduate scientists the unique opportunity to share their discoveries with the scientific world through the peer review process. *Eureka* is an educational institution, with a diverse team of reviewers from many scientific backgrounds, exposing undergraduate students to the publication process often excluded from their traditional scientific education. Through clear and effective scientific writing, students will be able to work together with faculty members to contribute original research and promote science.

Our mission is to support and promote quality peer-reviewed research by undergraduate scientists across Canada.

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## From Gavin Bradley: *Jurassic Park* and Making Space for Your Art

November 2023

The question that every dinosaur paleontologist gets asked more than any other is, undoubtedly: “*could Jurassic Park really happen?*” I’ve been in the field for about 10 years now, first as a graduate student fresh off the plane from my home in Northern Ireland, and now as the enthusiastic instructor of our big, undergraduate MOOC<sup>1</sup>-based paleontology classes for the past five years. You might remember me from such courses (Paleo 200, 201, 202, 203 & 204). During that time, I’ve heard it hundreds of times from students, colleagues, friends, family— and most frequently from grade school students, when I do outreach talks for groups like our Science Summer Camps. You would think, by now, I’d be tired of it.

The thing is, though, it’s a *really* good question.

I mean, who doesn’t want to know if we could one day pop around the Edmonton Valley Zoo and check out the *Tyrannosaurus* paddock, or pet a baby *Chasmosaurus*? Maybe even go down to the humane society and adopt an *Ankylosaurus*?



Unfortunately, for those who are wondering, the short answer is no. We can’t resurrect dinosaurs (at least not in the “mosquito fossil with dino blood” method in *Jurassic Park*). However, every time someone asks me that question, I get to introduce them to concepts that I love teaching about, like deep geological time, the atomic clock, or the fossilization process, and point them towards some revolutionary fields where researchers approach the problem from a different direction. Like Evolutionary Developmental Biology, where they are attempting to “reverse engineer” modern birds into dinosaurs.

Another thing that I like about that question? *Jurassic Park* is, to me, a perfect blend of art and science. The science is present enough that people ask themselves “*could* that actually happen?”, and the fiction is engrossing enough that people wonder “*what if* that actually happened?”

As a writer of poetry and fiction in my non-academic life, I have been balancing art and science since my undergraduate degree and was lucky enough to publish my first book of poems, *Separation Anxiety*, in 2022. Poetry and paleontology might seem like polar opposites, but to me, they are simply two sides of the same coin. Both are ways of understanding the world around us, both give you an unusual vocabulary, and both require an imagination.

You see, no matter what data we gather from the dinosaur fossil record, we can never go back in time and see these incredible animals as they were in life. Although we can interpret the Dry Island *Albertosaurus* bonebed as evidence for social behavior in tyrannosaurs, we can’t see the pack work together to take down large prey. Although we can recreate the sound that *Parasaurolophus* made through its hollow head crest, we can only picture the effect a cacophony from a whole herd of the giant herbivores would have had on an approaching carnivore! At the end of many long days in the field and even longer nights in the lab working with fossils, a paleontologist still must rely, at least a little bit, on a touch of creative thought to connect the present to the past.

Paleontology aside, any undergraduate program in science is demanding, and when you're in the thick of it there's a temptation—maybe even a pressure— to focus on your subject and only that for the duration of your studies. During grad<sup>2</sup> school I was so worried about what my new lab mates and supervisor might think of my creative writing that I published my first poems under a pseudonym. I was already a gangly, red-headed Irishman with a cartoonish accent, coming from a totally different academic background— I didn't think I needed another reason not to be taken seriously! Despite that, as I kept writing and publishing, and met more scientists with interests in the arts— musicians, visual artists, actors —I became less and less worried about what people thought. I was more and more convinced that my hobby was making me a better-rounded scientist. In fact, when I got my first job out of grad school writing and editing content for the paleontology MOOCs, it was as much for my ability as a writer as it was for my expertise in fossils. The truth is, in the great, big scary question mark that comes after graduation, the more strings you have to your bow, the more appealing and unique your application will be for employers or grad schools.

More important than helping your future job prospects, though, is the art of doing something in your free time that has nothing to do with your program, simply *just to do something that has nothing to do with your program*. This time of year is rough, and part of self-care is making space in your life for things you enjoy and that give you satisfaction beyond school.

Part of self-care can be making space for your art.

This doesn't mean you have to become a poet. Maybe you pick up the guitar you've neglected for a while. Open your old sketch book and do some tracing. Finish crocheting that scarf you started in your first week of undergrad. Watch the film you've been meaning to see, or read the book a friend recommended, or go dancing, or pottery-making or just go belt out Disney songs at RATT<sup>3</sup> karaoke. When you make space for your art, regardless of whatever art you choose, you're able to give the rest of your brain a break and recharge yourself for whatever comes next.

The fact that you, busy undergraduate students, have found the time to create *Eureka* is an incredible achievement, and a testament to your ability and determination to make the most of your time here. But somewhere between studying, working, and putting together undergraduate science journals, I hope you make some time to pick up an old hobby or discover a new one; perhaps it'll bring you a “*Jurassic Park*” blend of your own.

Cheers,

**Gavin Bradley**

Instructor, Earth and Atmospheric Sciences

Science MOOC Coordinator, Faculty of Science - Earth and Atmospheric Sciences

University of Alberta

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<sup>1</sup> MOOC — Massively Open Online Course

<sup>2</sup> Graduate

<sup>3</sup> RATT — Room At The Top



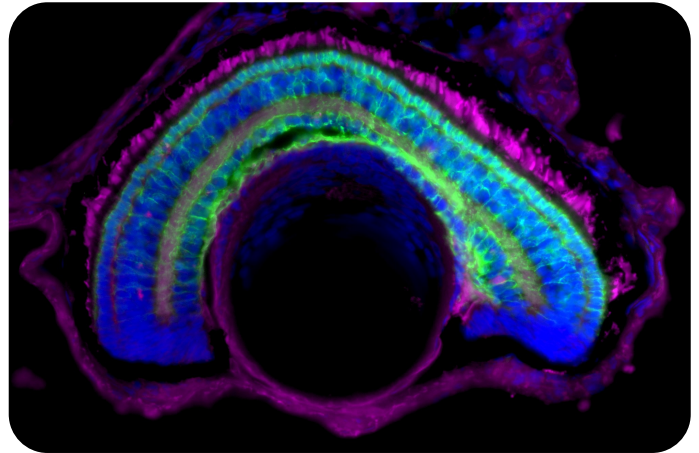
Allison Tran is an undergraduate student in the Faculty of Science who is studying biology. Her image titled *Sagittal section of a young *Xenopus laevis* retina labelled with fluorescent markers for nuclei, photoreceptor outer segments, and polysialic acid* is featured on the front cover of this issue.

### Have you made any interesting findings?

My project's objective was to investigate the effects of 8-keto-Neu5Ac, a polysialic acid (polysia) inhibitor, on the embryonic development and retinal neurogenesis of *Xenopus laevis* tadpoles. We hypothesized that if 8-keto-Neu5Ac prematurely terminated the natural binding activity of a polysialic acid named N-acetylneuraminic acid (Neu5Ac), then we would observe growth and retinal defects during tadpole development. Specifically, we hypothesized that 8-keto treatment would result in defects in photoreceptor outer segment development, due to the strong labelling of outer segments with wheat germ agglutinin (WGA), which binds to Neu5Ac and its precursor molecule, GlcNAc. To specifically locate and label Neu5Ac, we used a lectin called GFP-EndoN, which is a modified hydrolase for neuraminic acid with a fluorophore attached; we expected the fluorescence labelling to be found in the photoreceptor outer segments, like WGA. Although we did not observe a clear effect of 8-keto-Neu5Ac on the physical or retinal development of *X. laevis* tadpoles, we were amazed to find that the GFP-EndoN signalling was absent from the photoreceptor outer segments, and that it was instead strongly expressed in the Müller glia of the retina. Müller glia are the major glial cells in the retina that are tasked with supporting retinal neurogenesis and homeostasis. In summary, our fluorescence histochemistry strongly indicated that Neu5Ac is significantly more active in the Müller glia rather than the photoreceptor outer segments of the retina.

### Why is your research important?

Unlike many other vertebrate animals, existing literature supports that Neu5Ac is the only known polysialic acid expressed in humans due to their inability to convert Neu5Ac into another polysia, N-Glycolylneuraminic Acid (Neu5Gc). However, our current knowledge surrounding Neu5Ac, sialic acids, and consequently their natural



functions in Müller glia is limited. From the results of my project, we were able to specifically label the localization of Neu5Ac activity in the retina with GFP-EndoN. Following our discovery of Neu5Ac being more actively involved with Müller glia as opposed to the photoreceptor outer segments, our understanding of Neu5Ac's functional relationship with different retinal structures has improved. Reversely, we have also gained some insight into the functioning mechanisms of Müller glial cells using sialic acids. The results of our study narrow down the approach for future research in hopes of fully understanding the interactions between sialic acids and Müller glia, and their effects and roles in embryonic development, retinal development, and retinal neurogenesis.

### What part of research do you enjoy most?

The main reason why I decided to step into scientific research in my undergraduate years was because I had a passion for the science behind vision, which is arguably one of the most crucial human senses. I was excited to gain first-hand experience and learn more about this field. In Dr. Brittany J. Carr's lab, I learned a multitude of lab skills including fluorescence histochemistry, image analysis, and proper housing procedures for our *X. laevis* tadpoles. My favourite pastimes in that lab were caring for the tadpoles and capturing fluorescence images of the retina. In addition to these hard skills, my skill repertoire has also

expanded with a variety of soft skills, such as planning experimental designs, troubleshooting experimental designs, data analysis, and effectively communicating with other individuals in the field. Lastly, meaningful discoveries are never guaranteed; therefore, it always feels amazing when our laborious efforts are awarded new knowledge to advance our fields of interest.

**Do you believe all students can benefit from carrying out scientific research regardless of their educational backgrounds? If so, why?**

Regardless of their backgrounds and interests, I believe that anyone would benefit from participating in professional research at least once. The entire research

process is filled with trials and tribulations from both known and unknown variables, so growing one's character and expanding one's knowledge will always be inevitable. In other words, working in a lab over the summer of 2023 not only left me with lab-specific techniques but also with people whom I can connect to their shared interests, versatile problem-solving skills and a newly built confidence in myself. The benefits that I have listed here are only a few of the many, but they can be applied to almost every aspect of our daily lives. Furthermore, if someone can conduct scientific research and possibly advance the field that they are passionate about, then this achievement makes the other benefits they have received feel more rewarding.

Nicol San Juan is an undergraduate student in the Faculty of Science who is studying biochemistry. Her image titled *Now You See Me* is featured on the back cover of this issue.

### Tell us about your research!

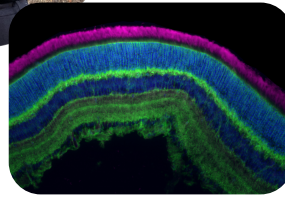
My lab (the Carr Lab) studies inherited retinal degenerations using genetically modified frogs as an animal model. Frog photoreceptors are much larger than other species, so dystrophy is easier to spot in frogs versus mice or rats. Age-related Macular Degeneration (AMD) is an inherited retinal degeneration, and *prom1*-null frogs develop a retinal degeneration similar to humans. A challenge with studying the development of AMD-like disease in frogs is how long it takes them to grow significantly large deposits of cellular debris, which cause retinal degeneration. I started in the lab as a summer student, where my project used flickering light to accelerate the shedding of outer segment tips and speed up retinal degeneration.



Now, for my BIOCH499 project, in the same lab, I am working on analyzing retinal polysialic acid expression and photoreceptor morphogenesis in various species. Polysialylated-neural cell adhesion molecule (PSA-NCAM) is expressed in the embryonic retina, and its expression becomes restricted in the adult retina, but the relationship between PSA-NCAM and the expression of Neu5Ac in Müller glia is unknown. The Willis Lab created a GFP-EndoN lectin, which was supposed to label photoreceptor outer segments but unexpectedly labelled the Müller glia. I've performed experiments testing GFP-EndoN in other species (mice, zebrafish, chicken and rats) at embryonic and adult stages to see whether the expression of Neu5Ac in Müller glia is consistent across species and developmental timescales. I use techniques such as immunohistochemistry and fluorescence light microscopy. Currently, I am working on creating a CRISPR deletion of the Neu5Ac polysia chain elongation enzyme (ST8SIA).

### Do you have any words of wisdom for aspiring undergraduate researchers?

Don't be afraid to try other labs, even if it seems beyond your current scope of knowledge. You'll never know what might pique your interest. Last summer, I applied into a microscopy-focused lab with no prior experience. Venturing into a new discipline allowed me to discover a genuine passion for microscopy that I might not have uncovered if I had stuck strictly to my undergraduate discipline (biochemistry). The best part of being an undergraduate in research is getting the opportunity to explore different research areas, and many research areas end up overlapping. Every research lab offers a range of opportunities and teaching styles, and being in a research lab allows you to get one-on-one mentorship



from your direct supervisor. Whether delving into wet or dry labs, working with cell cultures, or engaging with live organisms, experiencing these diverse environments firsthand is the most effective way to determine your preferences and interests.

### What part of research do you enjoy most?

What I enjoy the most about research is the challenge of learning about what has yet to be discovered. Research constantly presents puzzles that demand innovative solutions, pushing me to delve into literature, experiment with new methodologies, and think critically using knowledge from previous courses. I enjoy bench work, and there is no better feeling in this world than when you finally master a particular technique. I am finally at a point in my career where content learned from previous classes carries over into my experiments. I also love how everyone in research has different backgrounds and different areas of expertise. I enjoy the discussions during lab meetings

and listening to other approaches to a technique. I'm most interested in CRISPR-Cas9 (a form of gene editing) and am starting to learn how to set up CRISPR injections for my BIOCH499 project. Thanks to my PI, Dr. Carr, who is very supportive and considers my future goals when we discuss project design. She takes the time to explain and walk me through why we experiment a certain way; she's why I enjoy coming into the lab to do research.

**Tell us about a moment that has stood out to you in your research career!**

One of the best and unique parts about being in my current lab is that we share our space with the two other ophthalmology labs. This collaborative environment has enriched my research experience by providing opportunities to witness and engage in techniques beyond

the scope of my primary project. One afternoon, individuals from the neighbouring labs were dissecting cow eyes to collect photoreceptor outer segments. While my primary research focus is on *Xenopus laevis*, participating in dissecting a cow's eye was a fantastic experience. An interesting detail that many people may not know is that when working with eyes/photoreceptors, we work in the dark (using a red light) so that we do not photobleach the retina. That afternoon, I got to go into the dark room, and they let me dissect one of the cow eyes by myself. They also answered any questions I had about eye anatomy. I also got to enhance my fine motor skills by separating the different parts of the eye. Everyone in the Carr, MacDonald and Benson labs have been so encouraging, and they're always there to help me when I have questions. I am very grateful for all of them.

# Proteomic Analysis of Higher and Lower Altitude Cultivars of *Coffea arabica* Reveals Differences Related to Environmental Adaptations and Coffee Bean Flavour

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## ABSTRACT

**Background** Coffee ranks among the most popular beverages in the world and is considered an important global commodity, contributing substantially to the economies of many developing nations. Coffee beans are mainly harvested from two species of coffee plants, *Coffea arabica* and *Coffea canephora*, of which the former species produces beans which are almost universally considered to be superior due to their rich and balanced flavour. *C. arabica* is also known to be less resistant to disease and more sensitive to climatic variables such as temperature, precipitation, and oxygen availability, each of which vary with altitude.

**Methods** To better understand the impact of altitude on coffee plants, we performed a comprehensive proteomic comparison of two distinct cultivars of *C. arabica*, the high-altitude cultivar Rwanda Shyira (RS) and the lower-altitude cultivar Brazil Flor de Ipe (BFDI), using liquid chromatography MS/MS analysis.

**Results** We identified a total of 531 proteins, five of which - translation elongation factor 1- $\gamma$  (EF1 $\gamma$ ; AT1G57720), an NB-ARC domain-containing disease resistance protein (AT4G27190), fibrillarin 1 (AT5G52470), a pyruvate kinase family protein (AT3G52990), and a NAD(P)-binding Rossmann-fold superfamily galactose/glucose isomerase (AT2G33590) - exhibited statistically significant differences ( $p < 0.05$ ) in expressional intensity between the two cultivars. Notably, all of these proteins were expressed at a higher intensity in RS except for the NAD(P)-binding Rossmann-fold superfamily protein, which was higher in the BFDI.

**Conclusions** We propose that heightened expression of EF1 $\gamma$  in the RS cultivar may decrease the concentration of bitter flavonoids, thereby contributing to the improved flavour which is often noted among high-altitude coffee cultivars. Simultaneously, expressional intensity differences identified for the remaining four proteins may correspond to adaptations to cold, hypoxic, and disease stressors at different altitudes and in distinct geographic niches. Overall, substantial proteomic differences were identified between the two elevations, providing greater understanding of the effects of altitude on the *C. arabica* plant and the coffee produced from it, which has implications for the global market.

**KEY WORDS:** Coffee, Proteomics, Mass Spectrometry, *Coffea arabica*, Altitude Adaptation, Disease Resistance, Coffee Bean Flavour

## 1 | INTRODUCTION

Coffee is considered to be one of the world's most popular beverages, and its production, transport, and consumption is a major contributor to the global economy. In 2022, global production of coffee beans, harvested primarily from the crops of *Coffea arabica* and *Coffea canephora* (also known as *Coffea Robusta*), is estimated to have reached about  $1.04 \times 10^{10}$  kg,

amounting to a staggering average of roughly 1.3 kg per capita per annum (Livramento et al., 2017; United States Department of Agriculture, 2022). In the developing world, coffee is the second most important commodity following petroleum, and although coffee is primarily consumed in developed countries, it is harvested and processed almost exclusively in developing countries under a wide array of environmental conditions



(Livramento et al., 2017; Talbot, 2004; United States Department of Agriculture, 2022).

Coffee beans harvested from *C. arabica* are almost universally considered to have a stronger, richer, and more balanced taste as compared to the bitter *C. canephora*, consequently accounting for an estimated 55-70% of coffee production worldwide (Livramento et al., 2017; Olechno et al., 2021; United States Department of Agriculture, 2022). Unfortunately, *C. arabica* plants are simultaneously much more sensitive to disease and climatic conditions (Daviron & Ponte, 2005), meaning that the impact of these conditions on *C. arabica* warrants further investigation for the optimization of coffee production.

It is generally agreed that coffee produced from plants grown at higher altitudes has superior taste. This is thought to be the result of climatic conditions that result from higher elevation, primarily lower temperature, as well as lower precipitation and higher humidity, and possibly lower oxygen availability and sunshine hours, rather than as a direct result of higher elevation itself (Avelino et al., 2005; Decazy et al., 2006; Joët et al., 2010; Vaast et al., 2005). Although each of these factors likely plays at least some role, the prevailing hypothesis is that delayed ripening of the beans due to decreased temperatures results in prolonged accumulation of flavour precursors such as sugars, flavonoids, trigonelline, and chlorogenic acids; as a result the beans have a richer and less bitter flavour (Bertrand et al., 2006; Livramento et al., 2017; Vaast et al., 2005).

Although the direct effects of environmental conditions on flavour are undoubtedly substantial, proteomic differences between cultivars resulting from prolonged adaptation to distinct climatic niches are also likely to play a major role in various metabolic processes that underlie certain aspects of coffee plant and thus bean quality. These include disease resistance, homeostatic adjustments to environmental conditions, and possibly synthesis or catabolism of flavour precursors. Despite the importance of the coffee industry to the global economy, relatively few proteomic studies comparing coffee cultivars or even *Coffea* species have been conducted (Garrett et al., 2013; Livramento et al., 2017; Marques et al., 2022). Although the chemical differences between cultivars growing at different altitudes have been studied extensively (Avelino et al., 2005; Decazy et al., 2006; Pereira et al., 2020; Worku et al., 2018), we are aware of only one study investigating the effects of altitude on *C. arabica* bean proteomics. In this study, the authors compared beans harvested from relatively similar altitudes at the same Brazilian plantation and found virtually no explainable

proteomic differences attributable to elevation or elevation-driven environmental parameters (Livramento et al., 2017).

In this study, we take advantage of the substantial improvements in MS/MS technology, computational power, and proteomic database availability that have occurred in recent years to perform a comprehensive comparison of the proteomes of *C. arabica* beans harvested from two highly distinct cultivars. Specifically, the Rwanda Shyira (RS) cultivar grown at high elevation in the Nyabihu district of Western Rwanda, and the Brazil Flor de Ipe (BFDI) cultivar grown at comparatively low elevation in the Minas Gerais province of Southeastern Brazil. Here we report the first instance of a proteomic difference which could be linked to the superior taste of high-altitude *C. arabica*, as well as several major differences which imply adaptation of the RS cultivar of *C. arabica* to abiotic and biotic stressors resulting from high elevation - including hypoxic, cold, and disease stresses.

## 2 | METHODS

### 2.1 Protein Extraction

*C. arabica* beans obtained from Roasti Coffee Co (Sherwood Park, AB) were frozen with liquid nitrogen and ground into a fine powder using a mortar and pestle. Each biological replicate consisted of approximately 10 beans ground into 100 mg fractions (n = 3). Proteins were extracted using 1:3 (w/v) tissue to extraction buffer containing 50mM Tris-Cl (pH 8.0), 4% SDS in HPLC-grade water. DTT was added to each sample to a final concentration of 10 mM. Samples were vortexed and incubated on a heat block for 10 minutes at 95 °C. Samples were centrifuged at maximum speed for 5 minutes at room temperature, and the supernatant was then transferred to a fresh 1.5 mL Eppendorf tube. Samples were centrifuged again for 5 minutes at room temperature, and the supernatant was transferred again to a fresh 1.5 mL Eppendorf tube to ensure no particulates. Samples were diluted 1:5 sample to water to ensure SDS was diluted enough to be compatible with the Bradford assay. A Bradford assay was performed on the samples to determine protein concentrations ( $R^2 = 0.991$ ; data not shown). This allowed us to normalize the sample volumes to 250  $\mu$ L with extraction buffer (50mM Tris-Cl (pH 8.0), 4% SDS in HPLC-grade water) so that there was a maximum protein concentration of 100  $\mu$ g. 10 mM DTT was added to each sample, vortexed, and incubated for 10 minutes at room temperature. Samples were alkylated with 30 mM iodoacetamide and incubated for 30 minutes in the dark at room temperature. Samples were digested overnight with 1:100 sequencing grade trypsin (V5113; Promega) to make

**Table 1:** Environmental and Bean-Specific Parameters pertaining to the two investigated unroasted bean cultivars of *Coffea arabica*.

<b>Environmental Parameters of Growing Region</b> <small>(Kabirigi et al., 2015; Roasti Coffee Co, 2023; WorldData.info, 2023a, 2023b)</small>	Parameters	Brazil Flor de Ipe (BFDI)	Rwanda Shyira (RS)
	Region of Origin	Sul de Minas, Minas Gerais, Brazil	Shyira, Nyabihu District, Rwanda
	Altitude (m above sea level)	1100-1150	1850-2300
	Oxygen Availability (% sea level)	88	77-81
	Daytime Temperature Range (°C)	26-30	15-18
	Annual Precipitation (mm)	1500-1800	1100-1600
	Relative Humidity (%)	63-78	68-86
	Sunshine (h/day)	5.5-7.7	4.2-7.8
<b>Bean-specific Parameters</b> <small>(Roasti Coffee Co, 2023)</small>	Varietal	Yellow Bourbon, Caturra	Red Bourbon
	Collection Process	Natural	Washed

proteome peptide pools. The peptide pools were then acidified with formic acid to a concentration of 5% (v/v), and were then dried by vacuum concentrator. Peptides were desalted using ZipTip C18 pipette tips (ZTC18S960; Millipore). The peptides were dissolved in 3% acetonitrile (v/v), 0.1 % TFA (v/v) and eluted with 60% acetonitrile (v/v), 0.1 % TFA (v/v). Samples were dried by vacuum concentrator prior to LC-MS/MS analysis.

## 2.2 Liquid Chromatography MS/MS

*C. arabica* peptides were analyzed using a Fusion Lumos Orbitrap mass spectrometer (Thermo Scientific) with data-independent acquisition (DIA). Unlike data-dependent acquisition (DDA), DIA allows for fragmentation and subsequent analysis of all peptides, which means there is no bias for proteins with a certain mass to charge (m/z) intensity. For each replicate, 2 µg of the re-suspended peptide was injected using Easy-nLC 1200 system (LC140; Thermo and an Acclaim PepMap 100 C18 trap column (Cat# 164750; Thermo Scientific) followed by a 15 cm Easy-Spray PepMap C18 analytical column (ES906; Thermo Scientific) warmed to 50°C. Peptides were then eluted at 1.2 µL/min with a segmented solvent B gradient of 0.1 % (v/v) FA in 80 % (v/v) ACN (A998, Fisher) from 4 to 41 % solvent B (0 - 21 min). The FAIMSpro was used with a fixed gas flow of 3.5 L/min with a CV setting of -30, -50, -70. A positive ion spray voltage of 2.3 kV was used with an ion transfer tube temperature of 300°C and an RF lens setting of 40%. All precursor signals were recorded in the Orbitrap using quadrupole transmission in the mass range of 300–1,500 m/z. Spectra were recorded with a resolution of 120,000 at 200 m/z, a target value of 4E5 and the maximum cycle time was set to 3s. Precursor signals were selected for fragmentation with a charge state from +2 to +7 and a signal

intensity of at least 1E4. A dynamic exclusion list was used for 30s, and maximum parallelizing ion injections were activated. Data-dependent MS/MS were recorded in the Orbitrap using quadrupole isolation with a window of 2 Da and higher-energy C-trap dissociation (HCD) fragmentation with 30% fragmentation energy.

## 2.3 Mass Spectrometry Data Analysis

Raw data was processed using MaxQuant software version 1.6.14.0 (15; <http://www.maxquant.org/>). At the time of writing there has not been a publicly available *C. arabica* proteome sequenced. We instead searched our MaxQuant output against the *C. canephora* proteome (<http://www.coffee-genome.org/>). Carbamidomethylation of cysteine residues was inputted as a fixed modification (it will be present), while oxidation of methionine was included as a variable modification (it may or may not be present). The fixed modification accounts for cysteine residue interacting with iodoacetamide, while the variable modification allows us to increase our search space of potential sequences for methionine as it can be readily oxidized. We then performed further analysis with Perseus software v2.0.10.0 (16; <https://maxquant.net/perseus/>). Reverse hits and contaminants were removed, resulting in 531 hits. We performed a log2(x) transformation to determine fold-change between protein expressions. We further filtered based on proteins that had measurements above the detection threshold in at least 3 of the samples across both test groups, resulting in 71 hits. Doing this ensured that threshold values were present in multiple samples and not individual unique events. Data was then median normalized, and imputed with a normal distribution function.

We then performed a one-way ANOVA followed by unpaired Student's T-tests on these 71 hits. 5 of these had  $p$ -values  $<0.05$ , which we considered as the threshold for statistical significance.

## 2.4 Bioinformatics

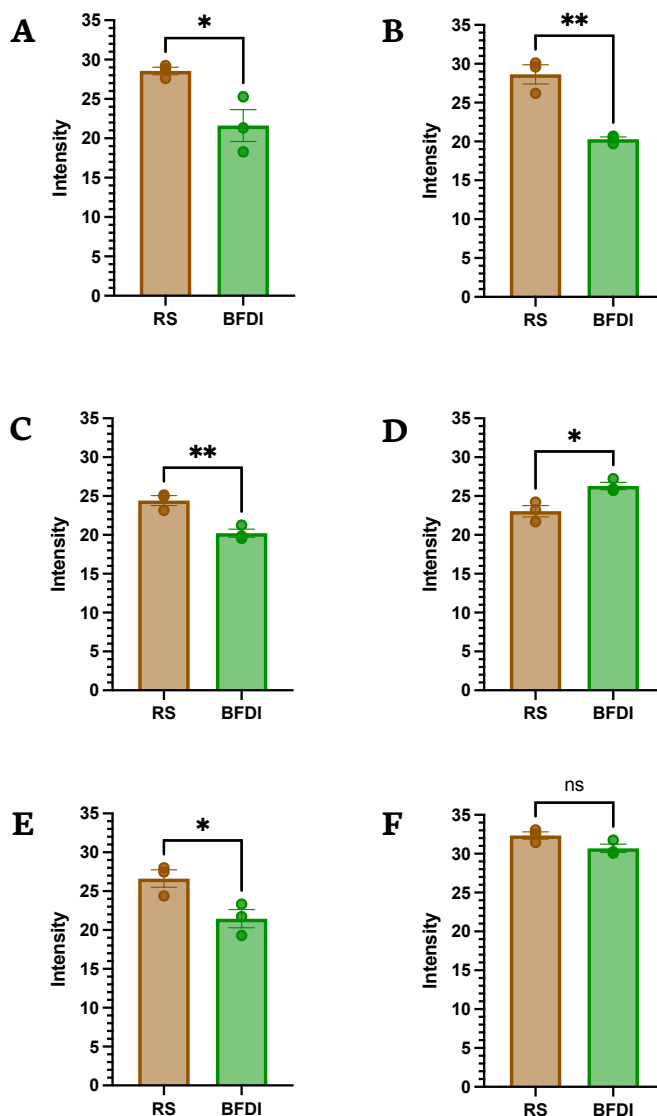
After obtaining significant hits, we queried the fasta headers against the *C. canephora* proteome (<http://www.coffee-genome.org/>) to obtain their complete protein sequences. We then BLASTed these protein sequences in Phytozome (Goodstein et al., 2012) against *Arabidopsis thaliana* Araport11 to obtain AGI locus codes. It is important to note that sequence similarity does not always translate to exact function, but the number of resources for the *C. canephora* proteome is limited as it has not been sequenced nearly to the extent of *A. thaliana*. The AGI locus codes allowed us to utilize resources such as TAIR (Berardini et al., 2015) to determine gene ontology, molecular functions of the proteins and the developmental stages in which proteins are expressed. Predicted subcellular localization information was obtained using SUBA5 (Hooper et al., 2022) and Ensembl (Cunningham et al., 2022). All figures were made using GraphPad Prism 9.3.1.

## 3 | RESULTS

We selected coffee beans harvested from two *C. arabica* cultivars, Rwanda Shyira (RS) and Brazil Flor de Ipe (BFDI), which were grown in two regions with considerable differences in altitude and, as a result, several environmental variables including temperature, precipitation, humidity, oxygen availability, and sunshine hours (Table 1). Following MS/MS we identified a total of 531 proteins, five of which exhibited statistically significant differences in expressional intensity between the RS and BFDI cultivars (Figure 1). These proteins have various molecular functions related to flavour precursor turnover and responses to both biotic and abiotic stressors such as cold, hypoxia, and disease (Table 2).

The highest difference in expressional intensity was observed for an NB-ARC domain-containing disease resistance protein (AT4G27190), which had significantly higher expression in RS relative to BFDI ( $p = 0.0027$ ; Figure 1B). This homolog was identified by performing a BLAST search with NB-ARC domain-containing protein (Cc00t22090.1), identified via coffee genome hub (see Table 2), against the *A. thaliana* proteome, and was selected because it had the highest query coverage (74%) and identical molecular function. Localized to the cytosol and plasma membrane, NB-ARC is

composed of three key domains: a nucleotide-binding (NB) domain, and two ARC domains, ARC-1 and ARC-2, which are



**Figure 1: Differences in expressional intensity of proteins with the highest identified expression variability between *Coffea arabica* cultivars Rwanda Shyira (RS) and Brazil Flor de Ipe (BFDI).** Significant differences in expression between RS (brown) and BFDI (green) were identified for Translation Elongation Factor 1- $\gamma$  (AT1G57720; **A**), an NB-ARC Domain-Containing Disease Resistance Protein (AT4G27190; **B**), Fibrillarin 1 (AT5G52470; **C**), Pyruvate Kinase Family Protein (AT3G52990; **D**), and an NAD(P)-Binding Rossmann-fold Superfamily Protein (AT2G33590; **E**), while the difference in expression of Galactose-Mutarotase-like Superfamily Protein (AT3G47800; **F**), despite being the next highest of all proteins queried in this study, was not statistically significant. Proteins with statistically significant differences in expression were identified using a one-way ANOVA followed by unpaired Student's T-tests using Perseus v2.0.10.0, with  $p < 0.05$  indicating statistical significance. Bars represent the mean values of three biological replicates, shown as points about the mean, while error bars indicate standard error of the mean of these replicates.

well conserved across plant species (Hooper et al., 2022; van der Biezen & Jones, 1998; Van Ooijen et al., 2008). The ARC domains have been found to regulate several important proteins, from which they received their name: apoptotic protease-activating factor-1 (APAF-1), R proteins, and *Caenorhabditis elegans* death-4 protein (CED-4; Takken et al., 2006; Van Ooijen et al., 2008)). NB-ARC is also an ATPase domain (Van Ooijen et al., 2008).

The second highest difference in expressional intensity was observed for the gamma chain of translation elongation factor 1 (EF1- $\gamma$ ; AT1G57720), which had significantly higher expression in RS relative to BFDI ( $p = 0.0289$ ; Figure 1A). In addition to its canonical roles in translation, EF1- $\gamma$  is known to be involved in copper ion binding through an as-of-yet unidentified motif (Kung et al., 2006) and, interestingly, contains a glutathione-s-transferase (GST) domain (Koonin et al., 1994). Indeed, EF1- $\gamma$  functions as a soluble cytosolic GST in several species of plants, and rice

EF1- $\gamma$  was also shown to produce GST activity when expressed ectopically in *Escherichia coli* (Jain et al., 2010; Kobayashi et al., 2001).

A smaller but significant difference in expressional intensity was also observed for fibrillarin 1 (AT5G52990), which had higher expression in RS relative to BFDI ( $p = 0.007$ ; Figure 1C). Fibrillarin is a highly conserved methyltransferase involved in processing of primary ribosomal transcripts, and is pivotal for ribosome biogenesis (Rodriguez-Corona et al., 2015). Fibrillarin is a core component of the small nucleolar ribonucleoprotein (snoRNP) that methylates rRNA (Tollervey et al., 1993) and is also involved in early steps of ribosomal transcription initiation (Yildirim et al., 2013). It is primarily localized in the fibrillar center and dense fibrillar component of the nucleoli where active rDNA transcription and rRNA processing take place, and is also found in Cajal bodies in the cell nucleus.

**Table 2:** Proteomic Data Pertaining to Five Proteins with Significant Variation in Expressional Intensity between *Coffea arabica* cultivars Rwanda Shyira (RS) and Brazil Flor de Ipe (BFDI)

FASTA header for <i>C. canephora</i> [Coffee Genome Hub] MaxQuant (Cox & Mann, 2008) Perseus (Tyanova et al., 2016)	% coverage of peptide sequences	NCBI Accession No. (NCBI)	Top BLAST hit for <i>A. thaliana</i>	AGI [Phytozome] (Goodstein et al., 2012)	Molecular Functions [TAIR] (Berardini et al., 2015)	Subcellular Localization [SUBA5] (Hooper et al., 2022)
<b>Cc00t22090.1</b> NB-ARC domain- containing protein	2.6	CDP20838	NB-ARC domain- containing disease resistance protein.	AT4G27190	ADP binding, regulation of APAF-1, R proteins and CED-4	Cytosol, plasma membrane
<b>Cc10t09370.1</b> Elongation factor 1-gamma 2	34.7	XP_027093104	Translation elongation factor EF1, gamma chain	AT1G57720	Copper ion binding glutathione transferase (GST) activity	Cytosol, mitochondrion, vacuole, cell wall, plasma membrane
<b>Cc02t02560.1</b> Mediator of RNA polymerase II transcription subunit 36a-like	6.1	XP_027103727.1	Fibrillarin 1	AT5G52470	RNA binding, histone H2AQ104 methyltransferase activity, mRNA binding, rRNA methyltransferase activity, snoRNA binding	Nucleus
<b>Cc02t10330.1</b> Pyruvate kinase 1, cytosolic	29.6	XP_027111400.1	Pyruvate kinase family protein	AT3G52990	mRNA binding, magnesium ion binding, potassium ion binding, pyruvate kinase activity	Plastid, cytosol
<b>Cc00t08940.1</b> Epimerase domain- containing protein	32.9	CDP19334.1	NAD(P)-binding Rossmann-fold superfamily protein	AT2G33590	oxidoreductase activity (acts on CH-OH group of electron donors, NAD or NADP as acceptor).	Nucleus, cytosol, mitochondrion, plastid, plasma membrane

Another small but significant difference in expressional intensity was observed for pyruvate kinase 1 (AT3G52990), a cytosolic enzyme which was the only protein identified in our dataset to have higher expression in BFDI relative to RS ( $p = 0.0205$ ; Figure 1D). The cytosolic pyruvate kinase (cPK) is a critical glycolytic enzyme which catalyzes the transfer of phosphate from phosphoenolpyruvate (PEP) to ADP for the synthesis of ATP during glycolysis (Wulfert et al., 2020). In addition to their roles in cellular energy homeostasis, different isoforms of cPKs are known to be differentially regulated by allosteric effectors, and have different enzyme subgroup associations and expression patterns in *A. thaliana* (Wulfert et al., 2020).

A substantial difference in expression was also identified for an NADP-Binding Rossmann-fold superfamily protein (AT2G33590), which had higher expressional intensity in RS relative to BFDI ( $p = 0.0334$ ; Figure 1E). Primarily localized to the cytosol, this protein contains domains involved in the catabolic processing of galactose to glucose. Interestingly, another protein in our dataset, which was identified to have only a slight (not statistically significant) difference in expression between RS and BFDI ( $p = 0.081$ ; Figure 1F), is a galactose mutarotase-like superfamily protein (AT3G47800) which is also involved in the catabolic conversion of galactose to glucose (Berardini et al., 2015; Hooper et al., 2022; Thoden et al., 2001).

## 4 | DISCUSSION

*4.1 Increased expression of elongation factor 1- $\gamma$  in the high-altitude C. arabica cultivar Rwanda Shyira may suggest a novel explanation for the superior taste quality of high-altitude coffee.*

Glutathione-s-transferases (GSTs) such as EF1- $\gamma$  are responsible for the transfer of glutathione groups to xenobiotic compounds with the intent of decreasing toxicity, promoting further metabolism, or directly facilitating the excretion of the targeted compound (Hayes et al., 2004). Interestingly, GST concentration and activity has been shown to increase in the saliva of individuals fed with diets rich in coffee, which contains flavonoids, and broccoli, which contains isothiocyanates (Sreerama et al., 1995). This implies that GSTs might be responsible for the detoxification of these compounds in human saliva (Sreerama et al., 1995). Several GSTs participate in the detoxification of isothiocyanates in *Drosophila melanogaster*, while in the taste buds of *Rattus norvegicus* GSTs are localized to Type II cells - which are known to be involved in the detection of bitter, umami and

sweet molecules, providing further support for a role for GSTs in the detection and detoxification of bitter compounds across several species (Gonzalez et al., 2018; Schwartz et al., 2023). Detoxification of bitter or otherwise unpleasant-tasting compounds in the taste buds is adaptive not only due to the reduction of toxicity, but also because the decreased concentration of the free molecule results in decreased binding to bitter molecule receptors. The lack of a strong bitter sensation indicates to the organism that the consumed amount of the bitter food is tolerable. In contrast, a strong bitter taste may indicate that binding to receptors remains high because detoxification enzymes have been saturated, suggesting that the consumed dose is unacceptable and could be toxic (Dagan-Wiener et al., 2019; Gonzalez et al., 2018; Schwartz et al., 2023). This phenomenon has been described extensively for Proline Rich Proteins (PRPs), which are present in saliva and are able to bind and sequester polyphenols - simultaneously detoxifying them and reducing their free concentration, which has the effect of reducing their binding to bitter taste receptors (Matsuo, 2000).

Recently, Schwartz et al. found that GSTs are able to bind isothiocyanates and flavonoids, as well as caffeine. The authors further demonstrated that GSTs are able to transfer glutathione to isothiocyanates in *ex vivo* human saliva samples, but whether this same mechanism extends to flavonoids remains to be elucidated (Schwartz et al., 2022). While the effect of glutathione transfer to these substrates on perception of bitterness remains unknown, it is likely that this mechanism of detoxification simultaneously prevents the binding of the bitter molecules to their receptors on gustatory cells, as described for PRPs, meaning that increased GST activity in the saliva can reasonably be hypothesized to decrease the sensation of bitterness. Concordantly, the heightened expression of the GST domain-containing EF1- $\gamma$  in the RS cultivar of *C. arabica* could potentially be associated with modification of endogenously occurring flavonoids and other bitter molecules, possibly reducing the bitterness of coffee brewed with beans of that cultivar.

Coffee quality is typically associated with stronger non-bitter flavours, and a corresponding reduction of the overall bitter taste; *C. canephora* beans, for instance, are more bitter and therefore considered inferior (Olechno et al., 2021). It is generally agreed that coffee beans harvested from plants grown at higher altitudes are superior in quality, and this is thought to be mainly due to the fact that lower temperatures delay the ripening process and thus allow flavour precursors to accumulate for a longer period of time, thereby enhancing the flavours of the beans (Avelino et al., 2005; Livramento et al.,



2017; Vaast et al., 2005). Although a number of studies have investigated the effects of altitude on metabolite composition in coffee, little is known about the effects of this variable on protein composition, and the few studies performed on this subject have failed to identify proteins which are known or even speculated to be associated with coffee flavour (Livramento et al., 2017).

Here we present the tantalizing conjecture that heightened expression of EF1- $\gamma$  in the RS cultivar of *C. arabica* may decrease the concentration of bitter molecules such as flavonoids and caffeine through the function of its GST domain, thereby contributing to the improvement of taste quality of this high-altitude cultivar by decreasing its overall bitterness. Since GSTs are not known to interact with other, non-bitter flavour precursors, this proposed mechanism might improve the overall taste of the resulting coffee. Although caffeine, flavonoids, and other bitter molecules found in coffee are not known to be cytotoxic in plants, flavonoids are thought to have antifungal properties (Galeotti et al., 2008), while caffeine is known to protect plants against predation by insects and other herbivores, as well as infection by bacteria and fungi (Baumann & Gabriel, 1984; Frischknecht et al., 1986; Mohammed & Al-Bayati, 2009; Nathanson, 1984). Given that stresses from herbivory and bacterial and fungal colonization are thought to be reduced at lower temperatures and thus higher altitudes (Desaint et al., 2021; Wu et al., 2021), bitter antimicrobial and anti-herbivory compounds may not be necessary in high concentrations at such altitudes and could be repurposed for alternative functions, thereby potentially explaining the adaptive value of the postulated selective breakdown of caffeine and flavonoids by high-altitude *C. arabica*. Although this preliminary postulation is interesting, it remains uncertain whether heightened EF1- $\gamma$  expression is a property of all high-altitude cultivars of *C. arabica* or only the RS cultivar being investigated in this study. Clearly, further research is required to validate and refine this conjecture.

#### 4.2 Increased expression of NB-ARC domain-containing disease resistance protein in the *C. arabica* cultivar Rwanda Shyira may reflect increased effector-triggered immunity as an adaptive response to lower temperatures at higher altitudes.

In *A. thaliana*, the NB-ARC domain-containing protein functions as a regulator of immunity and disease resistance through the regulation of resistance (R) proteins (Berardini et al., 2015; Cunningham et al., 2022; Van Ooijen et al., 2008; Zhu et al., 2010). Of the two ARC domains found in NB-ARC, the ARC-2 domain contains a highly conserved methionine-histidine-aspartate sequence (Van Ooijen et al.,

2008; L. Wu et al., 2014). This has been identified as being a key regulator of R proteins in *Nicotiana benthamiana*, and these R proteins activate effector-triggered immune (ETI) responses in response to the expression of avirulent (Avr) genes by invading pathogens (Van Ooijen et al., 2008; L. Wu et al., 2014).

The role of elevation in the development and expression of plant defenses remains controversial, with certain studies finding positive correlations between elevation and plant defense and others reporting the opposite. Wu et al. speculate that these inconsistent findings are likely due to other elevation-dependent abiotic factors such as temperature, rather than elevation itself (R. Wu et al., 2021). Interestingly, Cheng et al. identified that *A. thaliana* increases ETI signalling at temperatures ranging from 10°C to 23°C, but shifts to increasing pattern-triggered immunity (PTI) at temperatures ranging from 23°C to 32°C. This phenomenon is likely due to the co-evolution of plants with bacteria, since it has been found that pathogenic bacteria secrete Avr-factors at lower temperatures - forcing plants to increase R proteins and therefore ETI signalling and in order to respond to these Avr (Cheng et al., 2013; Van Dijk et al., 1999). These findings explain the greatly increased expression of the NB-ARC domain-containing disease resistance protein in beans of the RS cultivar of *C. arabica*, since the Shyira region of Rwanda from which these beans were harvested has a lower temperature range (15-18°C; Table 1), at which R protein expression and ETI signalling, which are regulated by the NB-ARC proteins, are prevalent.

Interestingly, three putative R proteins (all of them RGA3) were found in our initial 531 protein hits (prior to filtering, see Methods), but most of these had expression intensities below the detection threshold. One RGA3 protein, Cc00t11180.1, however, was expressed at an intensity above the limit of detection in one replicate of the RS cultivar, suggesting that further MS analysis with a longer gradient might allow for increased detection of at least some of these proteins and permit further analysis.

#### 4.3 Increased expression of fibrillarin in the high-altitude *C. arabica* cultivar Rwanda Shyira provides insights into the role of this protein in *C. arabica* immunity and cold stress responses.

In plants, evidence exists for fibrillarin involvement in several epigenetic nucleolar mechanisms. Specifically, fibrillarin is capable of methylating histone H2A while bound to rDNA (Loza-Muller et al., 2015), and in RNA polymerase I-mediated transcription, the protein complex FACT (facilitates chromatin transcription) interacts with methylated histone

H2A to reorganize nucleosomes in the active promoters for rRNA (Tessarz et al., 2014). Moreover, plant fibrillarin is also capable of interacting with histone H2A to carry out rDNA promoter methylation (Loza-Muller et al., 2015). Fibrillarin is also shown to have ribonuclease activity (Rodriguez-Corona et al., 2017) and is part of a protein complex that possesses endonuclease activity required for proper processing of rRNA (Dragon et al., 2002). Intriguingly, fibrillarin has been found to play an indispensable role in the infection process of several plant umbraviruses, which co-opt and utilize the protein as an essential component of translocatable viral ribonucleoproteins (RNPs). In particular, the ORF3 protein of the groundnut rosette virus has been shown to directly interact with fibrillarin to facilitate nuclear localization and assembly of movement-competent infectious RNP particles, which are required for systemic infection of the plant (Sang et al., 2007). Furthermore, fibrillarin knockdown suppresses the long-distance movement of groundnut rosette virus particles and prevents systemic infection of the plant, suggesting that lowered expression of fibrillarin could be a potential host defense mechanism against viruses with nucleolar replication phases, such as the groundnut rosette virus.

Fascinatingly, the prevalence and distribution of infection with groundnut rosette virus is substantially lower in South America relative to the African continent (Naidu et al., 1998; Okello et al., 2014; Thottappilly, 1992) which could be at least partially explained by the decreased expressional intensity of fibrillarin in the BFDI cultivar. Whether decreased expression of fibrillarin is common among South American *C. arabica* cultivars or is unique to the BFDI cultivar is unclear, however, and further proteomic investigations into South American cultivars will therefore be necessary to validate this conjecture.

Plant fibrillarin has also been shown to act as part (subunit 36a) of a multi protein complex - mediator of RNA polymerase II transcription - which provides an interface for communication between transcription regulation proteins and core promoters (Bäckström et al., 2007). Interestingly, the mediator16 subunit of the transcriptional coactivator complex has additionally been shown to regulate cold-responsive gene expression in *A. thaliana* (Hemsley et al., 2014). Due to its high altitude, the Shyira region of the Nyabihu district of Rwanda has a daytime temperature range of 15-18°C, considerably lower than the Sul de Minas region of Brazil (Table 1). This may explain the heightened expression of fibrillarin in the RS cultivar relative to BFDI (Figure 1C), since the RS cultivar of *C. arabica* may require higher fibrillarin expression to appropriately modulate Cold On-Regulated

(COR) genes in response to lower temperatures (Hemsley et al., 2014).

Taken together, these data might place fibrillarin at the center of a fascinating evolutionary trade-off in which the RS cultivar of *C. arabica* increases the expression of fibrillarin in response to temperature stresses, but is consequently more susceptible to systemic infection by umbraviruses such as the groundnut rosette virus.

#### 4.4 Decreased expression of pyruvate kinase 1 in the high-altitude *C. arabica* cultivar Rwanda Shyira raises questions about the levels and mechanisms of cold adaptation in coffee plants.

Low temperatures can strongly affect plant metabolism and respiration rate, and photosynthesis is known to be strongly suppressed in coffee leaves at temperatures below 13-18°C, which greatly limits the supply of ATP for cellular metabolism (Partell et al., 2010; Ramalho et al., 2003). Plants growing at such temperatures might therefore be expected to increase expression of respiratory enzymes such as pyruvate kinase. As discussed previously, daytime temperatures in the Nyabihu district of Rwanda are considerably lower than in the Sul de Minas region of Brazil (Table 1), and our findings regarding the relative expressional intensities of cPK (Figure 1D) are therefore challenging to understand. Interestingly, Partell et al. found that a relatively cold-resistant genotype of *C. arabica*, Catucaí IPR 102, exhibits increased activity by respiratory enzymes such as malate dehydrogenase and pyruvate kinase, in addition to possessing higher concentrations of soluble sugars involved in osmoregulation and membrane stabilization (Partell et al., 2010). Although Partell et al. did not investigate cPK expression levels, it is conceivable that the cPK enzymes of cold-resistant plants are optimized for higher enzymatic performance at lower temperatures, allowing the organism to catalyze reactions with a lower overall amount of the enzyme. This could indicate that the RS cultivar of *C. arabica* is cold adapted, which is reasonable given its environment, and therefore exhibits a lower expression of cPK since its cPK is optimized for higher enzymatic activity at lower temperatures. This is only speculative, however, since the enzymatic activity of RS cPK is not known, and further investigations comparing the cPK enzyme kinetics of cold-adapted and non-adapted strains in cold temperatures will therefore be necessary to shed more light on this unexpected finding.

#### 4.5 Increased expression of a galactose/glucose isomerase in the high-altitude *C. arabica* cultivar Rwanda Shyira could be an adaptive response to hypoxic stress.

The identified NADP-Binding Rossmann-fold superfamily protein is known to respond to hypoxic and water-deprivation conditions (Berardini et al., 2015; Hooper et al., 2022), possibly explaining the increased expression of this protein in the RS cultivar since the Shyira region of Rwanda is at a substantially increased elevation and consequently has decreased oxygen availability (Table 1). This protein primarily acts as an oxidoreductase which oxidizes CH-OH groups using NAD and NADP as electron-accepting cofactors, but also contains an NAD-dependent epimerase/dehydratase domain which is known to be involved in the conversion of UDP-galactose to UDP-glucose during the process of galactose metabolism (Allard et al., 2001; Berardini et al., 2015; Thoden et al., 2001). Both domains play roles in different forms of substrate isomerization, suggesting that AT2G33590 may be a galactose/glucose isomerase. Curiously, a galactose mutarotase-like superfamily protein (AT3G47800), which is also a cytosolic epimerase involved in the catabolic processing of galactose to glucose via UDP-galactose/glucose conversion (Berardini et al., 2015; Thoden et al., 2001), exhibited relatively similar levels of expressional intensity between the RS and BFDI cultivars (Figure 1F).

Given that the galactose mutarotase-like protein is not known to be associated with hypoxic stress and appears to have similar expression levels in RS and BFDI, while the hypoxia-associated NADP-binding Rossmann-fold protein has increased expression in high altitude-grown RS, it is conceivable that the latter protein is overexpressed under hypoxic conditions in order to generate a larger pool of glucose molecules which can be metabolized through various pathways to offset the effects of hypoxic stress. While intriguing, this preliminary conjecture ought to be validated through measurement of enzyme kinetics and glucose production under these varying conditions of altitude and oxygen availability.

## 5 | CONCLUSIONS

Overall, these findings serve as a starting point for further studies on the effects of endogenous glutathione-S-transferases (GSTs) on coffee bean flavour and the function of *Coffea arabica* immunoregulatory proteins, as well as research into the modifications of enzyme kinetics in response to low temperatures and hypoxia. In subsequent proteomic work, longer MS gradients will permit the detection of a broader

range of protein expressional intensities and thus allow for qualitative identification of other proteins which might be associated with our existing findings (such as the RGA3 or galactose mutarotase-like superfamily proteins). MS/MS analysis of other *C. arabica* cultivars would provide further insight into our results and allow us to determine if the presence of these proteins is exclusive to RS and BFDI or generalizable to all *C. arabica* cultivars from different elevations. These results would allow us to better understand whether the proteomic effects of altitude explain the superior yield, taste, popularity, and market performance of certain cultivars of *C. arabica*. Ultimately, our findings contribute to understanding the role that these various proteins play in the taste quality of *C. arabica* beans, and the responses this organism has to abiotic and biotic stressors caused by high elevation - providing further insights which could improve the production and quality of one of the most popular commodities worldwide.

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## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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# The effect of sunrise timing and mountain aspect on the dawn chorus of Swainson's Thrush

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## ABSTRACT

This study centers on the Swainson's Thrush (*Catharus ustulatus*), uniquely identified by a buffy eye ring, uniform brown back and tail, and a distinctive flute-like song with an ascending pitch. With a prevalent presence in woodlots, parks, and fruiting trees, they breed in coniferous forests with dense undergrowth, displaying notable vocalization and behaviour patterns. This research aims to explore the impact of pre- and post-sunrise timing and mountain aspect (North, East, South, West) on the dawn chorus of Swainson's Thrush. The hypothesis posits that those exposed to earlier sunlight will sing prior to those experiencing delayed sunlight. Specific predictions are made for each mountain aspect, considering temperature, sunlight exposure, and resulting bird activity levels. To test the hypothesis, five audio recording units (ARUs) are set up across the mountain's four sides. Three recordings are made before and after sunrise for each side, grouped as North, South, East, and West. Results reveal a statistically significant interaction between direction and time since sunrise, while direction alone does not significantly impact song frequency. Time since sunrise and Julian's days also influence singing behaviour. The study shows the intricate relationship between environmental factors, sunrise timing, and mountain aspect on the dawn chorus behaviour of Swainson's Thrushes. These findings underscore the need for further investigation into additional factors influencing the dawn chorus, contributing to avian ecology and conservation understanding. By unraveling these complexities, conservation efforts can be better informed and aligned.

**KEY WORDS:** Swainson's Thrush (*Catharus ustulatus*), Mountain Aspect, Direction, Time Since Sunrise, Dawn Chorus

## 1 | INTRODUCTION

Vocal communication facilitates survival, social organization, and reproduction in many species (Smith and Harper 2003). In bird species, singing is a common vocal behaviour that serves many functions including the establishment of territories, attracting mates, warning others of predators, coordination of group behaviour, and identification of individuals. The sounds made by birds can be divided into two categories: calls and songs. A call is often a brief and simple vocalization that indicates flight or danger. During the mating season, a song is a lengthy and complicated vocalization (Smith and Harper 2003). Morning singing is a common occurrence among birds and is a crucial aspect of avian behaviour. During the breeding season, male birds sing more to attract females, leading to increased vocal activity. Morning singing helps males establish dominance and fitness with potential mates, while also aiding

in the establishment and maintenance of territory boundaries, as sound carries farther and clearer in quieter morning air (Naguib et al., 2019).

The right timing of behaviour is essential for survival and reproduction in many organisms including birds. This is because many behavioural and physiological processes follow a 24-hour rhythm based on a circadian rhythm. Many studies on circadian rhythms concern animals living at temperate latitudes, where night and day are distinct (Da Silva and Kempenaers 2017). However, at northern latitudes, animals in spring and summer experience more limited changes in light intensity between day and night or even continuous daylight. Animals living in the Arctic have to cope with the absence of light cues to time behaviour. This is where they may rely on circadian clocks' entrained behavioural rhythms during the part of the year with sunsets and sunrises. Furthermore, in mountainous areas, the light environment can be very

different from that at lower elevations, and the different faces of the mountains have different timing of when sunlight first hits (Maphisa et al., 2016). Factors for this phenomenon include the angle of the sun, altitude, and latitude, which can have significant effects on the circadian rhythms of animals living in these areas (Cassone 2014). According to a study published in the journal *Atmosphere* 2021, the effects of shade on animals are more apparent in mountainous places where the topography creates strong gradients in light intensity and shadows. The timing of spring leaves in a high-altitude forest in the Rocky Mountains was found to be substantially impacted by local topography and the availability of shadows from nearby mountains. Because of variations in light availability, the researchers discovered that trees growing on South-facing slopes tended to leaf out earlier than trees growing on north-facing slopes (Hrach et al., 2021). According to these studies, the amount of light and shadows in mountainous regions influences many species' growth and life cycles as well as their circadian rhythms and the start of their daily activities.

The Swainson's Thrush (*Catharus ustulatus*) is the target species in this study. They are readily distinguished from the other *Catharus* species by their buffy eye ring, cheek and uniformly brown back and tail. They carry a flute-like song but spiral upward in pitch when they sing. It is phonetically described as whip-poor-will-a-e-zee-zee-zee (Mack and Yong 2020). Both sexes give a variety of distinctive call notes, including a soft "whit", and a frog-like flight call during migration season. They are often very common in woodlots, parks, and fruiting trees to pluck berries, and they tend to breed in mountains in coniferous forests with extensive leafy undergrowth (Mack and Yong 2020). Their population density is relatively high in Northern America, which makes it easier for detection. The best method to identify these species is by their songs. The Swainson's Thrush makes an excellent bird for this study because it has a unique and complex song, and researchers can gain insights into the behaviour, social structure, and communication patterns of Swainson's Thrush populations. Another reason why Swainson's Thrushes are a good species to investigate when looking at the dawn chorus is that they are the first birds to sing in the morning. The dawn chorus is an important phenomenon when it comes to understanding avian behaviours. The dawn chorus occurs when birds begin to sing before sunrise and cease to decrease song activity for the rest of the day, and is related to mate attraction, territorial defence, and mate guarding (Pérez-Granados et al., 2018).

Researchers can utilize autonomous recording units (ARUs) to listen to Swainson's Thrushes. They often install the

equipment in suitable habitat locations where it is known that birds are present. The ARUs may be programmed to record at particular hours of the day or night, and the recordings may subsequently be evaluated (Shonfield and Bayne 2017). By employing techniques such as using ARUs, researchers can determine if a Swainson's Thrush is present in a given area. ARUs are small weather-resistant units that are strategically positioned in areas where the animals under study are likely to be located. The microphones on the devices can detect and record a wide range of noises, including animal vocalizations, ambient noise, and even weather patterns (Yip et al., 2021). ARUs are used by researchers to collect information about animal behaviour, population dynamics, and habitat usage. Researchers may also identify species and establish the position and movement of wildlife by studying the sounds collected by the ARUs (Shonfield and Bayne 2017). Additionally, fieldwork is not necessary to identify large acoustic data sets when utilizing programs like Wildtrax to identify Swainson's Thrush singing activity. Audio data from ARUs can be processed by using platforms such as Wildtrax. Wildtrax is an online platform for managing, storing, processing, sharing, and discovering biological and environmental sensor data, which has thousands of recordings for our target species, the Swainson's Thrush. There are several transcription methods for identifying birds from recordings. Manual listening is a transcription approach that includes listening to recordings and manually transcribing the sounds heard on platforms such as Wildtrax. The platform can aid in the identification and categorization of sounds, making the process quicker and more precise. A newer and faster transcription method is spectrogram analysis which transforms recorded sounds into visual representations known as spectrograms. These spectrograms can be examined for patterns resembling distinct animal vocalizations and can be used to identify the presence or absence of Swainson's Thrushes on the recordings (Yip et al., 2021).

The purpose of this study is to answer the question, does before and after sunrise timing and mountain aspect (North, East, South, and West) influence the dawn chorus of Swainson's Thrush? In this study, we hypothesize that if the Swainson's Thrush starts singing when sunlight appears, then those that experience sunlight first will sing before those that experience sunlight later. We predict that Swainson's Thrushes on the East-facing side of the mountain would likely have more songs before sunrise due to earlier exposure to sunlight. This may lead to increased vocalization activity levels due to a head start on finding food and breeding partners. As for the South, we predict that South-facing slopes generally receive more sunlight throughout the day, leading to warmer temperatures.

This might result in higher levels of bird activity, including song production. We predict that the West-facing mountainside receives sunlight later in the day, and Swainson's thrushes may start singing later in the morning compared to those on the East-facing side. However, they might continue to sing for a longer period into the afternoon or evening due to the extended sunlight exposure. Lastly, we predict North-facing slopes typically receive the least amount of sunlight and are generally cooler. Swainson's Thrushes on these slopes might exhibit lower song production and activity levels. Before sunrise, song production might be minimal, as the birds would likely wait for warmth and more sun later in the day.

## 2 | METHODS

### 2.1 Experimental Design

To assess our research question, we would first need to determine if the data is decently balanced with the different sides of the mountain and locations of the Swainson's Thrush species. The experimental setup will comprise five ARU locations, situated across all four sides of the mountain. For each side, three recordings will be transcribed both before and after sunrise. Furthermore, the mountain's sides will be grouped into four categories based on their cardinal direction: North, South, East, and West.

### 2.2 Study Area

To address our research question, we will be using 20 study locations. These locations were carefully selected based on the quality of the recordings transcribed and their ability to provide a representative sample. After reviewing multiple locations, we selected 120 recordings that were obtained from these 20 research locations. For each location, it was essential to have three recordings both before and after sunrise, which may or may not contain Swainson's Thrush songs. Some other potential locations were excluded from the study due to technical issues that affected the audio recordings, such as poor sound quality or interference. Some of the study locations we used were WLNP 1-2 (South), WLNP 2-4 (East), WLNP 19-8 (North), WLNP 1-3 (East). The research locations are in Waterton Park, a small town in southern Alberta. The topography of the park is distinguished by high mountains, valleys, and several lakes. The park's climate is influenced by both continental and maritime air masses at elevations ranging from 1,290 meters to 2,910 meters above sea level,

resulting in unpredictable and rapidly changing weather conditions (Britannica 2017). Since Waterton Park has a variety of habitats, including grasslands, wetlands, forests, and meadows, it offers the Swainson's Thrushes and other bird species a variety of environments. This makes it a great study location to examine how the mountain aspect and the amount of time since sunrise affect the Swainson's Thrush dawn chorus. The diversity of habitats present in the area provides an opportunity for researchers to investigate how different surroundings influence the behaviour and vocalizations of Swainson's Thrushes. Furthermore, as the area serves as a breeding ground for the species, it also serves as a crucial stopover site for Swainson's Thrushes migrating from North America to their wintering grounds in Central and South America (Bégin-Marchand et al., 2021).

### 2.3 Sound processing and Data Collection

The Swainson's Thrush species' sound recordings were processed using Wildtrax. Wildtrax is a platform that tracks wildlife movement and behaviour and may be used to characterize various aspects of an ecosystem (Yip et al., 2021). For example, it can provide information on which habitats are used by different wildlife species, and it can help us understand which regions of an ecosystem are vital for species. It can also be used to make conservation and management decisions (Greenberg et al., 2019). Wildtrax displays sound files as spectrograms that can be visually inspected for target data, and there are thousands of playback recordings at various ARU locations of the Swainson's Thrush accessible. A spectrogram is a visual representation of the spectrum of frequencies of the strength of a signal over time at various frequencies present in a waveform (Yip et al., 2021). This approach allows for a balanced representation of the Swainson's Thrush dawn chorus in relation to time since sunrise and mountain aspect direction. Vision scanning enables faster clip categorization than manual transcription, which requires you to actively listen for songs and calls (Yip et al., 2021). There were 449 recordings that were uploaded to Wildtrax, and 120 recordings were transcribed for the 5 ARU locations. The ARUs recorded Swainson's Thrushes singing at each location per side throughout the morning hours (before and after sunrise). The Wildtrax platform includes the time of sunrise and coordinates that provide information on the mountain aspect, which aids us in answering the research question.



**Table 1.** The negative binomial regression table illustrates the relationship between Swainson's Thrush singing behaviour and various predictor variables, including time since sunrise, cardinal directions (with East as the reference category compared to North, South, and West), the interaction between direction and time since sunrise, the quadratic relationship between direction and time since sunrise, Julian days, and the quadratic relationship between Julian days.

Negative binomial regression				Number of obs = 120		
Dispersion: mean				LR $\chi^2$ (13) = 55.11		
Log likelihood = -205.78708				Prob > $\chi^2$ = 0.0000		
				Pseudo R <sup>2</sup> = 0.1181		
total_voc	Coefficient	Std. err.	z	P >  z	[ 95% conf. interval ]	
tss	-.0155877	.0072861	-2.14	0.032	-.0298682	-.0013071
c.tss#c.tss	-.0000632	.000105	-0.60	0.547	-0.0002691	.0001426
direction4id						
N	.5553578	.8158178	0.68	0.496	-1.043616	2.154331
S	.8585411	1.455935	0.59	0.555	-1.995039	3.712121
W	.2530542	.7560106	0.33	0.738	-1.228699	1.734808
direction4id#c.tss						
N	.0054243	.0091119	0.60	0.552	-.0124347	.0232832
S	-.0827528	.0677212	-1.22	0.222	-.215484	.0499785
W	.0136246	.0103656	1.31	0.189	-.0066917	.0339408
direction4id#c.tss#c.tss						
N	-.000176	.0001446	-1.22	0.224	-.0004595	.0001075
S	-.0015635	.0008481	-1.84	0.065	-.0032257	.0000986
W	.0000648	.0001369	0.47	0.636	-.0002036	.0003331
julian	1.188782	.4482888	2.65	0.008	.3101518	2.067412
c.julian#c.julian	-.0033714	.0013201	-2.55	0.011	-.0059588	-.000784
_cons	-102.8911	37.9974	-2.71	0.007	-177.3647	-28.41761
/lnalpha	.9774263	.2168904			.5523289	1.402524
alpha	2.657608	.5764096			1.737294	4.065447
LR test of alpha=0: chibar2 (01) = 349.92				Prob >= chibar2 0.000		

## 2.4 Statistical Analysis

The research design will include time since sunrise and the side of the mountain as our predictor variables, the number of songs by Swainson's Thrushes as the response variable, and Julian days as our covariate. We incorporated Julian days as our covariate. Julian days represent a straightforward method for quantifying the present date, established by counting the elapsed days since a chosen arbitrary date. Additionally, RStudio was used to determine the number of recordings per direction, sunrise time (adjusted daylight saving hours were taken into account), time since sunrise, and Julian days for the transcribed recordings at each location. RStudio is a tool that is used in data analysis to import, plot, and model data (RStudio 2020). In RStudio, a negative

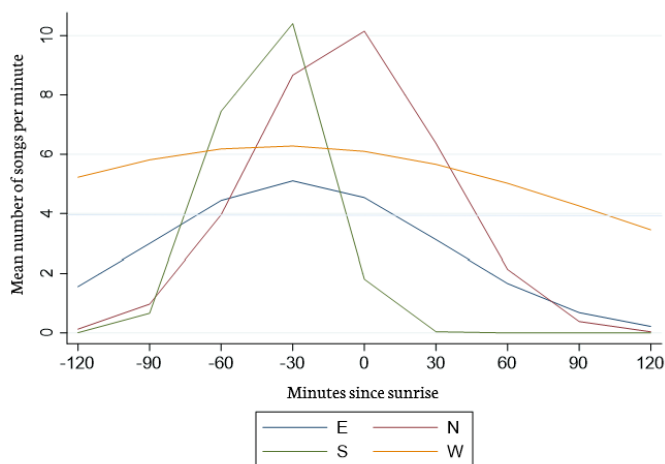
binomial regression and a Likelihood Ratio test (LRT) will be performed. Negative binomial regression is particularly useful for predicting the number of occurrences of an event when the data is characterized by a large number of zeros or when the data follows a negative binomial distribution (Ross and Preece 1985). The negative binomial regression model will be used in this study to examine the relationship between the number of songs produced by Swainson's Thrushes (response variable) and predictor variables such as mountain aspect (North, South, East, and West) and time since sunrise. The negative binomial regression model is appropriate for this study because there are a lot of zeros in our data, and this model is designed to handle count data with a high proportion of zeros, also known as zero inflation. In addition, the Likelihood Ratio test (LRT), which is a statistical test based on the ratio of two models' likelihoods, is used to compare the quality of fit of two models (Vuong 1989).

The LRT is used to test whether the model's capacity to explain variance in the response variable, which is the number of songs produced by Swainson's Thrushes, significantly improves with additional predictor variables (Vuong 1989). In this study, the LRT was used for testing to see if there was a significant interaction between our two predictor variables, time since sunrise and mountain aspect. If the LRT result is significant, it would suggest that the model including both predictors is better at explaining the variation in Swainson's Thrush song frequency.

### 3 | RESULTS

The negative binomial regression graph illustrates the temporal pattern of Swainson's Thrush singing in four cardinal directions: South, North, East, and West (Figure 1). In the South direction, the highest mean number of songs occurs before sunrise, which is about 11 songs per minute. Similarly, the North direction also exhibits a high number of songs immediately before sunrise, which is about 10 songs per minute; the number of songs decreases rapidly right after sunrise in both these directions. The East direction, although not as song-rich as the South and North, still reaches its peak song activity before sunrise. It can be seen that the East direction exhibits a maximum mean of 5 songs per minute before sunrise and then it slowly declines to 0. Lastly, the West direction displays a more curved pattern, characterized by an increased mean of songs before sunrise, approximately 6 songs per minute, followed by a gradual decline after sunrise, reaching about a mean of 3 songs per minute after sunrise. Additionally, the Southern direction experiences its peak prior to sunrise, followed by the Western, Eastern, and Northern directions. At the -120 time point, the Western direction exhibits the highest number of songs per minute, while both the Northern and Southern directions have minimal songs per minute. Meanwhile, the Eastern direction demonstrates a substantial number of songs per minute at the -120 mark, averaging around 2 songs per minute. Overall, in Figure 1, the graph reveals that all directions exhibit a high mean of songs per minute prior to sunrise, followed by a decline after the onset of sunlight. The South-facing direction demonstrates the highest mean number of songs per minute before sunrise, experiencing a rapid decrease after sunrise. In contrast, the East, North, and West directions maintain a relatively high mean number of songs before sunrise but exhibit a more gradual decline toward zero following sunrise. The Likelihood Ratio test (LRT) displayed that there is a significant interaction between the predictors, direction and time since sunrise (tss). This is showcased in Table 2, the LRT chi-square statistic is

34.37 with 6 degrees of freedom, and the p-value ( $\text{Prob} > \chi^2$ ) is 0.0000 for Swainson's Thrushes singing. The p-value represents the probability of observing a test statistic, which is 34.47. Since the p-value is 0.0000, it indicates that the observed test statistic is highly unlikely to have occurred alone. This suggests that the interaction is strong and influences the response variable.



**Figure 1.** The negative binomial regression graph illustrates the relationship between the mean number of songs per minute (y-axis) sung by Swainson's Thrushes in four cardinal directions (North, East, South, and West) and the minutes since sunrise (x-axis). This graph depicts the differing singing patterns of Swainson's Thrushes in relation to the minutes since sunrise across different cardinal directions.

The interaction between tss and direction in relation to the number of songs per minute by Swainson's Thrushes is assessed using the p-values (Table 1). Table 1 examines the relationship between time since sunrise and cardinal directions (North, South, East, and West), specifically at direction4id#c.tss, there is no statistically significant correlation for North ( $p=0.224$ ), South ( $p=0.065$ ), and West ( $p=0.636$ ). Additionally, direction East was used as a reference in direction4id#c.tss when comparing the other three directions. By using East as the reference category, we will know how the response variable, the number of songs produced by Swainson's Thrushes, differs in the North, South, and West directions compared to the East. Furthermore, the coefficient is positive for North and West, which tells us that total\_voc increases as you change from East to other directions. On the contrary, the negative coefficient for the South suggests that the total\_voc decreases when transitioning from the East to the South. Additionally, the negative coefficient for tss indicates that there is an inverse relationship between the variable and the response variable, which is the number of songs. As the value of tss increases, the value of the response variable is expected to decrease. Furthermore, Julian's

coefficient is positive, which means that as the value of the Julian variable increases, the value of the response variable is expected to increase as well (total\_voc). Moreover,  $c.julian \# c.julian$ , a quadratic relationship, has a negative coefficient. This interaction implies that as the value of Julian increases, the strength of the relationship between Julian and total\_voc decreases. Furthermore, it shows that it is statistically significant because  $p < 0.05$ .

**Table 2 .** The Likelihood Ratio test displays the significance of the interaction between direction and time since sunrise. The table presents the LR chi-square statistic (34.37) with 6 degrees of freedom, and the  $\text{prob} > \chi^2 = 0.0000$ , indicating a significant difference between the null and alternative models in explaining the variation in total\_voc in Swainson's Thrushes songs.

LR $\chi^2(6) = 34.37$
Prob $> \chi^2 = 0.0000$

## 4 | DISCUSSION

The research question in this paper is, does before and after sunrise timing and mountain aspect affect the Swainson's Thrush (*C. ustulatus*) dawn chorus? We see that time since sunrise plays an important role in the mean number of songs per minute in each direction (Table 1). The South direction exhibits the highest number of songs prior to sunrise but experiences a sharp decline immediately following sunrise. This shows that the birds are most vociferous in this direction before sunrise. The observed trend may be attributed to a range of variables, such as favourable habitat conditions and environmental factors that promote increased vocalization activity in the South-facing direction during the early morning hours. Swainson's Thrushes tend to thrive in dense vegetation, especially in wooded areas like coniferous forests, which are often found in Southern directions due to greater sunlight exposure (Da Silva and Kempenaers 2017). In this study, Waterton Park has a variety of vegetation, which makes it an ideal habitat for Swainson's Thrushes. The graph indicates a sharp decline in the average number of Swainson's Thrush songs per minute as the day progresses, eventually falling to zero. This decrease in song production might be explained by a change in the bird's activity as the day goes on. The reason for this is attributed to the singing behaviour of Swainson's Thrushes during the early morning hours, which is commonly referred to as the "dawn's chorus". Consequently, a decline in the number of songs is observed as the day progresses. The North-facing direction shows the same pattern, however, the peak of the number of vocalizations is closer to the sunrise time

than the South-facing direction. The peak number of songs per minute is about 10 before sunrise and then it gradually decreases, not as steeply as the North-facing direction. In addition, the West-facing direction is distinct from the other directions, though there are more songs per minute before sunrise, the mean number of songs does not drop to zero after sunrise, implying that Swainson's Thrush birds in the West-facing direction continue to exhibit some level of vocalization throughout the day. Similarly, the East-facing direction exhibits some singing before sunrise but does not sing frequently before sunrise in comparison to the other directions, it peaks at about 5 songs per minute before sunrise and gradually drops to 0 as the day progresses. This may be because the West-facing direction may be facing away from a sparser forest or open field, which may have fewer food sources and mate prospects; we might observe this pattern. To find mates or protect their territory, the birds in this direction may need to vocalize continuously throughout the day. Similarly, the North-facing direction could be facing toward a colder climate, which would require the birds to conserve energy and vocalize less frequently. Additionally, the North-facing direction may have a different habitat or food source availability, which could explain the steeper decline in vocalization after sunrise compared to the other directions. In Table 1, the interaction between direction and time since sunrise square represents that Swainson's Thrushes singing songs are more likely to occur in certain directions (East, North, South, and West). The East direction serves as the intercept and reference point for other directions. This is done to facilitate a comparison between the frequency of Swainson's Thrushes songs in directions such as North, South, and West, with that of the east direction.

Patterns seen in Figure 1, include that East, North, South and West all have a high frequency of songs per minute by Swainson's Thrushes before sunrise. This suggests that there is a peak in singing activity before sunrise, regardless of the direction in which the Thrushes are singing. After sunrise, the number of songs in the South direction decreases rapidly, whereas the number of songs in the North, East, and West directions gradually declines. This implies that there might be some differences in the singing patterns of Swainson's Thrushes in different directions after sunrise. However, based on the negative binomial model, all directions have a p-value greater than 0.05, which means that there is no statistically significant difference in the singing patterns of Swainson's Thrushes among the different directions. However, the LRT showed a significant interaction between tss and direction. It implies that factors such as Julian's days, and direction (North, South, West, compared to East), are important in

understanding the singing behaviour of Swainson's Thrushes. We hypothesized that if Swainson's Thrushes start singing when sunlight appears, then those that experience sunlight first will sing before those that experience sunlight later. Upon examining the negative binomial model presented in Table 1, it can be deduced that direction did not significantly influence the quantity of songs performed by Swainson's Thrushes. Nevertheless, Julian's days and the time since sunrise were crucial factors in determining the song frequency per minute. Moreover, the LRT revealed a statistically significant interaction between the time since sunrise and direction, as evidenced in Table 2. These findings support my initial hypothesis.

In the research paper, spatial and temporal variation of bird dawn chorus and successive acoustic morning activity in a Mediterranean landscape conducted in southeastern Brazil, investigated the effects of environmental factors and time-related variables, such as Julian day and time since sunrise, on the dawn chorus in a Neotropical bird community by analyzing the singing activity of 66 bird species. The study found that temperature, humidity, wind speed, light intensity, and time-related variables can influence bird singing behaviour during the dawn chorus, with temporal variation being one of the primary results (Farina et al., 2015). The authors observed a significant correlation between the dawn chorus onset time and the time of sunrise. As the season progressed, the dawn chorus started earlier, following the pattern of earlier studies. Similarly, in my study, it was found that Swainson's Thrushes were also influenced by time since sunrise. We discovered that they tend to sing more songs before sunrise than after sunrise. Additionally, the research paper also reports that following the dawn chorus, the bird acoustic activity decreased but then increased again later in the morning (Farina et al., 2015). This pattern was observed across all studied habitats. The authors hypothesized that this secondary peak in acoustic activity may be related to factors such as foraging activity (Farina et al., 2015). In correlation to my results, the gradual decline in the number of songs after sunrise may be due to environmental factors such as vegetation due to the mountain aspect as mentioned previously; this may be a plausible explanation for why we see a gradual decline in the number of songs after sunrise, and why some directions show a rapid decline in songs after sunrise. Furthermore, in another research paper, the researchers investigated how the slope aspect influences plant growth, development, and reproduction in mountain habitats (Yang et al., 2020). Plants on South-facing slopes produced more seeds and allocated more resources to reproduction, whereas plants on North-facing slopes committed more resources to

vegetative development and survival (Yang et al., 2020). These patterns were found in a wide variety of plant species, indicating that the slope aspect has a major influence on plant growth and reproductive strategies in mountains. The environment is extremely important to any species since it influences behaviour and ecology, especially in birds (Yang et al., 2020). The data in our study was taken in Waterton, Alberta, where the vegetation is diverse and comprises, ecological zones ranging from prairie grasslands to alpine tundra. The results did not show that direction played a significant role in Swainson's Thrushes singing behaviour, which was unexpected as different aspects of the mountains had different levels of vegetation. A potential reason for this outcome might be the absence of location as a random effect, which would necessitate controlling for the maximum number of Swainson's Thrushes at each site. Factoring in location as a random effect would have enabled me to manage location-specific influences, especially those related to the predictor variable of interest, such as the mountain direction. Regardless, the LRT demonstrated that both time since sunrise (tss) and direction had an impact on the response variable.

There were three significant limitations in this study. To begin, one limitation of this study was individual variability. Individual differences in singing behaviour can influence how Swainson's Thrushes respond to various environmental cues and factors. For instance, some individuals might be more vocal than others in general. This heterogeneity among individuals might have reduced our ability to detect significant effects in our study. To address this issue, we could use capture-mark-recapture methods to track individual birds collect detailed information on their singing behaviour over time and improve statistical accuracy (Mclean et al., 2018). Secondly, seasonal variability in Swainson Thrush's dawn chorus behaviour is another limitation. Factors such as migration, breeding behaviour, and food availability can influence the dawn chorus and may confound the results. Therefore, a study that only looks at days over a short span of time to limit variation due to different seasons may be necessary to understand the effects of sunrise timing and mountain aspect from other influencing factors. Lastly, poor weather and background noise. The accurate detection and analysis of the Swainson's Thrush's dawn chorus could be hindered by background noise, such as other bird species vocalizing and anthropogenic sounds. This may lead to difficulties in distinguishing Swainson's Thrushes songs from the environment, potentially reducing the study's findings. Employing techniques like advanced audio recordings could minimize the impact of background noise (Naguib et al., 2019). The implications of this research are significant in the study of

avian ecology and behaviour. Understanding how environmental elements like sunrise timing and mountain aspect influence the Swainson's Thrush's dawn chorus will help us understand the species' vocalization patterns, mating behaviour, and migration patterns. There are also broader consequences, such as shedding light on the complex interactions between avian species and their surroundings, resulting in more informed conservation efforts (Bégin-Marchand et al., 2021). Additionally, studying the dawn chorus of Swainson's Thrush can enrich our understanding of how birds synchronize their activities with diurnal cycles and how they adapt to changes in their environment. As for future research, one possible research direction could be to explore how other environmental factors, such as temperature, humidity, and wind conditions interact with sunrise timing and mountain aspect to influence the Swainson's Thrush dawn chorus (Farina et al., 2015). This would provide a more holistic understanding of the intricate relationships between birds' vocalization and their environment. Another potential research direction we could take is considering other *Catharus* species, like Hermit Thrushes that display similar patterns of dawn chorus behaviour as Swainson's Thrushes in response to sunrise timing and mountain aspect. Expanding the study to include more closely related species may offer a broader perspective on how ecological and environmental factors shape avian patterns. Another direction for future investigation could involve examining the impact of human-induced disturbances, such as habitat fragmentation or noise pollution, on the Swainson's Thrush dawn chorus. This could help identify potential threats to the species and contribute to conservation initiatives (Farina et al., 2015). Additionally, in the future, we could better understand the entire population of Swainson's Thrush's dawn chorus in each direction and how it fluctuates throughout the day by considering the maximum or average number of Swainson's Thrushes observed while singing. This may make it easier for us to identify peak singing periods and any variations in the number of Swainson's Thrushes singing over time (Henderson 1977). We may also learn more about the spatial distribution of Swainson's Thrushes and how it relates to things like habitat and environmental conditions by comparing the overall number of Swainson's Thrushes in various directions (Henderson 1977).

## 5 | CONCLUSIONS

We hypothesized that if Swainson's Thrushes start singing when sunlight appears, then those that experience sunlight first will sing before those that experience sunlight

later. We found that our results supported our hypothesis and suggested that direction and time since sunrise had a statistically significant interaction in the LRT. However, it is worth noting that direction alone did not influence the mean number of songs. Time since sunrise and Julian days also influenced their singing behaviour as can be seen in Table 1. This study sheds light on the intricate relationship between environmental factors, such as sunrise timing and mountain aspect, on the dawn chorus behaviour of Swainson's Thrushes. The findings highlight the need for more investigation into additional factors that may influence the dawn chorus as well as the potential implications of these behaviours in the broader context of avian ecology and conservation, even though they offer insightful information about this species' vocalization patterns. We can aid in the conservation of biodiversity by deepening our awareness of these intricate relationships.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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# Breaking the Ice: Exploring the Link Between Glaciers and Mental Well-being

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## ABSTRACT

**Background** Glaciers are integral in maintaining hydrological cycles, moderating oceanic levels, and preserving valuable ecosystems. Cryospheric regions are often overlooked in evaluating the environmental factors affecting mental health. This study investigates the potential influence of glacial presence and melt behaviour on global mental health, particularly among marginalized communities.

**Methods** National suicide rates of general population and specific age categories were gathered from World Health Organization between 2012-19. Glacial data was sourced from the World Glacier Monitoring Service, and Randolph Glacier Inventory. Wilcox testing was conducted to identify mean suicide rates across countries with and without glaciers. Pearson and Spearman correlation testing were employed to identify relationships between melt rate indicators and suicide rates.

**Results** Over the entire eight-year duration, countries with the existence of glaciers revealed a notably higher suicide rate (p-value of 0.0001). Children aged 5-15 years old demonstrated a consistently higher suicide rate amongst countries with glacial bodies (p-value between 0.020-0.037). A positive correlation between regional suicide rates and glacial area was revealed, except in low-latitude countries. Although melt rate variability showed no significant correlation with suicide statistics, Greenland was the only country to demonstrate a negative relation among all populations.

**Conclusions** To address the ongoing impacts of the climate crisis, further research is necessary to develop an inclusive framework that acknowledges the unique challenges faced by communities living in cryospheric regions. This study is the tip of the iceberg, recognizing the importance of inclusivity in addressing the mental health implications of climate change in these environments.

**KEY WORDS:** glaciers, cryosphere, suicide rates, mental health, environmental factors, climate change

## 1 | INTRODUCTION

The paper explores correlations between climate change and its potential impact on mental well-being, emphasizing the value of natural environments. Recent research highlights these associations, particularly in urban areas, and their broader effects on cognition and mood (Graddini, 2022; Nutsford et al., 2013; Maes et al., 2021; Bratman et al., 2012). Likewise, protected areas have been found to improve general well-being (Reining et al., 2021;

Buckley et al., 2019; Wood et al., 2017). However, the rapid severity of climate change (Bellard et al., 2012; Dale et al., 2001) has led to increased negative consequences affecting both physical and mental health (Clayton, 2021; Cianconi et al., 2020; Berry et al., 2010). Solastalgia, or emotional distress due to environmental changes (Albrecht et al., 2007), exemplifies the complex interplay between mental health and climatic factors, with potential differential effects on populations.



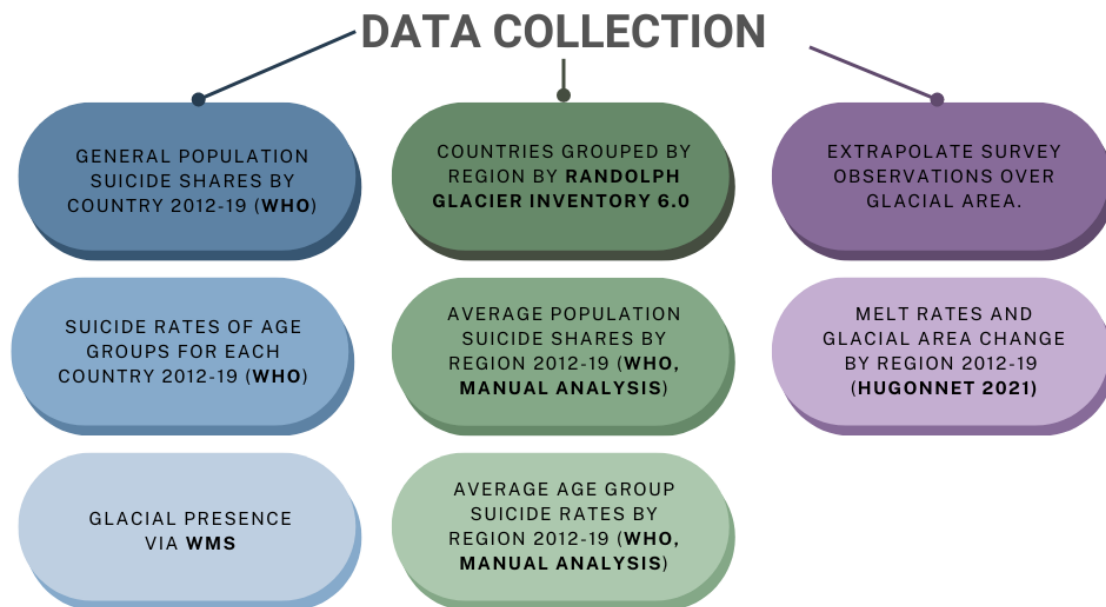
Certain groups, including low socioeconomic individuals, women, children, and Indigenous populations are often disproportionately impacted by climate change (Cianconi et al., 2021; Benevolenza & DeRigne, 2019; Clayton & Manning, 2018). Youth are especially vulnerable to mental health challenges related to the climate crisis. Many studies have attributed this link to a fear of the unknown future and the incredible responsibility to devise a solution (Vergunst & Berry, 2022; Cianconi et al., 2021; Majeed & Lee, 2017). Shifts in societal values have encouraged environmental perspectives on mental health to inform policy (Lawrance et al., 2021; Cunsolo et al., 2016; Gagné et al., 2014). Earlier attitudes often downplayed or ignored the connections between environmental factors and mental health focussing primarily on the physical consequences of climate change and environmental degradation.

Cryospheric environments are crucial components of the Earth's planetary system, demonstrating complex relationships with global health and society (Stefánsson et al., 2021; Su et al., 2019; Rasul & Molden, 2019; Hovelsrud et al., 2011). Cryosphere is derived from the Greek roots *kryó* signifying cold and *sphaíra* meaning globe. These regions are categorized due to the predominance of frozen hydrology, seasonally or year-round, such as glaciers and sea ice. Approximately 13% of the Earth's surface is identified as cryospheric through remote sensing and field observations (Barry & Gan, 2022; IPCC, 2019). They are key in balancing many climatological processes, driving hydrological cycles,

maintaining sea levels, and sustaining intricate ecosystems (Ren et al., 2019; DeBeer et al., 2016; Bentley, 1984). Cryospheric regions are also important in the greater network of water, acting as the largest storage of fresh water (Yonghian et al., 2020).

Cryospheric environments as a source are often overlooked in evaluations of the effects of the environment on mental health. However, the consequential natural disasters, hydrological stresses, and rising sea levels tied to them are often highlighted (Talukder et al., 2021; Milner et al., 2017). The exclusion of this essential component illustrates a discrepancy in fully understanding the relationship between society, environment, and health. This demonstrates the importance of recognizing the cryosphere beyond a climate indicator and approaching environmental research with a comprehensive approach to avoid such limitations to the policies shaped by current research (Carey et al., 2016).

Many Indigenous cultures and alpine communities hold a unique relationship with the cryosphere through historical and spiritual significance. The Inuit in the Arctic have traditionally relied on ice and snow for food, shelter and transportation. Many Buddhist communities in Tibet consider mountains and glaciers sacred and are often sites of monasteries. Indigenous cultures in the Andes see glaciers as deities or protective entities and are central to spiritual ceremonies and rituals. This connection reaches beyond melt cycles and the natural repercussions of the changing



**Fig.1: Data Collection.** Included elements and associated sources of each data set. Blue corresponds to glacial presence, green to suicides rates corresponding to regional melt observations, and purple to the melt rate and glacial size extrapolation.

environment (Ruiz et al., 2020; Burnasheva, 2020; Allison, 2015; Cunsolo et al., 2015; Jurt et al., 2015). Furthermore, frozen regions are delicate ecosystems highly susceptible to the effects of climate change, magnifying the threats to nearby populations. Indigenous values are driven by their traditional way of life. The disappearance of the surrounding cryosphere jeopardizes their cultural identity by compromising food security, traditional customs, spiritual teachings, and physical health (Cunsolo et al., 2015; Cunsolo et al., 2013; Hovelsrud et al., 2011; Dewailly et al., 1989). Understanding the relationships to cryospheric regions from Westernized **and** Indigenous perspectives is essential in understanding the complex influence of the climate crisis from a historical, scientific, economic, and sociocultural outlook on the environment. This carries the intricate relationship between nature and human life into developing reflective global policy and protecting the planet for a prosperous future (Gagné et al. 2014). It is worth acknowledging the limited research studies conducted on smaller Indigenous communities across Northern Canada that are directly affected by climatological implications in the cryosphere (Cunsolo et al., 2015; Cunsolo et al., 2013). However, current research has yet to bring meaningful policy change and raise awareness among the global population about the importance of this ecosystem. We hope this project can showcase the crucial role of frozen environments on a global scope and give a voice to these marginalized communities by encouraging discussions about environmental policy through mental health.

This research project assesses the potential influence of glacial presence and melt tendency on suicide rates across the world. We analysed suicide rates from 2012-19 to avoid potential skew due to elevated mental health challenges caused by the COVID-19 pandemic. Suicide rates are a quantitative measure requiring a level of categorization and investigation in contrast to a diagnosis process. As with other conditions, the reliability of statistics is highly linked to the quality and accessibility of resources and national perspectives on mental health (Tøllefsen et al., 2012). We looked at various age groups (>70 yrs., 50-70 yrs., 15-50 yrs., 5-15 yrs.) to gain insight into the consequences on specific populations, particularly vulnerable individuals such as youth. We expect a continuation of the witnessed negative trend in mental health due to environmental factors linked to climate change. This may be particularly detrimental to younger generations, due to a sense of responsibility to create a solution for climate change or anxiety associated with an

uncertain future. We believe this association is likely to be more prevalent among the cryospheric regions, as they are incredibly delicate environments and highly susceptible to climatological evolution. They also act as indicators of the deteriorating condition of our planet and exhibit accelerated rates of change (Haeberli et al., 2007). Researchers have noted changes in the role they play in ecological balance and human life across all domains (Talukder et al., 2021; Milner et al., 2017, Allison, 2015). Human populations are expected to adapt to this change in their surroundings often without warning or direction (Kääb et al., 2005, Cunsolo et al., 2015; Cunsolo et al., 2013). This could also amplify the perception of climate change and begin to outweigh their beauty. To the best of our knowledge, there is no current research evaluating the impact of cryospheric regions on mental health, particularly with respect to glacial bodies. Limited research has tied negative effects of the changing cryosphere to the mental well-being of Indigenous populations in Northern Canada (Cunsolo et al., 2015; Cunsolo et al., 2013). This study seeks to fill the gap in the literature, providing insights into the potential mental health consequences of cryospheric changes in populations worldwide. It is crucial to recognize the essential role of these environments and the effects on vulnerable populations to investigate the implications of climate change, from its origins to its outcomes. Embracing an inclusive approach that considers the intricacies between nature and humans is key to developing sustainable policies for a hopeful future.

## 2 | METHODS

### *Data Collection*

We compiled data sets and completed the initial analysis using Excel following the process outlined in Figure 1. To investigate the potential impact of glacier presence on global mental health, we compared suicide rates of countries across the world with and without glacial formations. We gathered data from the World Health Organization (WHO), including annual suicide shares for nearly every country and US territory worldwide between 2012-19. Additionally, we used suicide rates from various age groups provided by WHO (70+ yrs, 70-50 yrs, 50-15 yrs, and 15-5 yrs) to identify trends amongst these specific populations. We manually confirmed the existence of glaciers through the World Glacier Monitoring Service (WGMS) database and the Randolph Glacier Inventory (Pfeffer et al., 2014). Our objective to address the relationship between glacial melt behaviour and

the mental health of the related region involved a more extensive analysis. We organized countries by region categorized by the Global Terrestrial Network for Glaciers (GTN-G) Glacier Regions, see Figure 1 of the Appendix. For ease of analysis, multiple glacial regions may have been combined into a single data set, see Table 1 of the Appendix for affected areas. The annual melt rate and glacier area loss data were sourced from a recent Nature publication (Hugonnet et al., 2021). This data employed field observations, satellite data, and extrapolation techniques to analyze decades worth of melt data. We used the rates aligning with the 2012-19 period to match our WHO suicide data. We calculated the average suicide rates for the overall population and individual age groups across all included nations for each year. In data sets consisting of multiple regions, the glacial data was averaged.

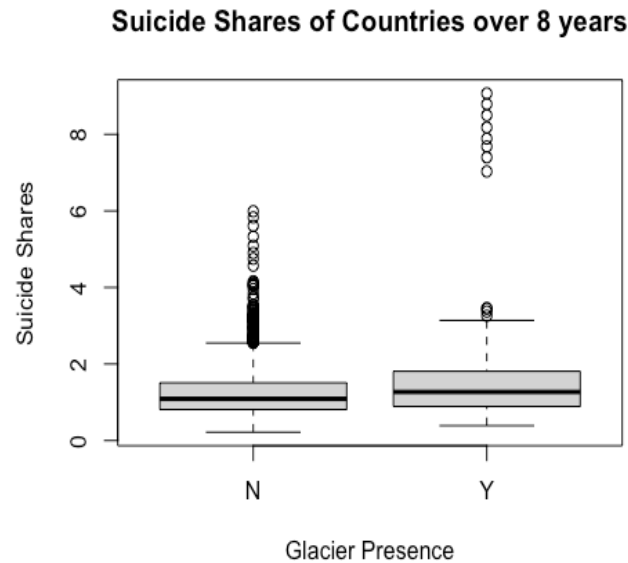
### Ethics

Considering ethical implications, this study aims to minimize negative effects on communities impacted by suicide through reporting and disseminating information responsibly and sensitively. From an ethical standpoint, this study would like to consider the individuals we may affect. Additionally, we prioritize the well-being of communities in proximity to glaciers, striving to avoid further marginalization and harm. Our ethical conduct prioritizes the benefit of these populations and amplifies their valuable perspective throughout this study. We also hope to encourage conversations around frozen environments and mental health through the inclusion of these groups.

### Data Analysis

We used the R programming language with tidyverse, ggpubr, dplyr, ggsignif packages for statistical analysis following the pipeline visualized in Figure 2. We began by conducting Levene and Shapiro testing for annual suicide data of the overall population and each age category to identify equal variance and normality. We performed a non-parametric Wilcoxon test to analyze the mean suicide rate between countries with and without glaciers. This process was repeated for each age group annually. The eight-year period of each nation's general population suicide shares was combined into a single data set to investigate an overall trend across a greater temporal scale. A p-value of  $<0.05$  was used to determine the statistical significance between means. Box plots were generated to visualize the relationship. We calculated the average glacial melt rate by extrapolating the

rate of a smaller observed region to the entire glacier area. Pearson and Spearman correlation testing was conducted between averaged suicide data and indicators of glacial melt behaviour for each region across the eight-year duration. Depending on the distribution of the data set, we performed the appropriate test across the overall population rates and individual age groups with the glacial area loss and calculated melt rates. A p-value of  $<0.05$  was considered significant and the correlation variable determined whether the variables

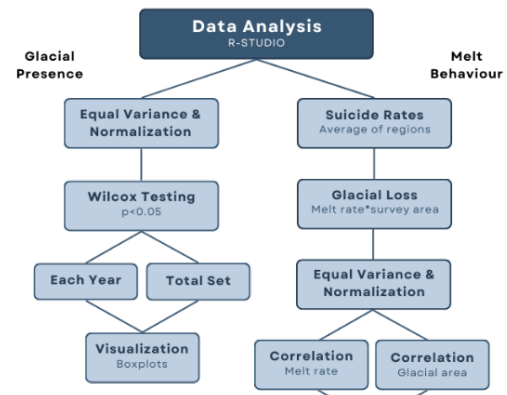


were positively or negatively related. The results were plotted to demonstrate the relation between variables.

**Fig. 2: Data Analysis.** A flowchart illustrating the analysis process followed using R to determine results for each data set.

## 3 | RESULTS

Wilcoxon testing of annual death shares (the proportion or percentage of total deaths within the



**Fig. 3. Box plot illustrating significantly higher mean suicide shares of countries with glaciers across the entire 8-year period 2012-19.**

population attributed to suicide) insignificant results. The compiled data set containing suicide shares (the proportion of total deaths attributed to suicides within a specific population) of countries during the entire eight-year period demonstrates a statistically higher mean in suicide rates of countries **with** glacial formations holding a p-value of **0.0001**. Suicide rates refer to a specific measure of the frequency of suicides within a population, often expressed per a certain unit of population and provides a standardized metric for understanding the prevalence of suicide in a way that accounts for differences in population sizes. Figure 3 illustrates these results as a box plot.

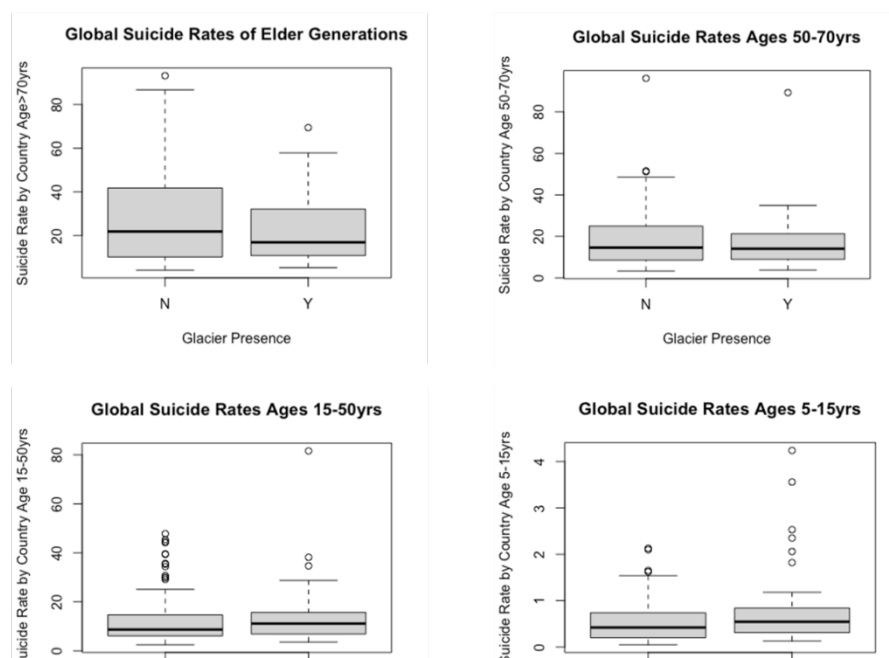
The plot in Figure 4A demonstrates a slightly higher mean suicide rate of individuals 70 years old and above in countries **without** glaciers consistent each year, however, Wilcoxon testing concluded this difference was insignificant. This discrepancy began to **equalize** in the population aged 50-70 years old, with an insignificant difference between suicide rates demonstrated by Figure 4B. Though statistically insignificant, Figure 4C illustrates a slightly higher mean amongst individuals ages 15-50 years old for countries **with** glacial bodies visually verified each year. The youngest population aged 5-15 years old indicated a statistically significant **p-value between 0.037-0.020** each year. As displayed in Figure 4D, a higher mean suicide rate was identified among countries **with** glacial formations.

Overall, each country demonstrated a unique relationship between melt behaviour and suicide rates with many inconsistencies present amongst different age groups. Melt tendencies were analysed based on two factors, glacial area size in and the annual melt rate in . No prevalent trends could be identified regarding annual melt variability. Greenland was the only nation to demonstrate a negative correlation between glacial melt rates and suicide rates amongst each population, however, considered statistically insignificant by Pearson correlation testing. This is illustrated by linear plots, corresponding to each population, shown in Figure 5.

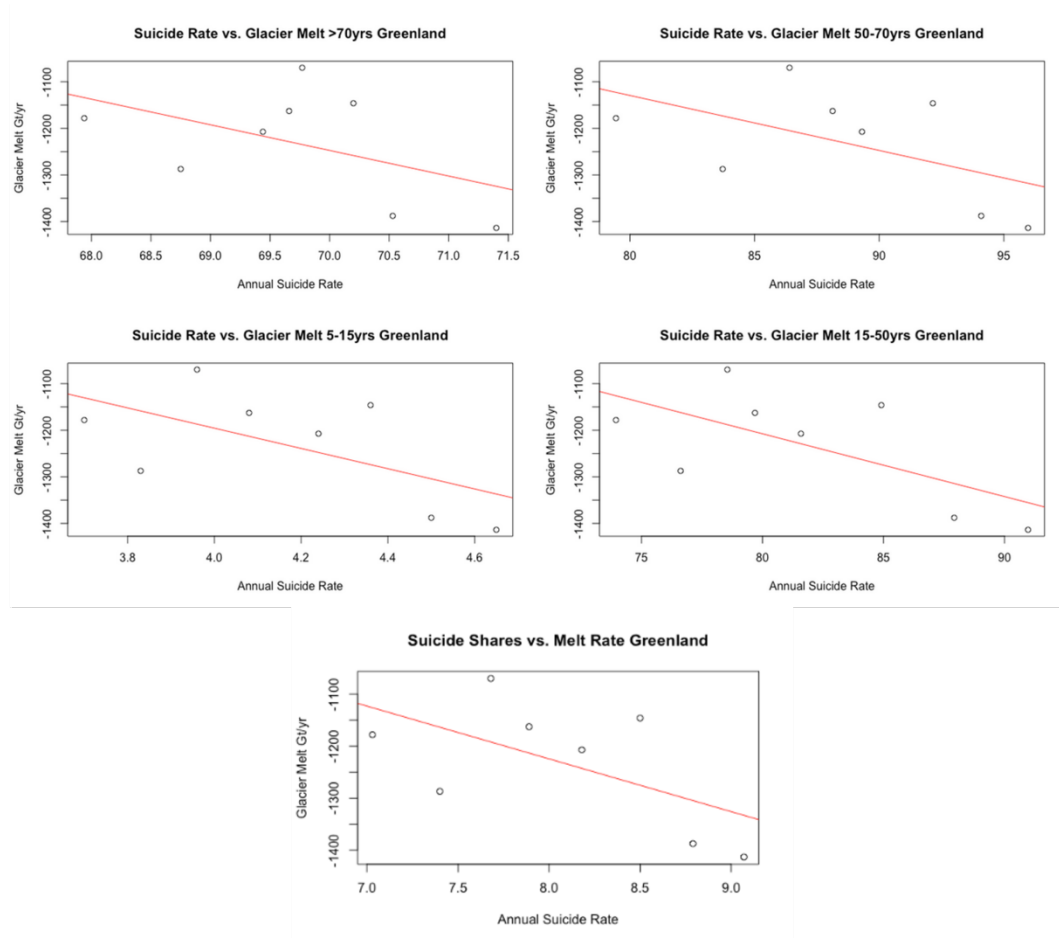
Each region consistently revealed a statistically significant relationship positively correlating glacial area with suicide rates of the general population, excluding the region of low latitudes. This relationship was prevalent throughout many of the age categories, with few exceptions. Figure 6 demonstrates the results of each region.

## 4 | DISCUSSION

As the climate crisis worsens, humans are experiencing a growing negative influence on their mental health. While for many, nature has traditionally acted as a sanctuary of safety and peace, the deteriorating state of our



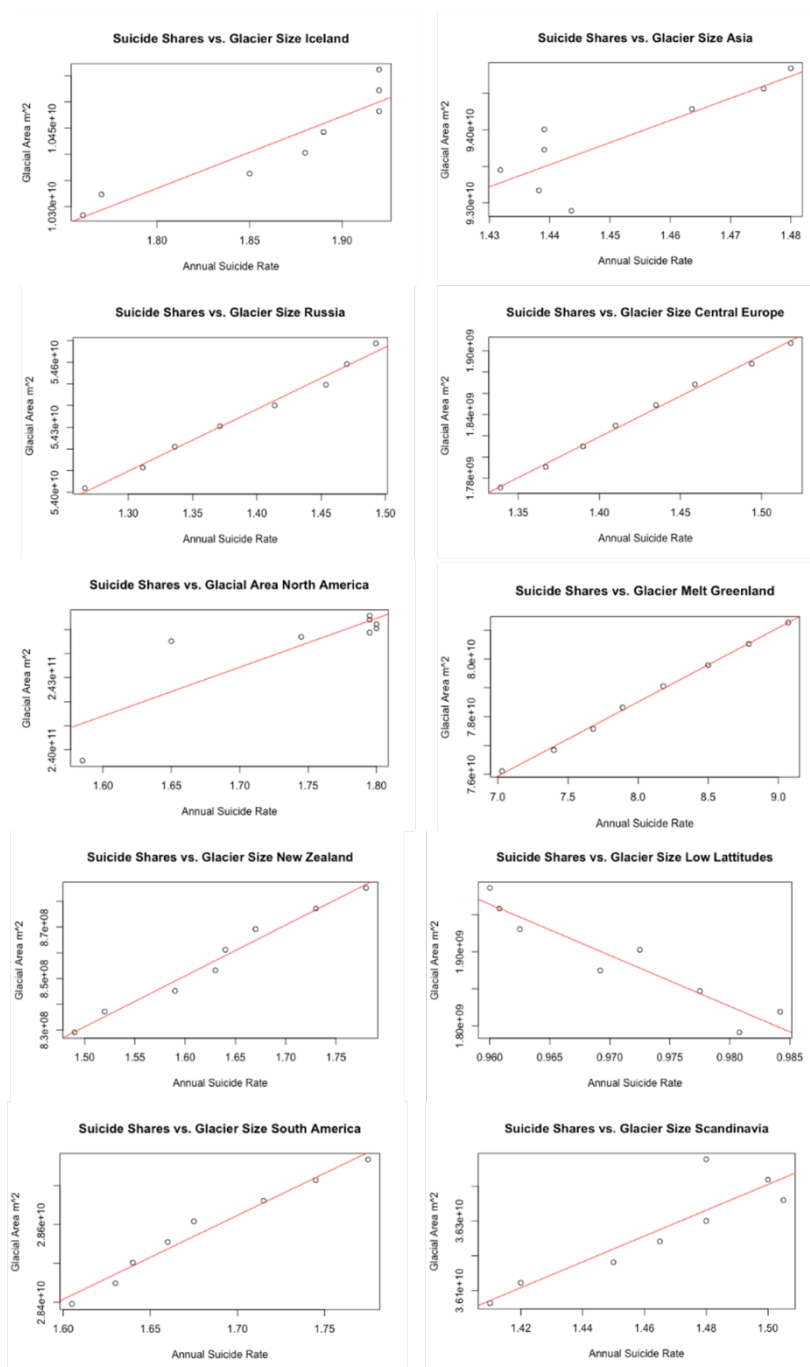
**Fig. 4A-D.** Box plot of illustrating mean suicide rates of various age groups between countries with and without glacial bodies in 2015, representative of trends present each year.



**Fig. 5A-E. Linear plots demonstrating the statistically insignificant negative correlation between melt rate variability and suicide rates consistent amongst the general population and age groups in Greenland between 2012-19.**

planet is threatening our physical, emotional, and mental well-being (Clayton, 2021; Cianconi et al., 2020; Berry, et al., 2010). Communities living in cryospheric environments are particularly vulnerable to the complex challenges to their physical and mental health related to rapid changes in their surroundings (Cunsolo et al., 2015; Cunsolo et al., 2013). The regions they inhabit are incredibly fragile, resulting in unpredictable weather patterns, natural disasters, food and water insecurity, and loss of traditional ways of life. It is essential to include these cryospheric regions in the evaluation of mental health in the context of climate change. By including these perspectives and experiences, we can gain insight into the direct challenges these populations face and how frozen features potentially affect the global population. This understanding can support sustainable and inclusive policy development for a prosperous future (Lawrance et al., 2021; Cunsolo et al., 2016; Gagné et al., 2014).

This study suggests that the presence of glaciers may have a negative impact on global mental health over a longer period, particularly among youth. We believe this could be due to various factors such as amplified losses associated with these highly vulnerable climatological indicators, the disastrous consequences resulting from unforeseeable changes in this environment, and the vital role these features play in human society. It is important to acknowledge the unique cultural connection between glaciers and their nearby population, whether it is spiritually recognized by Indigenous peoples in Northern Canada, or a source of national identity like in Iceland, where a funeral was held for a melted glacier (Guardian News and Media, 2019). Our results show a notable rise in suicide rates in children ages 5-15 years old with glacial formations each year. This supports previous research identifying the disproportional effects of climate change on youth (Vergunst, F., & Berry, H. L., 2022). This is concerning as children are highly susceptible to



**Fig. 6A-J. Linear plots demonstrating significant correlation relationships between glacial area and suicide rate between 2012-19 for each glacial region.**

changes in their environment lacking the coping mechanisms, knowledge, and resources to manage the stress and mental distress associated with the changing climate. The positive correlation identified between suicide rates and glacial areas of many of the analyzed regions is likely due to

unrelated trends in decreasing suicide rates globally through public health efforts specifically aimed at improving mental health and reducing incidence of suicide (Naghavi, 2019). Concurrent to suicide rates, climate change is worsening at alarming rates resulting in accelerated glacial loss. This is an

example of where correlation does not imply causation as the correlation is a result of external variables we could not account for in our statistical framework due to the scope of our study. The region consisting of nations at low latitudes demonstrated the reverse relationship; this should be investigated further. Greenland showed a negative correlation variable between melt tendency and suicide rates across the general population and specific age groups. Though statistically insignificant, this trend is potentially due to the significance glaciers have to national identity and the population's proximity to frozen regions. Overall, these interpretations offer initial insights into the potential influence of glaciers on mental health across the world.

### *Limitations*

While research has explored the indirect consequences of the cryosphere, the direct impacts on individuals and marginalized communities worldwide should not be overlooked. In light of these considerations, this study identifies several key limitations that deserve attention when interpreting its findings. First, the reliance on suicide data collected by the World Health Organization introduces potential limitations in capturing the full scope of suicide rates worldwide. Inconsistent data quality, as evident in Figure 2 of the Appendix, except for Greenland, was present in the final dataset. Additionally, the quality and accessibility of mental health resources and individual countries' approaches to reporting may lead to variations in the accuracy of data. These disparities in data quality pose challenges that are difficult to address comprehensively within the scope of this initial evaluation. It's crucial to note that suicide rates are not the sole indicators of mental health, emphasizing the importance of including all mental health conditions in policy advocacy, resource allocation, and stigma reduction efforts.

Second, the unique nature of cryospheric regions, often remote and hostile, limits the consistency and availability of data. These environments require a deep understanding of geomorphology and the broader systems influencing glacial behavior, making it challenging to generalize findings from limited field observations to a global context. Furthermore, while satellite data is valuable for assessing large-scale and long-term changes, it lacks the precision to account for the intricate internal dynamics of cryospheric environments. The study could not consider numerous variables related to these environments, such as their specific economic, industrial, societal, and cultural

connections, which might impact the results. While this quantitative analysis couldn't pinpoint the explicit causes of reported mental health outcomes, it does highlight potential relationships that warrant further investigation

To address limitations of this study, we suggest conducting a focused study across a region with a well-researched glacial body such as the Juneau Icefield. This would ensure consistent field observations with a comprehensive understanding of the geomorphology dictating melt behaviour and standardized access to adequate mental health resources and diagnosis procedures. This design would enable researchers to interview the nearby population and determine the factors directly impacting communities, thus providing more reliable mental health statistics and informing scientists for accurate interpretations. We would recommend investigating whether years of increased melt affect mental health instability in later years, to identify a potential trickle-down effect that takes time to reach a noticeable impact. To mitigate bias, analysis strategies should account for external factors and alternative environmental variables. A holistic approach should examine the environment as a larger system, exploring how each element could lead to potential influences on mental health. This framework could be employed on a national scale and applied globally to identify trends and study the effects on various regions. A larger systemic perspective is important when it adequately represents individuals. Asking inhabitants of countries containing substantial glacial formations directly if and how glacial melt and climate change affects their mental health would be valuable in establishing the validity of these claims. This complex issue of glacier impact on mental health is often overlooked due to the challenges associated with cryospheric regions. Through focused design accommodating diverse populations, we can develop policy strategies to address environmental conservation in the context of mental health.

## **5 | CONCLUSION**

This study aims to highlight the importance of including frozen regions and marginalized populations in assessing the impact of the climate crisis on mental health. These regions are essential in sustaining life on Earth and represent a component of human identity. By conducting initial studies such as this, we hope to encourage prioritizing cryosphere research and informing policies to protect these



environments as part of our human identity. The cryosphere deserves as much reverence as forests and oceans, despite seeming untouched and remote. Future research studies are not only an academic pursuit but an ethical imperative, as they must give voice to marginalized populations, shedding light on the unique and disproportionate mental health burdens they bear in the face of climate change.

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**Dr. Shereen Hamza is a Teaching Assistant Professor in the Department of Physiology at the University of Alberta, and is an Integrative Physiologist.**

**Emma Elder: Thank you for joining me today! Can you tell us a bit about yourself and your current role at the university?**

Shereen Hamza: I'm a Teaching Assistant Professor in the Department of Physiology. By training, I went through the University of Alberta for my Bachelor's degree in Physiology & Developmental Biology and then went on to do a PhD in physiology. I then went on to complete postdoctoral training at the University of Mississippi Medical Center. I have a lot of family ties here in Edmonton, so I returned to the University of Alberta in 2012 and have been here ever since.

**You refer to yourself as an Integrative Physiologist. Can you explain what that means?**

Yeah, integrative physiology means that, rather than studying an isolated organ system or isolated cell type, you always have the perspective that everything is connected with each other. When we study physiology from this approach, usually we're interested in how different systems work together to keep us healthy and to maintain normal function. Communication between systems can be compromised and lead to disease, or perhaps that communication is altered, which can also contribute to the initiation or perpetuation of a disease process. So, integrative physiology is really thinking about how things work together as opposed to in isolation. That's not to say that we don't do focused work, but we always have to

**"She completely surprised me and said yes."**

take a step back and say, "Okay, how is this functioning in the context of an intact system?" It can get pretty complicated, but the value in it is really high;

ultimately, if we're talking about a translation of what we're doing to improving human health, then, we're not talking about isolated systems anymore. It gets even more complex because it's not just the physiology, but also the social determinants of health, and things like trauma and other factors that come into play.

**As a physiology student myself, that's a really great explanation. When you were an undergraduate student, did you engage in research? What was that like?**

When I think of myself way back when I was an undergraduate student, I was not very well informed or savvy with respect to navigating the education system; I feel like students these days know a lot more than I did. I liked the process of investigation and working with my hands, so I was looking for

**"I was shown how to do different surgical techniques - literally holding my hands in their hands to demonstrate how to tie a suture for the first time."**

opportunities to gain lab experience. I emailed different professors, and either they were completely full or didn't have funding at the time. I would even walk into labs and speak to the supervisors directly, but I wasn't having any luck. There was a homeostatic physiology course I wanted to take, but to register you needed a form signed by a professor, so I took the form to the course coordinator Dr. Susan Jacobs. I noticed the sign on the door read 'cardiovascular physiology' and thought, "oh, that's kind of interesting." I went in and we had a brief meeting to sign the form, and as I

was getting up to leave, I just blurted out “would you be willing to have an undergraduate volunteer in the summer?” She completely surprised me and said yes.

I started in the summer, and mostly washed glassware and observed graduate students and post docs conducting their experiments. I was making sterile saline for the other trainees and providing support here and there. One of my jobs was to take a photocopy card, go down to the library, and take out the big volumes of journals to photocopy them for our lab

**“I wouldn’t say that I chose it. A lot of it was by chance.”**

database. When I started to build relationships with people in the lab, observing the experiments turned into supporting set up and organizing instruments. Shortly after that, I was shown how to do different surgical techniques – literally holding my hands in their hands to demonstrate how to tie a suture for the first time. Truly, it was baby steps. By the end of that summer, I stayed on to do a fourth-year research project which culminated in my first publication.

**I’m so glad you mentioned your naivete as an undergraduate. At Eureka, our mission is to encourage young scientists to engage in research, and we provide a platform and the resources to do so. Speaking of starting in research, after taking a few of your courses, your love for renal and cardiovascular physiology is evident. How did you end up choosing that research area?**

You know, I wouldn’t say that I chose it. A lot of it was by chance. The topic of my first publication was on portal hypertension, which is a condition that occurs with chronic liver diseases. In that context, there is a lot of cardiovascular dysfunction that happens, including in the kidney. Many of my projects involved developing novel techniques...one even involved using a vibrator to directly and evenly infuse substances into the kidney, believe it or not. When I was finishing my PhD and chatting with my mentor about next steps, an

email had popped up on her computer. It was an advertisement posted by Dr. John Hall – the renowned renal physiologist and editor of Guyton and Hall’s Textbook of Medical Physiology. He was looking for two post-doctoral fellows in renal and cardiovascular physiology. My mentor read the advertisement, and she said they were describing exactly me. I thought, “I’ll just submit an application, why not.” After a two-day intensive interview process in Mississippi, I received the offer by email when I was at the airport on my way back to Edmonton.

**I’m going to shift gears a little bit. It’s no secret that you’re a student favorite in the Department of Physiology – can you tell us more about your transition from a research setting to the classroom?**

Well, to start, it’s a pleasant surprise that my courses and teaching are so well received, and I can play a small part in a student’s journey. It’s humbling, actually, because when I think about it, I’m just me. Again, teaching is not something that I planned, but I always enjoyed it. As a graduate student, we used to run review sessions for second-year undergraduates in physiology before midterms and final exams. It was great, because it gave us the chance to review all the foundational physiology concepts, and I enjoyed guiding younger peers. I frequently supervised undergraduates in the lab, and my PhD mentor always

**“It comes easily to me; for example, I might be driving or washing the dishes and think of an idea for a lecture topic or exam question, or even an idea for an entire course.”**

made sure that each of her graduate students had a guest lecture in her homeostatic physiology course. When I came back from my post-doc in Mississippi in 2012, it coincided with her retirement, and I later stepped in to coordinate that course. That was my first real experience with being a coordinator and teaching regularly, instead of guest lectures here and there.

Over the years, there have been opportunities that have presented themselves, or I have stepped up and offered to coordinate courses that were at risk of being lost because there was no longer a faculty member to facilitate it. That is how I wound up with a lot of teaching over the years, and I have discovered that I not only enjoy it, but I have a lot of ideas. It comes easily to me; for example, I might be driving or washing the dishes and I think of an idea for a lecture topic or exam question, or even an idea for an entire course. It's a privilege interacting with students and seeing them develop skills, grow, and succeed. I haven't completely let go of research, but these days it's in more of a collaborative capacity as opposed to being primarily a researcher with a busy lab and lots of students.

**During the pandemic, you received the Remote Teaching Award for your online lecture videos. In fact, a lot of students describe listening to your lectures to that of a podcast because they're so smooth and easy to follow. What was it like being an instructor during those unprecedented times of COVID? Has it impacted the way that you run your courses now?**

Yeah. Big impact, big change, and it totally changed some aspects of my teaching philosophy to begin with. But it was scary, really scary. At the time I had heard of Zoom but hadn't actually used it. I also had never done any online teaching before, and most of my courses had two midterms and one big final exam – all written.

**“The pandemic really changed my teaching philosophy.”**

In the Winter 2020 semester, when we shifted to credit or no credit instead of the typical grading scheme, I received tons of emails from students. They

were worried and devastated because a lot of them needed this grade for their GPA so they could apply to professional programs. Many of these emails were alarming just on the human level; students were saying they don't see what the point was and how they don't know how to go on, things like that. It was really worrying.

**“Instinctively, I felt I needed to connect with these students. I sat in front of my camera and made the first video ever. It was short and unscripted, but from the heart. I acknowledged that yes, this is a scary situation, and I am scared too.”**

but from the heart. I acknowledged that yes, this is a scary situation, and I am scared too. Then I asked students, “What was your intention when you signed up for this course?”. For some, it was to get an A+ and go do whatever they were planning to do with their career, but if their real intention was to learn something and use it later in some capacity, does it really matter if we have a grade or not? After providing some reassurance, the final projects that I received were very well done, and they produced some really inspired work.

During the summer of 2020, I knew that the fall semester would still be online, so I started teaching myself online course design and bought some resources to study on my own. I watch a lot of YouTube, and I enjoy when YouTubers post on a regular schedule and have consistent branding – so that's exactly what I did. I created a YouTube channel for each of my courses, and I started creating a brand through my content. I played around with intro and outro music, and did weekly introductions where I would appear on video just to create that sense of connection and community with my students. Although, it was weird in those early days of the pandemic to look into the camera lens and see myself. I was not prepared for that, and it took some getting used to. I have kept assessments the same since then,

So, instinctively, I felt I needed to connect with these students. I sat in front of my camera and made the first video ever. It was short and unscripted,

creating problem-based questions and scenarios that have real-world applications. Students really enjoy these assessments and got more out of it because they could just focus on learning the concepts and how to use the information to solve a problem, rather than

**“You’re trying to do something that hasn’t been done before. There’s no instruction manual.”**

stressing about memorization. The pandemic really changed my teaching philosophy with respect to assessments, and I saw what was possible and what worked better for students. It takes a long time and a lot of effort to evaluate that type of work, but it’s an investment on everyone’s part, so I think it is worthwhile.

**I’ll say from a student perspective, after taking a few of your courses, I have never learned so much in a class, truly. I think about the earlier years of my undergrad where I would memorize a wad of information, spit it out on a test, and then forget about it. I could not agree more with your approach, and I thank you for doing things this way.**

That’s the important thing, and it kind of goes back to the teaching philosophy. Like, what’s my goal here? What do I want to help people to achieve? Is it just to get good grades? Well, yeah, that’s possible. It’s also possible to assess students in ways that might make my workload a lot easier. But, if the ultimate goal is to help people develop skills and form a solid knowledge base that they can use in other ways, it’s totally worth the investment.

**Nothing but high praise for you, Dr. Hamza. Looking at your career thus far, what do you think has been the greatest challenge? I know it’s a heavy question.**

Yeah, there’s a lot of big challenges. In my early graduate student and postdoc days, we were trying to do things that people hadn’t done before, also from a

technical perspective. A large part of the work that I’ve done in the lab has involved developing new methods which can be really frustrating. It can also be really lonely, because you’re trying to do something that hasn’t been done before. There’s no instruction manual. My lab books were my best friend because they turned out almost like a journal – I would kind of talk to the lab book, *“I tried this today and it didn’t work for the 110<sup>th</sup> time. This is what I’m going to come back and try tomorrow.”*

Most of the time, things are going to fail. This was also true when I was in Mississippi; we were trying to develop a method for recording renal sympathetic nerve activity in conscious mice. It had been established in rats, but no one had published it in mice before. That also involved a lot of trial and error, a lot of frustration, and a lot of tears. I remember driving home on the highway and sitting with my frustration in the car. As a young person, you’re looking forward to when your ‘real life’ is going to start, so every time an experiment doesn’t work you can’t help but take it to heart; you feel like it is another delay in all the other things you want to achieve.

**“The need to innovate has always been a challenge, but a welcome one.”**

Also, when you’re teaching at the advanced levels, there’s always new information coming out, and you’re always innovating. You have to be able to keep up with it and help students make sense of it, and often we can’t make sense of things. There is no answer. So, that is also a challenge. Life throws things in your path and you have to figure out what to do. How do you edit a video? How do you start a YouTube channel and post videos? Now this is routine for me, but at the time it was a huge learning curve. So, I would say the need to innovate has always been a challenge, but a welcome one.

**You know, it’s really comforting to hear that professors were also struggling to develop a routine during COVID and they were also scared.**



Yeah, most of my videos were recorded at two or three o'clock in the morning!

**Now that I think back, it was pretty dark in the background in some of those videos so I'm not surprised! As a final question, what advice would you give to undergraduate students who are interested in science, but they don't really know what to do in terms of a career?**

I would say a couple of things. First, give yourself a break, and don't worry because you're still young! There are lots of opportunities, and you don't have to have everything all figured out now. I don't have everything figured out now. You know, you have this

**“Grades aren’t always the best reflection of our knowledge, our abilities, and our potential.”**

idea in the back of your mind of, *what am I going to be when I grow up?* That's still evolving for me. I have a lot of colleagues who share that sentiment as well. So, I would say, relax, and don't feel like you have to be rushed. The other thing I would say is don't be afraid to try. You don't know if something is going to be a good fit unless you actually immerse yourself in that experience. A great way to try is to be open to being a volunteer. Be open to experiences, and also don't be afraid to admit when something isn't quite the right fit.

Another thing that comes up from time to time is grades. Grades aren't always the best reflection of our knowledge, our abilities, and our potential. The reality is that grades have some importance for applications and awards and such, but sometimes this can lead students down the path of working for a grade rather than building their knowledge and developing their skills. In physiology we see a lot of students interested in a career in medicine or dentistry. If you apply to a program and don't get in, it could just be that you have a different destiny, a different purpose. And even if students do wind up in those programs, they might find after a very long road and maybe after a lot of money and heartache, that it's not where their heart lies. My advice to students is to be open to alternative careers – ones that may not even exist yet. There are tons of opportunities in the developing world of science.



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