WHOLE-BODY INTERINDIVIDUAL RADIATION-INDUCED BYSTANDER EFFECT IN HOUSE CRICKETS (*ACHETA DOMESTICUS*)

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TITLE: Whole-Body Interindividual Radiation-Induced Bystander Effect in House Cricket (*Acheta domesticus*)

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Lay Abstract

This thesis explores the field of non-targeted low dose ionizing radiation using a cricket model to understand radiation-induced bystander effect (RIBE) on an interindividual level. RIBE is when irradiated individuals cause unirradiated individuals to respond as if they have been irradiated, oftentimes leading to shifts in development. This work has implications for environmental radiation protection and outlines how irradiated individuals could indirectly affect unimpacted regions. I showed examples of interindividual RIBE using whole-body endpoints, where interactions with irradiated individuals will cause a developmental shift in unirradiated individuals. I demonstrated how indirect interactions, where irradiated crickets who never occupied the same space as unirradiated crickets can still mediate RIBE. I showed that irradiated cricket eggs can also mediate bystander signal transfer. These contributions further the field of environmental radiation protection and RIBE, demonstrating the potential widespread impacts of RIBE to ecosystems.

Abstract:

Ionizing radiation is an area of concern in environmental stress and protection. Although the targeted effects of ionizing radiation on DNA and the impacts on the health of human and vertebrate species are well understood, there is a paucity of data when exploring non-targeted effects of ionizing radiation, particularly on invertebrates. One such effect is radiation-induced bystander effect (RIBE), the signal mediated transfer of irradiated effects from irradiated to unirradiated individuals. Here, I am one of the first to translate *in vitro* RIBE research and demonstrate them with *in vivo* endpoints using house crickets (*Acheta domesticus*). I demonstrated the effect of RIBE on development, showing how interactions with irradiated individuals can shift development of unirradiated individuals, resulting in higher average growth rate at maturation in the population, oftentimes due to faster maturation time. Further research suggests some of the possible mechanisms of signal mediation in RIBE in the absence of direct interactions. I demonstrate that RIBE can be mediated to unirradiated individuals through soiled housing materials or biophotons, shifting development. Lastly, I demonstrate that irradiated cricket eggs can cause RIBE, and that these exposed eggs can signal and mediate RIBE, delaying maturation of unirradiated juveniles. My research suggests widespread environmental ramifications for RIBE, suggesting a need to shift our focus away from targeted effects to more holistic models of radiation protection to fully understand the implications of ionizing radiation on the environment and individuals.

Dedicated to my parents, who sacrificed everything to give me anything.

Thank you.

Acknowledgements

I am not one for nostalgia, so the acknowledgements might bother some people for being short and curt, for that I apologize.

I am extremely appreciative of every single person whose paths I've crossed during my time here at McMaster, from first year undergrad all the way to the end of my PhD, a decade in the making.

Likewise, for the friends I have made throughout my childhood from when I first moved to Canada all the way up until now, for making me who I am today.

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To my parents, who uprooted their entire lives just to give me a chance at a better one.

I hope that I make you proud.

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Acronyms

ALARA As Low as Reasonably Achievable

ANOVA Analysis of Variance

BER Base Excision Repair

Bq Becquerel

Ci Curie (unit)

CEZ Chornobyl/Chernobyl Exclusion Zone

DSB Double Stranded Break

GJIC Gap-Junction Intercellular Communication

Gy Gray

HR Homologous Recombination

ICRP International Commission on Radiological Protection

LNT Linear No Threshold

LT Linear Threshold

MMR Mismatch Repair

NER Nucleotide Excision Repair

NHEJ Non-Homologous End-Joining

NTE Non-Targeted Effects

RIBE Radiation-Induced Bystander Effect

ROS Reactive Oxygen Species

SIT Sterile Insect Technique

SSB Single Stranded Breaks

Sv Sieverts

Declaration of Academic Achievement

I, Xiaobing Li, declare that this thesis and the works presented here are my own.

All chapters, unless otherwise referenced, are my original ideas and designed with guidance from Dr. Carmel Mothersill, Dr. Dave Rollo, Dr. Colin Seymour, and Dr. Jon Stone.

Animal husbandry of experiment animals, irradiation, and data collection was all performed by me. Data analysis and interpretation was performed by me with guidance from Dr. Katie Pelletier for chapter 2 and Dr. George Samuel Long for chapter 3 and 4.

As this thesis is a sandwich thesis, chapters 2-4 are written in style of manuscripts, with some published and others in preparation. The writing and publishing process are completed by me with guidance from Dr. Carmel Mothersill, Dr. Dave Rollo, and Dr. Colin Seymour. The rest of the thesis is my own writing with editorial feedback from Dr. Carmel Mothersill, Dr. Dave Rollo and Dr. Tamara Fuciarelli. Chapter 6 within the appendix was written and published by me with guidance from Dr. Dave Rollo.

Animal husbandry of the cricket colony and maintenance of the lab were performed by Xiaobing Li, Dr. Tamara Fuciarelli, Susan Marsh-Rollo, undergraduate thesis students, lab techs, and volunteers from McMaster university.

Preamble

Dear reader,

This thesis is prepared in a "sandwich" format with chapters 2 through 4 being written in the style of journal articles. These chapter should be viewed as a stand-alone piece with its own introduction assuming no prior knowledge of the other chapter, as such there may be overlap in introduction and methodology of these papers. There is a general introduction and conclusion chapter which introduces and summarizes chapters 2 – 4, and their relevance.

Readers might notice that a published article is tucked away in the appendix (chapter 6), this is not me trying to hide anything. Quite the opposite, this publication does not fit the theme or hypotheses of the main thesis and is therefore not discussed within the main body of this work. However, I felt it necessary to still include as it was work done during my graduate career, and my first published work.

All figures, tables, and references for each chapter are presented at the end of each chapter. Chapter 2 and 6 are represented *verbatim* from published articles, with copyright information present on the first page of the chapter. Citation, page formatting, figure and table numbers were adjusted to fit the overall style of the thesis.

I hope that you will have as much fun reading this as I did writing it.

Chapter 1

1. Introduction

Invertebrates account for a large fraction of the earth's biomass, and are critical to most ecosystems as food sources, detritivores, and pollinators (Yang & Gratton, 2014). Yet, amidst the sixth massive extinction event, invertebrate, particularly insect extinction has been largely ignored in favour of larger, more noticeable animals. In radiation research, the primary research goals on insects often focus on reducing insect populations for agriculture and zoonotic diseases, and rarely on the impacts of radiation on insect population and ecosystem health (Benedict, 2021). There also exists a paucity of data regarding non-DNA and non-targeted effects (NTE) of ionizing radiation, with most studies focusing on the immediate targeted effects of ionizing radiation on DNA for medical and remediation purposes. There has been a concerted effort to shift towards more holistic models in radiation research, investigating beyond targeted effects and direct DNA damage to understand NTE of ionizing radiation. Insects proved to be excellent research species due to their quick generation times and large sample sizes, allowing for ease of understanding long-term, developmental impacts of ionizing radiation (Li & Rollo, 2021). Using the house cricket *Acheta domesticus*, this work aims to address this paucity of data by better understanding how insects respond to ionizing radiation, particularly beyond targeted effects, investigating -targeted effects of low dose ionizing radiation on a whole-body, interindividual level. Here, I examine the effects of radiation-induced bystander effect on *A. domesticus* development. Each individual

chapter for this work will have its own introduction, with this introduction serving as a general introduction and background on the field at large, and how this research fits in.

Perception of Radiation Throughout History

The public perception of radiation has gone through phases of fame and infamy, as such it makes sense that focus of radiation research fluctuates and is shaped in accordance with the public perception of radiation. This section focuses primarily on the public perception of radiation and how legislation around radiation safety came to pass, and not so much on medical benefits of radiation in cancer research and treatments. Radiation has demonstrated to be effective as a treatment for cancerous tumors and other diseases, but this research is not biomedically focused (Reed, 2011).

X-rays were discovered in 1895 by Wilhelm Conrad Roentgen, and within six months mass production and use had begun due to its potential medical abilities (Reed, 2011). Just a few years later Henri Becquerel, Pierre Curie, and Marie Curie discovered the radioactivity properties of uranium and radium, coining the term “radioactivity” (Mould, 2007; Reed, 2011). Radiation was viewed as a novelty and technology of the future, radium became all the rage as people abused its name in an effort to get rich (Gunderman & Gonda, 2015; Mould, 2007). As time went on however, the dangers of radiation became apparent with the death of Marie Curie, radiation burns for Henri Becquerel and Pierre Curie, and the infamous radium girls (Gunderman & Gonda, 2015; Mould, 2007). The public opinion of radiation took a nosedive, and by the 1920’s, guidelines were set in place by governments to protect the public, with research focused on understanding what makes radiation so dangerous, and how to harness its powers (Clarke & Valentin, 2009).

Unfortunately for the world, the dangers of radiation came to a head on August 6th, 1945, with the atomic bombing of Hiroshima, and then Nagasaki three days later. The aftermath of the atomic bombs saw a drastic shift in public perception and research, shifting focus to understand short and long-term effects of radiation damage on survival and reproduction. The reputation of radiation took further hits on April 26th, 1986, and on March 11th, 2011, with the nuclear reactor disasters of Chornobyl (Chernobyl) and Fukushima respectively. The public opinion of nuclear power and radiation as a whole has therefore been reasonably negatively skewed, as the deleterious effects of ionizing radiation are reported from these disasters. Nevertheless, research persisted despite public opinions, and we are now acutely aware of the benefits of radiation, and its undeniable role, from x-rays, radioisotopes, cancer treatments, to nuclear power generation.

Nuclear Accidents & Fallout

The Chornobyl disaster provided a unique opportunity for researchers to observe and examine long-term, chronic impacts of ionizing radiation on an ecosystem (Møller & Mousseau, 2006). Despite this opportunity however, there lacks continued interest and funding from governing body on the long-term effects of chronic radiation on non-human biota, leading to inadequate support for large longitudinal projects.

In media, the negative outcomes of Chornobyl and its inhabitants are now oftentimes downplayed, focusing primarily on highlighting the increased biodiversity in the area (*How Chernobyl Has Become an Unexpected Haven for Wildlife*, 2020; Orizaola, 2020). While it may be true that there is increased biodiversity and abundance within certain pockets of Chornobyl, that is caused primarily from the lack of anthropogenic

disturbances. Researchers continue to demonstrate negative correlations between radioactivity and abundance, particularly among terrestrial arthropods (Bezrukov et al., 2015; Møller & Mousseau, 2009, 2016, 2018). Looking at migratory birds, the picture becomes starker as elevated DNA damage levels are observed (Møller & Mousseau, 2015). Putting a further question mark on if the animals within and surrounding Chornobyl are actually adapting to elevated background levels of radiation or are they just suffering silently (Møller & Mousseau, 2015).

Ionizing and Non-Ionizing Radiation

Before diving farther, I feel it imperative to explain in brief what radiation is, what is ionization, what are the properties that make radiation so valuable/dangerous, and why I was and still am fascinated by it. The International Atomic Energy Agency defines radiation as "energy that moves from one place to another in a form that can be described as waves or particles" (*What Is Radiation?*, 2023). There are two major categories of radiation, non-ionizing and ionizing. Non-ionizing radiation refers to radiation that does not have enough energy to eject or "ionize" electrons from atoms or molecules, while ionizing is radiation has enough energy to do so (Desouky et al., 2015). Non-ionizing radiation is typically harmless, but intense or prolonged exposures may still result in damage to the body. The divide between non-ionizing and ionizing radiation happens within the ultraviolet spectrum, but there is no hard transition (Desouky et al., 2015).

Types of Radiation Dose Units

Radiation is measured through radioactivity and denoted in becquerel (Bq) units, where 1 Bq is 1 radioactive decay per second (Materials, 1999; US EPA, 2017). While useful to know and to measure when looking at radioprotection, the unit most researchers utilize is absorbed dose, or how much radiation is absorbed from the emitter. Absorbed doses are measured in gray (Gy), where 1 Gy is 1 joule of energy absorbed by 1 kilogram of biological matter (Materials, 1999; US EPA, 2017). Gray is widely used in both radioprotection and radiation research, and is the standard units used in reporting and setting radioprotection standards. However, different types of radiation means that not all Gys are created equal, as 1 Gy of gamma radiation could have more harmful effects than 1 Gy of alpha or beta radiation. To equalize the effects of radiation, particularly on human health, the equivalent dose, or sieverts (Sv), was created. Sv is calculated using a specified radiation weighting factor to equalize the effect different types of ionizing radiation could have on the body. The effective dose is then derived from the equivalent dose. Which aims to encapsulate the whole-body probability of increased cancer and tumor risks by accounting for equivalent dose of all exposed specified tissues and organs in a human body (Materials, 1999; US EPA, 2017).

Ionizing Radiation Damage

Majority of radiobiology research focuses on ionizing radiation protection, remediation, and radiation therapy, as they have the ability to induce DNA damage (Baskar et al., 2012; Desouky et al., 2015; Kryshev & Sazykina, 2015). There are six types of ionizing radiation, alpha, beta, positron, gamma, x-rays, and neutrons (Karmaker et al., 2021).

Neutrons differ from all other types of ionizing radiation as they can make other objects radioactive (Thomadsen et al., 2014). Excluding neutrons then the rest fall into one of two camps in how they ionize, direct or indirect. Alpha, beta, and positrons are all directly ionizing and have low penetrative properties, i.e. cannot travel far in air and will be absorbed by the top layer of skin, but they are electrically charged and can directly interact with biological matter (Karmaker et al., 2021). Gamma and x-rays fall under indirect ionization, as they are neutrally charged and do not interact with biological materials. They have high penetrative properties and interact with water and organic molecules within the body to generate free electrons and reactive oxygen species, which then can induce ionization within the body (Karmaker et al., 2021).

As the works presented here utilizes ionizing gamma radiation, I will summarize in brief indirect ionizing radiation, also known as electromagnetic or photon radiation (Hall & Giaccia, 2018). Both x-rays and gamma rays are considered photons, with the difference being where and how the photons are produced. X-rays are produced extranuclearly, free electrons are generated from machines and accelerated to high energies then abruptly stopped, with some of the kinetic energy being converted to x-rays. Gamma rays are produced intranuclearly via radioactive isotopes from the excess energy given as they return to stable forms (Hall & Giaccia, 2018).

Radiation energy is not deposited evenly within the body, and it is the size of the individual streams of photons, or packets of energy that determines if there is enough energy to eject electrons and be classified as ionizing, or if there is insufficient energy to eject electrons from the atoms, which is classified as non-ionizing, or simply excitation

(Hall & Giaccia, 2018). The ionization of atoms and generation of free electrons is the basis for radiation damage. There are two major ways in which DNA is damaged, the first being radiation is directly interacting with DNA, breaking the bone between the polynucleotide backbones. Or with indirect ionization, ionization of atoms generates reactive oxygen species (ROS) which can chemically change DNA, breaking the bonds and leading to DNA damage (Hall & Giaccia, 2018).

DNA Damage and Repair

By damaging DNA and cellular structures, ionizing radiation can lead to increased risk of cell death, elevated cancer and tumorigenesis risk, and increased somatic and germline mutation risk (Baskar et al., 2012; Desouky et al., 2015). Cellular radiation responses have been shown to utilize general stress response pathways, with questions regarding the uniqueness of ionizing radiation effects on the body, and if radiation can be grouped similarly to other stressors, as such it is often referred to as ionizing stress. (Mothersill et al., 2024; Mothersill & Seymour, 2014).

High doses of ionizing radiation directly correlate with DNA damage, including cytotoxic, carcinogenic, and mutagenic impacts on DNA and cells, with organisms exposed to ionizing radiation demonstrating increased induced DNA lesions and cell death (Lomax et al., 2013; Morgan et al., 1996). There are two main types of DNA damage that are induced via radiation, single stranded and double stranded DNA breaks. Single stranded breaks (SSB) are when one of the two phosphodiester backbones of DNA breaks. They are repaired through DNA excision repair mechanisms, three main excision pathways are currently understood: nucleotide excision repair (NER), base excision repair

(BER), and mismatch repair (MMR). NER is the main pathway used in mammals to repair lesions caused by acute radiation. BER is responsible for small non-helix-distorting base lesions. MMR primarily targeted mismatched bases during DNA replication and recombination but can also target and repair mismatched bases caused by DNA repair (Anindya, 2020; Lindahl et al., 1997).

Double stranded breaks (DSB) are of more interest to radiation researchers, as they are the more deleterious lesion type, and are the major cause of deleterious mutations and cell death after radiation (Rothkamm & Löbrich, 2003; van der Schans, 1978). DSB are when the phosphodiester backbones of both strands of DNA are broken, and are separated by 10 or less base pairs, making it harder to repair using SSB repair mechanisms due to the lack of reference strands (Mladenov & Iliakis, 2011).

There are two main pathways identified for DNA DSB repair, homologous recombination (HR) and non-homologous end-joining (NHEJ). HR uses intact homologues from undamaged DNA that shares extensive sequence homology with the damaged DNA. Genetic information is retrieved from the undamaged DNA and used to repair the broken base pairs. It can only be used in cases where a homologue is found, but it is typically error free and does not cause sequence insertions or deletions due to the extensive sequence homology (Mladenov & Iliakis, 2011; van de Kamp et al., 2021). Most of the time, when a homologue cannot be found, NHEJ is used. NHEJ induces ligation to the two sides of a DSB without the need for a homologue guide, however this leads to a higher rate of error in repair, resulting in sequence deletions or insertions (Mladenov & Iliakis, 2011; van de Kamp et al., 2021).

So far everything I've discussed regarding ionizing radiation has been centered around DNA damage, with both direct and indirect ionizing radiation falls under the targeted theories of radiation. Targeted theories postulates that ionizing radiation must hit a target, DNA, water, or other, to induce damage to the cells and DNA.

Targeted Theories of Ionizing Radiation

Using primarily data from survivors of atomic bombs supplemented with lab studies, the first of the targeted response models proposed was the linear threshold (LT) model, which postulates that since all organisms have a base level of radiation resistance, any exposure below a certain threshold will not increase DNA damage and health risks. Any exposure above the threshold would then cause increased risk, scaling linearly to the exposure (Christensen et al., 2014).

The second model proposed was the linear no-threshold (LNT) model. This model uses similar data as the LT model, except that it now extrapolates data for the low doses and assumes no base level of radiation protection (Christensen et al., 2014). LNT predicts that any and all damage will have a corresponding increase in DNA damage and health risk. This is believed to be the safest model, hence why it is used often in radiation protection. However, as research and data from low dose ionizing radiation becomes more prevalent, it is clear that the targeted theories do not fully account for the observed biological data at low doses, and other factors are at play in the low dose range (defined hereinafter as 0.5 Gy and below).

Non-Targeted Effects and Ionizing Radiation

At low doses of ionizing radiation, researchers have found that there are other factors at play beyond direct and indirect ionizing radiation which are causing the observed heightened biological responses. In 1992, Nagasawa and Little suggested that radiation could induce DNA damage in cells not directly targeted by ionizing radiation (Nagasawa & Little, 1992). This was one of the first amongst what would become a discipline of radiobiology focused on radiation- adaptation responses and NTE, shifting the paradigm of radiation away from targeted theories.

There are various co-existing and counteractive NTE, highlighting the diversity of biological responses at low doses (Desouky et al., 2015; Mothersill & Seymour, 2014). The effect I will be focusing on is the radiation-induced bystander effect (RIBE), RIBE fits with what was initially described by Nagasawa and Little as non-irradiated cells behaving as is they have been irradiated after interactions with irradiated cells. Studies found that non-irradiated cells seeded on cell media which previously housed irradiated cells would have higher mortality than those seeded from media on non-irradiated cells (Mothersill et al., 2018; Seymour & Mothersill, 2004). RIBE is currently defined as signals mediated transfer of biological effects from irradiated to non-irradiated cells.

Radiation-Induced Bystander Effect

Initial work for RIBE was performed in cells cultures, but research is now being conducted using various models at various biological organizational levels to better understand the mechanisms underlying RIBE, and to establish its impacts for radiation protection, particularly environmental radiation protection. A substantial amount of

research has been conducted focusing on intraindividual effects using single cell organisms, plants, subterranean invertebrates, as well as frog, mice, and fish (Mothersill et al., 2006; Reis et al., 2018; Rusin et al., 2019; Smith et al., 2011; Surinov et al., 2004; Yang et al., 2007). Through those experiments, it is now commonly understood that there are two main routes in which RIBE signals are mediated, gap-junction intercellular communication (GJIC) and extracellular soluble factors, with biophotons being recently suggested as a potential third type of signal mediators of RIBE signals (Azzam et al., 1998; Le, McNeill, et al., 2015; Le, Mothersill, et al., 2015; Seymour & Mothersill, 1997).

Gap-Junction Intercellular Communication

Gap-junctions are intercellular ion channels comprised of medium-sized families of hexamers, connexins in vertebrates and innexins in invertebrates. They are named for the ~ 2 nm extracellular "gap" which form when families of hexamers join between cells, facilitating direct cell-cell transfer of molecules and ions (Hoorelbeke et al., 2020). Gap-junctions are found ubiquitously in all solid tissues, with intercellular communication being regulated by the number of gap-junctions, which are in part mediated via phosphorylation (Goodenough & Paul, 2009). The first evidence of GJIC mediated RIBE was demonstrated in 1998 by the same lab who in 1992 suggested the concept of RIBE, and then further confirmed in 2001. The lab first demonstrated that reduced membrane permeability and blocked gap-junctions could prevent RIBE and later showed that RIBE was not inducible in cells that lacked gap-junctions entirely (Azzam et al., 1998, 2001). In these studies, and for GJIC in general, it is suggested that radiation stimulates gap-

junction associated protein expression, leading to higher numbers of gap-junctions and thus increased cell-cell communication, mediating RIBE (Azzam et al., 2003).

Extracellular Soluble Factors

At roughly the same time in Ireland, Mothersill and Seymour were asking similar questions and found extracellular/intercellular soluble factors' role in RIBE signal mediation (Seymour & Mothersill, 1997). Soluble factors are signaling molecules secreted by the cells into extracellular space that can be picked up by other cells. Soluble factors such as reactive oxygen species (ROS), cytokines, and exosomes play a major role in communication between both distal and neighbouring cells. Initial studies demonstrated that filtered media from only certain types of cells caused RIBE, while others did not, leading to the belief that RIBE stemmed from extracellular soluble factors (Seymour & Mothersill, 1997). This was later confirmed, resulting in the now commonly understood framework that both GJIC and extracellular soluble factors play a role in RIBE signaling (Mothersill & Seymour, 1998).

UV Biophotons

It has been demonstrated that all living organisms emit photons (indirect ionizing radiation) to a certain extent, and that physical damage/stressors can increase photon emission (Cifra & Pospíšil, 2014; Salari et al., 2025). The idea that photons, or packets of light being emitted by a biological system, can relay information is not necessarily novel, it has been suggested since the 1900's for inter and intracellular communication, coining the term biophotons (Cohen & Popp, 1997). However, more recent work by Ahmad *et al*.

in 2013 and follow up work by Le *et al.* in 2015 demonstrated that ionizing radiation can lead to increased UV biophoton emissions from human cell, impacting cells on another plate (Ahmad et al., 2013; Le et al., 2015). Further experiments by Le *et al.* in 2015 found that these secondary UV biophotons can induce apoptosis in neighbouring cells, and that by blocking and filtering out these biophotons, they can mitigate the apoptotic effect (Le et al., 2015). As such, UV biophotons have been suggested as a physical mechanism of RIBE, particularly as an indirect (no deposition of physical materials into a shared media) mechanism of signal transfer between individuals.

Interindividual Radiation-Induced Bystander Effect

As we uncover more and more the impact of RIBE, interest shifted to interindividual impacts and potential widespread environmental ramifications. Some of the first of these studies was conducted by Surinov *et al.* in 1998. The team discovered that cohabitating irradiated rodents with unirradiated individuals decreased the number of leukocytes within the blood of the unirradiated individuals (Surinov et al., 1998). Further work by this lab proved that bystander signals could be transmitted via volatile compounds within the urine, without any direct interactions required (Surinov et al., 1998). Over the next three decades, advances have been made focusing on interindividual RIBE in fish and amphibians, highlighting keystone species and the potential impacts of nuclear reactors on nearby bodies of water and its inhabitants (Mothersill et al., 2006). However, many of the studies utilized secondary clonogenic assays to illustrate the presence of RIBE, and there still exists a paucity of data at the organismal and population level, lacking

empirical data on how RIBE could impact individual development, sexual fitness, and overall survival.

Ionizing Radiation and Invertebrates

Invertebrates and insects play a major role in ecosystem and are critical for assessing environmental health. Yet, publication 124 from the Internation Commission on Radiological Protection (ICRP), Protection of the Environment under Different Exposure Situations, still suggests a framework which while an improvement from their last publications, primarily utilizing the LNT framework which fails to recognize the potential impacts of NTEs, as well as focusing primarily on vertebrates (Pentreath et al., 2014). As such the impacts of NTEs and RIBE, particularly amongst fallout exclusion zones and ecosystems, are still being underestimated.

Sterile Insect Technique

While it is true that there is a paucity of data regarding insects and radiation for environmental protection purposes, there is lots of data on radiation as a sterilization method and insecticide. Known as the Sterile Insect Technique (SIT), SIT refers to the mass rearing, irradiation and release of sterile insects (most often males, but sometimes both sexes) to “infested” areas as a biological method of controlling insect populations (Benedict, 2021; Pérez-Staples et al., 2021). While the released insects are sterile, they are still able to compete for and mate with wild insects, resulting in non-viable eggs or offspring, reducing local populations. SIT is often used for agricultural or disease

prevention purposes, targeting primarily mosquitos, flies, moths, and beetles (Benedict, 2021; Pérez-Staples et al., 2021).

Viewed using targeted theories of radiation, particularly through an anthropogenic lens, SIT is almost the perfect insecticide. It is environmentally friendly, leaves no chemical or physical residues, provides very little risks to non-target species, and is compatible with other forms of biological controls (Benedict, 2021; Pérez-Staples et al., 2021). However, attempting to investigate SIT through a non-targeted lens leads to some concerns, and the benefits of SIT, particularly of how it would not impact other species, comes under scrutiny. The question of both intraspecies and interspecies RIBE arises, and while this work aims to address intraspecies RIBE, the extent of interspecies RIBE, particularly on species of concern or importance, is still left unexplored. Recent work has shown heavy metal can induced bystander effect between two differing species of earthworms (Fernandes et al., 2020). While different than RIBE, it is enough to suggest that similar responses could be occurring between the irradiated species and other off-target species.

Cricket Model

This work therefore will aim to establish whole-body endpoints for RIBE using the house cricket (*Acheta domesticus*). My work aims to bridge the gap between current *in vitro* research and demonstrate the impact of RIBE on individuals using *in vivo* endpoints. The primary question this work aims to address is, can irradiated crickets induce RIBE and what does that look like, how are these biological effects being mediated between individuals, and what is mediating RIBE transfer between individuals.

The house cricket, *A. domesticus*, is a well-established model for development, behaviour, and radiation research since the 1980's. *A. domesticus* can be reared in high numbers, have a mean lifespan of 120 days, with juveniles undergoing a known number of molts. Maturation and adulthood are easily identifiable due to the presence of developed wings. Males and females are easily separatable due to the presence of an ovipositor (long needle-like structure at the base of the body of females used for laying eggs) and are sexually mature at ~ 50-60 days post hatch. These traits make it straightforward for collecting sex-specific maturation data such as time to, mass at, and growth rate to maturation.

Radiation Source

The radiation source used within this work is the McMaster Taylor Radiobiology Source, which is a high-dose Cesium-137 gamma emitting source. Irradiation is done at a fixed distance of 16.4 cm from the source. Due to natural decay of Cs-137 over time, our dose rate ranges from ~0.54 – 0.58 Gy/min but are relatively fixed within experiments. Dose rate plays an important role in determining the impact of radiation, with higher dose rates being typically more damaging, as such it's important to keep in mind both the dose, and the dose rate when considering any radiation research.

The are two primary dosages used within this work, the first being a relatively high dose of ~23 Gy (with a single group up to nearly 70 Gy) in chapter 2, and the other being 0.5 Gy for chapters 3 and 4. The first set of dosages were chosen to address the initial question of whether we can induce RIBE between individuals, and while not environmentally relevant, this dosage was chosen for a couple reasons. The first being

that *A. domesticus* are radioresistant compared to vertebrates used in radiobiology. 23 Gy is a dose where we begin observing negative morphometric and developmental impacts in *A. domesticus*, while still being sexually viable. Secondly, as we weren't certain that RIBE was inducible between individuals, we wanted to pick a high enough dose to induce RIBE and compare it to visible targeted effects. Later it was demonstrated that such high doses were not required, and as with intraindividual RIBE, low doses are enough to saturate RIBE. The dose used for the second and third study was 0.5 Gy, which was chosen as it is more environmentally significant, and is useful in demonstrating potential environmental ramifications of RIBE.

Equalizing External Stressors

When you attempt to categorize the number of stressors placed onto a research system beyond radiation, you soon realize that there are multiple stressors outside of radiation, such as handling and transport stress. As such, it is particularly important for radiation research to minimize or equalize the amount of non-radiation stressors. The biggest stressor is handling and transport, where research animals need to be manipulated (counted and sorted into proper treatment groups, placed into and removed from the radiation source), and transported to be irradiated. To equalize stress, all groups are transported, even the ones that are not receiving radiation exposure, while ensuring as equal of handling and manipulation as possible. By transporting the controls out of the lab setting and into the irradiation source, they have now become what is known colloquially as "sham" controls, or fake irradiated treatment groups. These shams experience the same stress of transport with the variation in temperature and environment, and get placed onto

the source, but never actually receive radiation. This way researchers can account for majority of the uncontrollable stressors, and ideally the only difference between treatments will be radiation exposure.

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Chapter 2

2. Investigation of presence and impact of radiation-induced bystander effect in *Acheta domesticus*

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2.1. Preface

The aim of this published manuscript was to demonstrate that radiation-induced bystander effect (RIBE) is transferrable between individuals using whole-body endpoints. This is

the first paper, to our knowledge, to demonstrate whole-body RIBE, and particularly is the first to demonstrate interindividual RIBE using *in vivo* endpoints. By using whole-body endpoints, we are no longer speculating about what the results could potentially mean for a population but can confidently say that irradiated individuals can alter development of an entire unirradiated population. We found that irradiated crickets stimulated development and accelerated the time to maturation, suggesting potential environmental ramifications.

2.2. Introduction

Anthropogenic activities have increased stress spanning ecosystems, communities, and among animal and plant species. Individuals exposed to ionizing radiation may reflect impacts on mortality, development, phenotype, life history aspects, behavior, and reproduction. Ionizing radiation is a stressor of particular interest as it can permanently induce DNA damages, mutations, and chromosomal instabilities, associated with tumorigenesis and apoptosis (Desouky et al. 2015).

Ionizing radiation is employed across multiple disciplines including medicine, research, and agriculture. Ionizing radiation is used in cancer therapy as it induces apoptosis and reproductive death of cancer cells and can reduce tumor mass (Baskar et al. 2012). It is also studied to determine long-term impacts of elevated radiation exposure from nuclear accidents and fallout on humans and ecosystems.

The fallout zones, or Chornobyl/Chernobyl exclusion zones (CEZ) of the 1986 Chornobyl incident remains to this day an area of interest and concern for both environmental and

radiation-based research. A 2020 report showed that radiation-induced effects on wildlife are still present within the CEZ. With the extend of the effects not fully known (Kovalchuk et al. 1999; Perino et al. 2019; Beresford et al. 2020).

Research examining risks of wildfires within CEZs have observed a ten-fold increase in certain radionuclides such as Cs137 in ground ash as compared to topsoil, possibly increasing the bioavailability of the radionuclides (Beresford et al. 2021). Considerable research investigates radionuclide uptake and impact are of plants, herbivore, and vertebrate populations (Kovalchuk et al. 1999; Mothersill et al. 2022). A study in 2009 investigating abundance of insects and spiders have found reduced abundance in Chornobyl, suggesting stronger ecological impacts of ionizing radiation than previously anticipated (Møller and Mousseau 2009). Yet potential concerns regarding radionuclide uptake from insects and invertebrates are less understood, despite their relative importance to food chains and ecosystems.

Beyond direct impacts, there are also indirect, or non-targeted effects (NTE) of ionizing radiation. Ionizing radiation can affect a wider area than intended, affecting cells, tissues, and organisms that were thought to have no radiation exposure (Mothersill and Seymour 2001). This pose concerns regarding not only its surrounding environments, but also impacts of radiation therapy (Mothersill et al. 2018). NTEs require care in administrating radiation and assessment of risk vs reward. It is now clear that radiation can affect much more than just the intended target (Seymour and Mothersill 2004).

The most common NTEs involve ionizing radiation production of radiation-induced bystander effects (RIBE) and genomic instability (Seymour and Mothersill 2004). The

mechanisms are thought to involve generation of reactive oxygen radicals and radiolysis, but the full pathway has yet to be fully understood (Lyng et al. 2000). This phenomenon known as RIBE refers to the detectable effect of non-irradiated individuals responding as if they had been irradiated (Mothersill and Seymour 2001).

RIBE can affect cell signaling and even animal behavior. Individuals affected by RIBE react similarly to irradiated counterparts, even leading to increased mortality or developmental shifts, despite no exposure to radiation (Mothersill and Seymour 2001). RIBE may even spread from irradiated to non-irradiated individuals, as well as within individuals and cellular organelles (Mothersill et al. 2006; Smith et al. 2011).

Individuals exposed to radiation can produce bystander signals and factors that affect non-exposed individuals, causing radiation like symptoms in non-irradiated individuals (Seymour and Mothersill 2004). RIBE experiments have observed mediated cell signaling in irradiated cells, communicating radiation effects to neighboring, non-irradiated cells, inducing apoptosis (Mothersill and Seymour 2001). In vitro experiments have observed similar outcomes on a larger scale, where irradiated individuals are able to communicate and affect non-irradiated individuals, leading to similar behavioral and developmental shifts to the irradiated counterparts, despite the lack of radiation (Mothersill and Seymour 2001).

Research has primarily focused on vertebrate populations due to anthropogenic and medicinal perspective and concerns of RIBE and NTEs in radiotherapy. Research looking at subterranean earthworms found inter-species RIBE (Fernandes et al. 2020). There is

still a critical lack of data on invertebrates, particularly in terrestrial invertebrate RIBE, which could uncover ecosystem level impacts of RIBE.

Initial research looking into RIBE focused on genomic instabilities and mutations in cells, studies introduced non-irradiated cells into medium of irradiated cells and found a higher mortality rate (Baverstock 2000). These initial studies suggests that factors, or better known now as bystander signals, are expressed into the medium, and can cause non-irradiated cells to behave as if they have been irradiated (Seymour and Mothersill 1997). Further research into bystander signaling has found RIBE within whole animal communication. Initial research found that irradiated mice and rats can have immunosuppressing effects on the non-irradiated animals that they're housed with (Surinov et al. 2004). Studies looking at rainbow trout suggests the release of bystander signals into the water by irradiated individuals, inducing RIBE in un-irradiated individuals swimming alongside (Mothersill et al. 2006). Stemming from the initial in vivo vertebrate research, some work has been done in plants and invertebrates of interest. Research looking at microbeam irradiation in Arabidopsis embryos suggests long-distance bystander effects, with direct damage in one part of the plant affecting the rest of the plant's development (Yang et al. 2007). Using bladders from radio-adapted frogs, research has shown cultured media of irradiated frog bladders can induce RIBE as well (Vo et al. 2022). Although most of the work has thus far been focused on vertebrate and plant species of interest, there's been an uptick in invertebrate RIBE research. Research looking into eutardigrades, and daphnia demonstrated saturability of bystander signaling, showing further proof interactions with irradiated individuals will lead to decreased

survival in non-irradiated populations (Fernandez et al. 2016; Reis et al. 2018). More recently, inter-species work has been done between earthworm species, and found system wide RIBE on multiple species of sub-terrestrial earthworms (Rusin et al. 2019).

On a cellular level, it has been determined that RIBE is signal mediated, and can be induced either through gap-junction intercellular communication (GJIC), extracellular soluble factors, or physical signaling involving electromagnetic signals (photon and acoustic signals) (Azzam et al. 1998, 2000; Ahmad et al. 2012). On a population and community level, it has been found that urine and exosomes from irradiated animals can also trigger RIBE in non-irradiated individuals (Surinov et al. 2005; Le et al. 2015). Insects have been studied worldwide, and at Chornobyl and Fukushima to understand multigenerational impacts of radiation exposure (Hancock et al. 2019). Biodiversity was reduced in radiation-contaminated areas and some understanding could be extrapolated to vertebrates. However, similar research is lacking for insects and invertebrates in these areas (Møller and Mousseau 2007, 2009, 2011). Insects are a likely source for understanding aspects of radiation impact, including RIBE.

Given rapid growth and short generations, Acheta domesticus (the house cricket) is ideal for studying radiation exposure of individuals and RIBE in populations. Using sub-lethal dosages (23.2 and 69.6 Gy), we are studying impacts of introducing irradiation individuals to non-irradiated individuals and populations.

2.3. Methodology

Study animals and experimental groups

Breeding colony

House crickets (*A. domesticus*) were generated from a main breeding colony (93 × 64.2 × 46.6 cm plastic terrarium) held at 29 ± 2 °C, with 1.5 cm thick Durofoam insulation and fans atop the enclosure to provide circulation with a 12h light/12h dark photoperiod. Egg carton shelters were provided alongside ad libitum water and food. Distilled water was provided in soaked cellulose sponges, Quicks Country Range Multi-Fowl Grower Rations (17.0% crude protein, 2.5% crude fat, and 4.0% crude fiber) was available in large petri dishes.

Egg collection

The breeding colony was provided was oviposition medium (Organic Garden Soil, Swiss Farms Products Inc., Maryville, USA) in small plastic containers (7 × 7 × 7 cm), 14 days after 95% of the colony reached sexual maturity. One container of oviposition medium was left to collect eggs for 24 h, at which point the medium was removed and incubated at 29 °C ± 2 °C. The medium was kept moist by daily spraying and partial covering with a plastic lid. Eggs hatched after approximately 12 days. Medium was removed 24h after initial hatching to ensure all juveniles were approximately the same age.

Irradiation

Crickets were irradiated at the Taylor Radiobiology Source at McMaster University. The Taylor Radiobiology Source consists of a nominal 74 TBq (2 kCi) 137Cs source in a shielded irradiator. An automated portway exposes the source with an on/off control in the exterior room. The current activity of the source (November 2003) is approximately 37 TBq (1 kCi), it was last calibrated by McMaster's Health Physics and Facility Management in January 2008. Due to known half-life of caesium-137 and the accurate

fields within the Taylor Source, exact doses can be obtained by placing and exposing subjects at specific distances for specific times. For juveniles, crickets were placed in cylindrical plastic vials (9.5 × 2.5 cm), with six vials irradiated at a time (10 juveniles per vial, 60 juveniles per exposure; 2 adults per vial, 12 adults per exposure). In general, dosimetry measurements during irradiations have proven counterproductive due to their interference with correct positioning of the subjects and inaccuracy related to the difficulty of having the same geometry for the dosimetry as for the subjects (with reference to the source fields). Here, position and orientations of the subjects was facilitated by placing specimens in a tube apparatus contained seven individual tubes tied into a circle (with the center tube remaining empty). These were placed equidistant from the circular opening beneath the source (16.4 cm, dose rate = 0.58 Gy/min). Crickets were confined in each tube, allowing some freedom of movement to reduce stress while ensuring sufficient restriction to calculate the received dose with confidence. Dosages were picked for experiments based on previous studies conducted in lab looking at mating success, sterility, as well as altered hydrocarbons levels (Fuciarelli and Rollo 2020, 2021). A male and female adult were placed in each vial for irradiation (12 adults per dose, 6 of each sex). All individuals were then transported back into lab and placed into their respective groups for the remainder of data collection. Experimental groups were housed in the same conditions as the colony. All groups, including non-irradiated groups, were brought to the Taylor Source in insulated boxes to maintain similar conditions across all groups, and to control for traveling and handling stress. This will be denoted as ‘sham’ for both experiments.

Experiments

Experiment 1: indirect observations of RIBE in crickets

All experimental groups were obtained from the same oviposition medium. To determine the presence of RIBE in insects, we mixed and raised irradiated and non-irradiated juveniles, and compared to irradiated and control group populations raised separately, but combined data.

Fourteen days after hatch, crickets were randomly selected and placed into one of the three groups, non-irradiated, irradiated (23.2 Gy at 0.58 Gy/min), and cohabitated (half non-irradiated, half irradiated).

Three hundred and sixty crickets were randomly selected, separated, and placed into one of the three groups. Groups were housed in plastic containers (24 × 12.5 × 16 cm, 120 individuals per group). In the cohabitated group, 60 juveniles were irradiated and then mixed with 60 non-irradiated crickets; 120 juveniles were irradiated for the irradiated group.

We combined non-irradiated and irradiated cricket data (denoted as non-cohabitated) to compare to the cohabitated group. This allows us to compare average growth rate and time to maturation, with all other parameters kept the same, the only difference being that in the non-cohabitated group, the two populations were separate and never interacted, whereas in the cohabitated data, the two sub populations were kept together while developing.

Experiment 2: direct observation of RIBE in crickets:

Following up on experiment 1, we aimed to directly compare and measure the impacts of RIBE on cricket development. By introducing adult irradiated crickets to a juvenile population, and by removing the adults before the non-irradiated juveniles matured, we can directly compare RIBE on the population.

Fourteen days after hatch, crickets were randomly selected and placed into one of 5 groups, new sham, newly irradiated 23.2 Gy, newly irradiated 69.6 Gy, previously irradiated 23.2 Gy, and previous sham.

Adults were collected a month after maturation, with some irradiated as juveniles (14 days post hatch), and others irradiated immediately before introduction to juvenile populations. These are denoted at previously irradiated (irradiated 14 days post hatch as juveniles) and newly irradiated (irradiated day of as adults) respectively and will aim to determine the presence of RIBE. New Shams were brought over to the source at the same time as the newly irradiated adults, and previous sham were brought over at the same time as the previously irradiated adults. All experimental juvenile populations were obtained from the same oviposition medium, and had adults introduced when they turn 14 days old. Seven hundred and fifty crickets were randomly selected, separated, and placed into one of the five groups. Irradiated and non-irradiated adult crickets (six males and six females) were placed into their respective group for 7 days from 14 days post hatch to 21 days post hatch (one previously 23.2 Gy male was accidentally left in container for an additional 3 days). Juveniles were housed and raised in plastic containers (24 × 12.5 × 16 cm, n = 150 per group).

Maturation

Experimental groups were monitored daily at approximately the same time for sexual maturity (adult molt) as indicated by expression of wings. Newly mature male and female crickets were immediately weighed with an Accuris analytical balance (readability of 0.001 g ± 0.002 g) and separated into group-dependent adult containers. Time (days) to maturation and weight (mg) were recorded and used to determine growth rate (mg/day).

Statistical analysis

All statistical analysis has been performed in R 4.2.2 (https://github.com/Xiaobing9/CricketRIBE). Figures were plotted using least square means calculating model with effect of group and sex using emmeans. Linear models were created to test the effects of group and sex on the three parameters of maturation weight (mg), maturation time (day), and growth rate (mg/day) in for both experiments. Type II ANOVAs on the linear model of the three parameters were performed, contrasts between estimated marginal means were calculated between groups within sex as well as between sex within groups using the emmeans (1.8.4-1) package in R. p Values were adjusted using Tukey's method for comparing a family of 5 estimates in the second experiment.

2.4. Results

Indirect observation of RIBE in crickets

An ANOVA was performed for maturation weight and found significant effect of sex ($F = 9.6635$, $p = .002086$) with no significant effect of group, and no significant interaction term of group and sex. No significant differences in estimated mean between

the groups within sex were observed. A significant difference in estimated means was observed between sex within the non-cohabitated group (females were estimated to be 39.65 mg heavier, p = .0009), but not within the cohabitated group (Figure 2.9.1). Significant differences in maturation time were observed in both group (ANOVA, $F = 59.5323$, $p < .001$) and sex ($F = 10.6449$, $p = .00125$), with no significant interaction term found between group and sex. Significant differences in estimated means in maturation time were observed in both cohabitated males and females (estimate of 5.82 and 5.41 days faster, respectively, $p < .0001$ for both) when compared to the non-cohabitated group. Significant differences in estimated means were observed within the non-cohabitated group (females were estimated to mature 2.36 days faster, $p = .0044$), but not within the cohabitated group (Figure 2.9.2).

Significant differences in growth rate were observed in both group (ANOVA, $F = 15.0357$, $p < .001$) and sex (ANOVA, $F = 12.6859$, $p < .001$), with no significant interaction term found between group and sex. Significant differences in estimated means of growth rate were observed in the cohabitated males (estimate of 1.13 mg/day higher, $p = .0007$) when compared to non-cohabitated. Significant differences in estimated means were observed within the non-cohabitated group (females were estimated to have 0.94 mg/day higher growth rate, $p = .0005$), but not within the cohabitated group (Figure 2.9.3).

Direct observation of RIBE in crickets

Significant differences in maturation weight were observed in both group (ANOVA, $F = 5.5365$, $p < .001$) and sex (ANOVA, $F = 92.2408$, $p < .001$), with no significant

interaction term between group and sex. Significant differences in estimated means were seen between females of previous sham and previous 23.2 Gy group ($p = .0001$) (Figures 2.9.4 and 2.9.5; Tables 2.8.2 and 2.8.3).

Significant differences in maturation time were observed in both group (ANOVA, $F = 91.111$, $p < .001$) and sex (ANOVA, $F = 97.361$, $p < .001$), with no significant interaction term between group and sex. Significant differences in estimated means were seen between groups within sex between new sham, new 23.2 Gy, and new 69.6 Gy groups ($p < .0001$, no difference between irradiated groups for both sexes); as well as between previous sham and previous 23.2 Gy males ($p = .0004$) (Figures 2.9.6 and 2.9.7; Tables 2.8.2 and 2.8.3).

Significant differences in growth rate were observed in both group (ANOVA, $F = 28.312$, $p < .001$) and sex (ANOVA, $F = 182.181$, $p < .001$), with no significant interaction term between group and sex. Significant differences in estimated means were seen between groups within sex between new sham, new 23.2 Gy, and new 69.6 Gy groups ($p < .0001$ and .0002 for females, $p = .0006$ and $p = .0007$ for males, no difference between irradiated groups for both sexes); as well as between previous sham and previous 23.2 Gy ($p < .0001$ and $p = .0396$ for females and males, respectively) (Figures 2.9.8 and 2.9.9; Tables 2.8.2 and 2.8.3).

Significant differences in estimated means were observed in all groups when comparing between males and females in all three parameters (females were larger, matured faster, and had overall higher growth rate) (Table 2.8.1)

2.5. Discussion

Radiation induced bystander effects (RIBE) are a growing cause for concern due to their broad implications across multiple fields of research. Current RIBE research focuses primarily on cellular impacts due to the focus on biomedical research, particularly cancer treatments (Hei et al. 2008; Prise and O'Sullivan 2009). However, more research is required in other areas of radiation bystander research to better understand widespread, long-term environmental effects of RIBE. There is a paucity of radiation research in lower organisms and invertebrates, by utilizing insect models, we aim to elucidate the presence of RIBE in terrestrial insects, and better understand its environmental and longitudinal impacts.

Initial objective of this study aimed to determine the presence of RIBE in *A. domesticus*. We aimed to understand how RIBE affects maturation of non-irradiated individuals in a cohabitated population with irradiated individuals. We found significant differences in growth rate in male crickets between non-cohabitated and cohabitated groups (Figure 2.9.3). When broken down, we found no significant contrast in maturation weight between groups, but a significantly faster maturation time in the cohabitated group in both sexes (Figures 2.9.1 and 2.9.2).

A. domesticus and others in the Orthoptera order are hemimetabolous, requiring multiple molts before reaching adulthood, and we could not determine a non-intrusive way to separate maturation data of irradiated and non-irradiated individuals (Horch et al. 2017). From the initial experiment we could only indirectly compare and confirm the presence of RIBE. Our second experiment aimed to address this concern by introducing adult

irradiated crickets into non-irradiated juvenile populations and removing them before the juveniles approach maturation to directly determine effects, and presence of RIBE on non-irradiated populations.

We found significant differences in growth rate once again, however this time in both sexes among the groups (Figures 2.9.8 and 2.9.9). In newly irradiated groups (23.2 and 69.6 Gy), where adults irradiated and immediately introduced into juvenile populations, both sexes had significantly higher growth rates when compared to new sham group, similar results were observed in the previous sham and previous 23.2 Gy group, where adults that were previously irradiated as juveniles were introduced to juvenile populations. Increases in overall growth rate were a result of accelerated time to maturation in all groups expect for previous 23.2 Gy females, who instead were the only ones who had a significantly higher weight at maturation instead (Figures 2.9.4–9).

Results from the second experiment confirmed our initial findings of RIBE in crickets, and its effect on development to adulthood. These results also agree with the current literature in saturability of bystander signals as we found almost no difference in any of the parameters between the new 23.2 and 69.6 Gy groups (Tables 2.8.2 and 2.8.3). Saturability of RIBE has been demonstrated in multiple difference species and suggests that the amount of bystander signals that can be generated is limited, which could have ramifications on using bystander signals as a biomarker in radiation detection (Seymour and Mothersill 2004; Fernandez et al. 2016; Reis et al. 2018).

We demonstrate that introduction of irradiated individuals, both adults, and juveniles of the same age, will affect development into maturation. There appears to be sex and time-

dependent effects as we saw shifts in how growth rate was affected in males and females, and between new and previous irradiation groups.

Sexual size dimorphism is present in various arthropods, and typically skew toward higher maturation weight through a higher number of larval instars in females (Esperk et al. 2007). *A. domesticus* demonstrates similar size dimorphism, where females on average have a higher maturation weight than males, but also have a faster time to maturation. While females having a higher weight at maturation is consistent with lots of arthropod research, females maturing faster than males is rarer, but has been observed in species where females don't have more instars (Esperk et al. 2007). This could be the case in *A. domesticus*, as it is unclear currently if there is sexual dimorphism regarding number of instars. But our previous research has demonstrated this accelerated time to maturation with a higher weight at maturation, and what we found has been consistent with our observations of colony crickets, where females will mature faster and a higher body weight than males, demonstrating higher overall growth rate as well (Li and Rollo 2022). In our initial experiment we found that cohabitated males caught up to females in all three parameters, and were no longer significantly different, this is contrasted from our second experiment where there was still a significant difference between the sexes (Table 2.8.1). This could be due to sex-dependent radio-resistance, previous study in rats have demonstrated sex specific response to radiation depending on location of radiation exposure, and we suggest there could be sex-dependent responses to bystander signals, where males and females react differently to the bystander signals (Kovalchuk et al. 2003). This could also be due to sex-dependent radiosensitivity, and while females are

typically more radiosensitive than males, previous work looking at NTE of radiation in A. domesticus suggests that males could be more radiosensitive to NTEs, but more work is required to fully understand sex-dependent radiosensitivity in crickets (Li and Rollo 2022).

2.6. Conclusion

Much is still unknown about the implications of RIBE on a population level, or how RIBE affects individuals who are in close contact with irradiated individuals. Our results demonstrate that those in close contact with irradiated individuals develop symptoms of irradiation despite never being irradiated, suggesting elevated levels of stress, and inducing developmental shifts from adolescence to maturation. Further work suggests that RIBE signals persists long after irradiation and can be transmitted well after the irradiation.

More research is required to understand the potential ramifications and holistic impact of RIBE. It is possible that numerous stressors other than radiation share stress pathways and RIBE may be impacted by multiple and interactive stressors (Rohleder 2012). More studies are required to separate effects of sex, as well as time of irradiation on RIBE. Our research on RIBE in *A. domesticus* are one of the first in invertebrates and suggests long-term ramifications of RIBE in terrestrial insects. Our research also raises concerns within agricultural research and sterile insect technique (SIT), where males are sterilized with radiation and released as a form of population and birth control, while SIT has so far been

applied to holometabolous insects, research in labs have shown the possibilities of SIT in hemimetabolous locust, a relative of the house cricket (Dushimirimana et al. 2010, 2012). While our results were conducted with direct gamma radiation in an isolated room, the presence of RIBE appears to persist long past initial radiation and raise concerns in nuclear fallout exclusion zones. This raise concerns in nuclear fallout fringe zones where irradiated insects are freely able to travel and could impart RIBE onto non-irradiated populations. We suggest that longitudinal impacts of RIBE need to be studied, to understand impacts of RIBE on a broader scale.

2.7. Acknowledgements

The authors would like to acknowledge Katie Pelletier for her help in the statistical analysis and figures of the data, without whom I'd still be stuck on google trying to generate a simple boxplot.

2.8. Tables

Table 2.8.1. Differences in estimated means within groups, between males and females.

Tukey's HSD Test Males vs Females P Values	Weight at Maturation (mg)	Significance	Time to Maturation (day)	Significance	Growth Rate (mg/day)	Significance
New Sham	0.0001	***	< 0.0001	***	< 0.0001	***
New 23.2	< 0.0001	***	< 0.0001	***	< 0.0001	***
New 69.6	0.0005	***	< 0.0001	***	< 0.0001	***
Previous Sham	0.002	**	< 0.0001	***	< 0.0001	***
Previous 23.2	<0.0001	***	0.0016	**	< 0.0001	***

Note: Females on average had significantly higher growth rate, faster time to maturation, and higher weight at maturation than their male counterparts. *$p < .05$, **$p < .01$, ***$p < .001$.

Table 2.8.2. Difference in estimated mean between groups of female crickets.

Tukey's HSD Test Female Groups	Weight at Maturation (mg)	Significance	Time to Maturation (day)	Significance	Growth Rate (mg/day)	Significance
New Sham - New 23.2	0.9212	N/A	< 0.0001	***	< 0.0001	***
New Sham - New 69.6	0.975	N/A	< 0.0001	***	0.0002	***
New 23.2 - New 69.6	0.9999	N/A	0.9868	N/A	1	N/A
Previous Sham - Previous 23.2	0.0001	***	0.522	N/A	< 0.0001	***

Notes: All females had significant difference for growth rate when compared with their respective shams, for the newly irradiated groups the difference came primarily from significantly faster time to maturation, while for the previous irradiated group the difference came primarily from significantly higher weight at maturation. There is no difference between the newly irradiated 23.2 and 69.6 Gy groups. *$p < .05$, **$p < .01$, ***$p < .001$.

Table 2.8.3. Difference of estimated mean between groups of male crickets.

Tukey's HSD Test Male Groups	Weight at Maturation (mg)	Significance	Time to Maturation (day)	Significance	Growth Rate (mg/day)	Significance
New Sham - New 23.2	0.9622	N/A	< 0.0001	***	0.0006	***
New Sham - New 69.6	0.9408	N/A	< 0.0001	***	0.0007	***
New 23.2 - New 69.6	0.9999	N/A	1	N/A	0.9999	N/A
Previous Sham - Previous 23.2	0.6919	N/A	0.0004	***	0.0396	*

Notes: All males had significant differences for growth rate when compared with their respective shams, for the all irradiated groups the differences came primarily from significantly faster time to maturation. There is no difference between the newly irradiated 23.2 and 69.6 Gy groups.
*$p < .05$, **$p < .01$, ***$p < .001$.

2.9. Figures

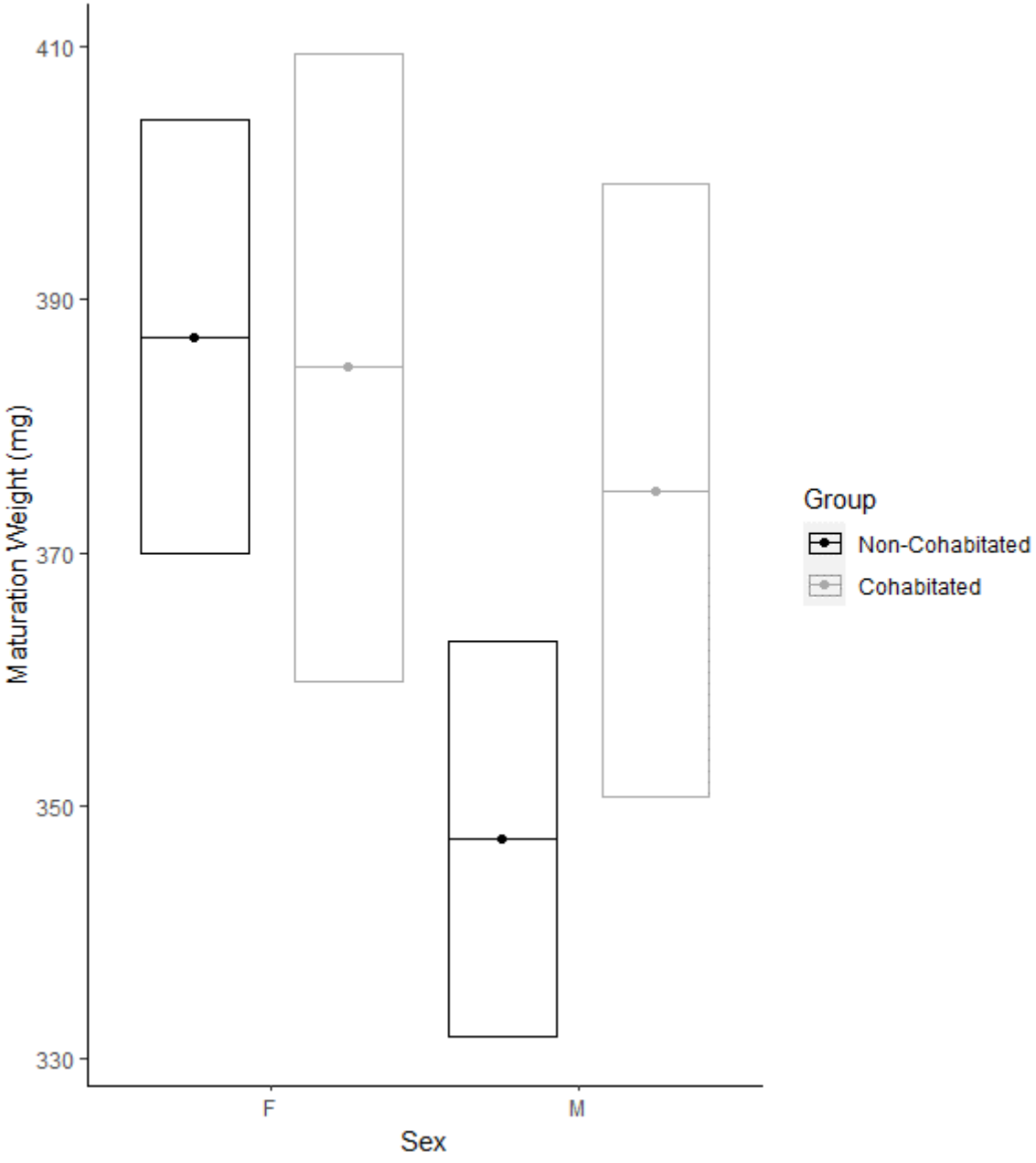

Figure 2.9.1. Weight at maturation (mg) of cohabitated and non-cohabitated crickets. No significant differences were observed between groups in either sex. Bars represent 95% CI calculated using least square means using emmeans.

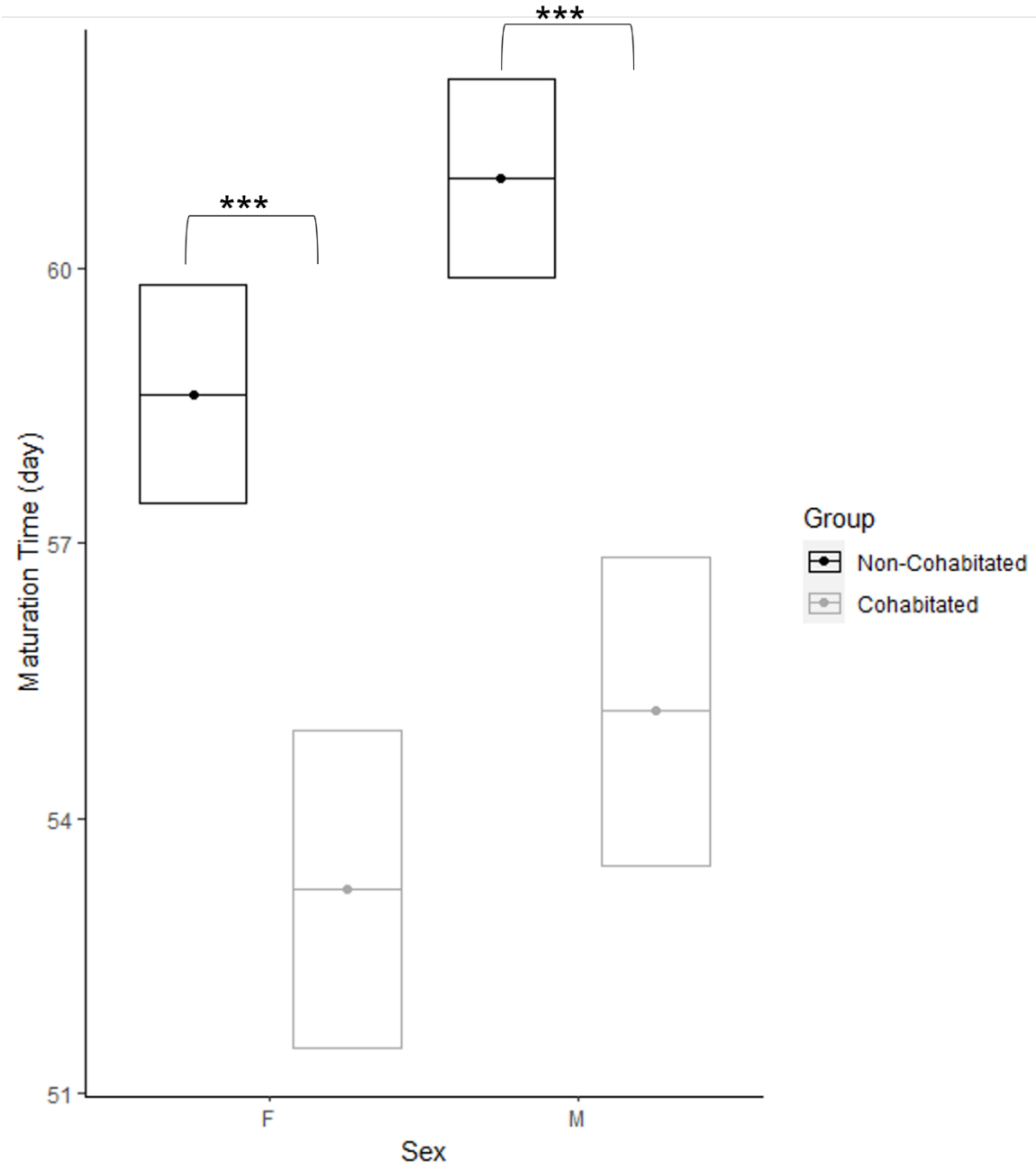

Figure 2.9.2. 95% CI of least square means of time to maturation (day) of cohabitated and non-cohabitated crickets. Cohabitated groups matured significantly faster than their non-cohabitated counterparts in both sexes. ***$p < .001$.

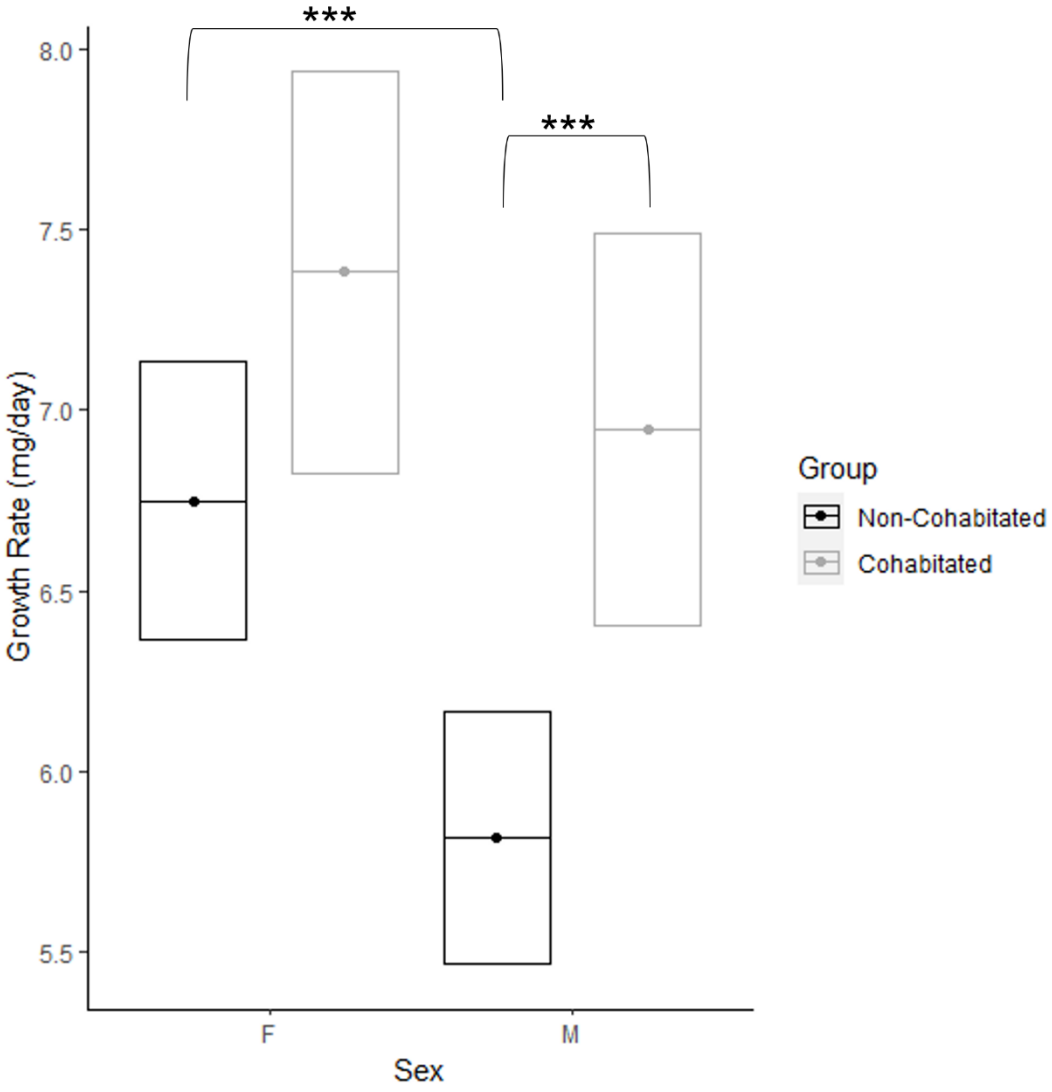

Figure 2.9.3. 95% CI of least square means growth rate to maturation (mg/day) of cohabitated and non-cohabitated crickets. Significantly higher growth rates were observed in cohabitated males when compared to non-cohabitated males. A significant difference in growth rate was also observed between sexes in the non-cohabitated group, but not within the cohabitated group. ***$p < .001$.

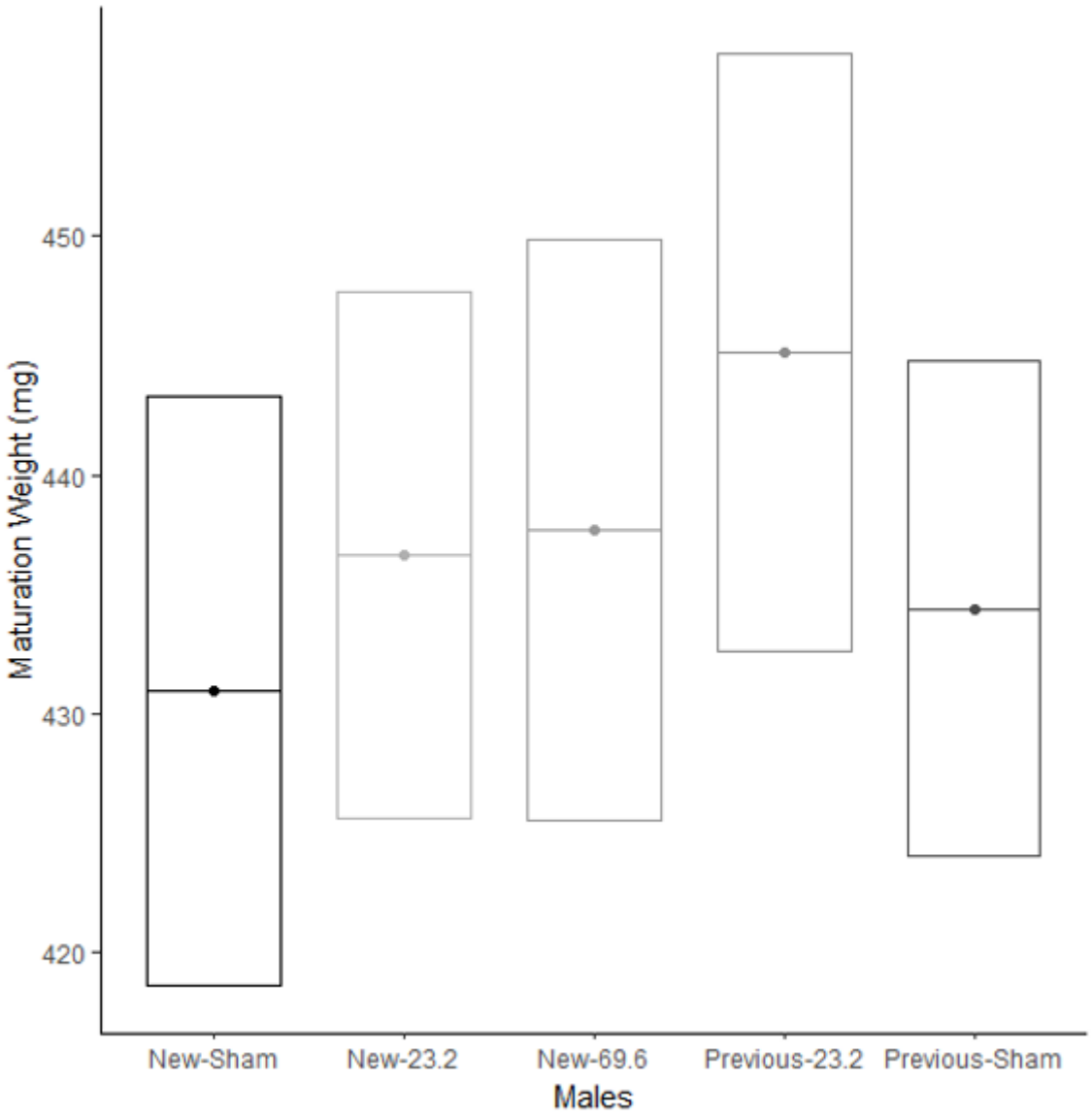

Figure 2.9.4. 95% CI of least square means of weight at maturation weight (mg) of male crickets. No significant differences were observed between relevant irradiation groups and their respective shams.

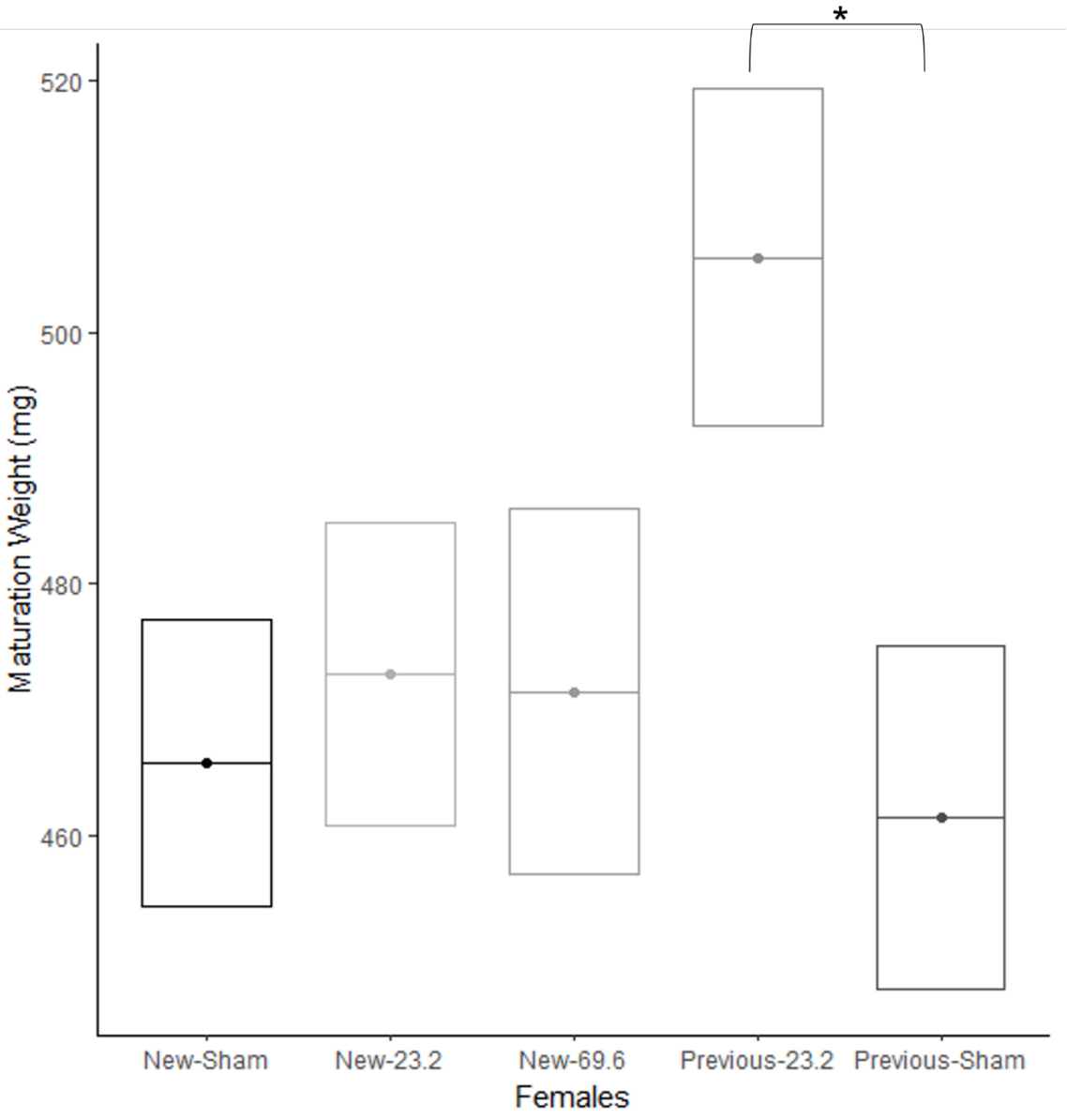

Figure 2.9.5. 95% CI of least square means of weight at maturation (mg) of female crickets. Previous 23.2 Gy females had significantly higher weight at maturation compared to their respective sham. *p < .05.

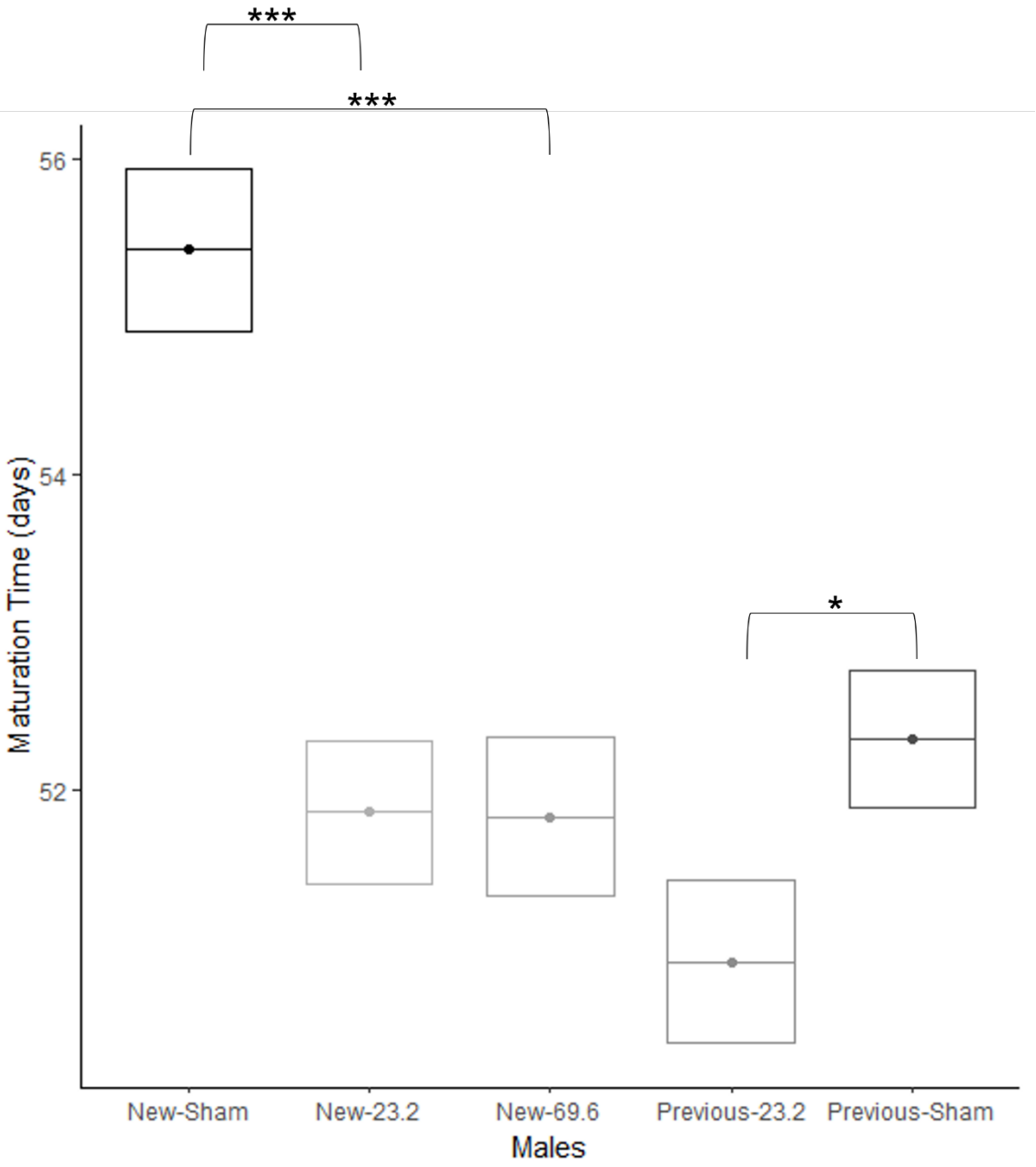

Figure 2.9.6. 95% CI of least square means of time to maturation (day) of male crickets. All irradiation groups had significantly faster time to maturation when compared to their respective shams. *p < .05, ***p < .001.

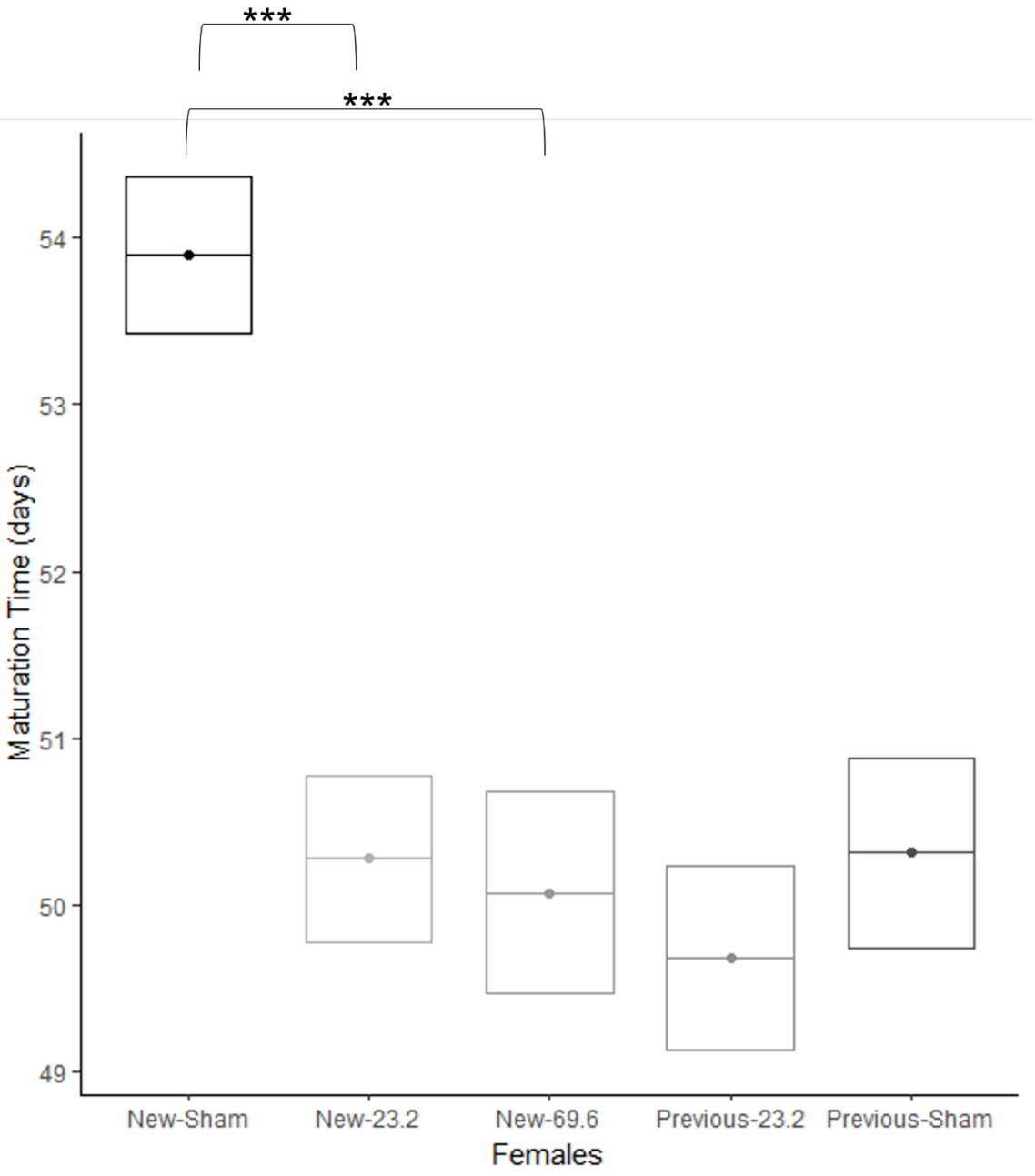

Figure 2.9.7. 95% CI of least square means of time to maturation (day) in female crickets. Both new irradiated groups had significantly faster time to maturation when compared to their sham, no significant differences were observed in the previous groups. ***p < .001.

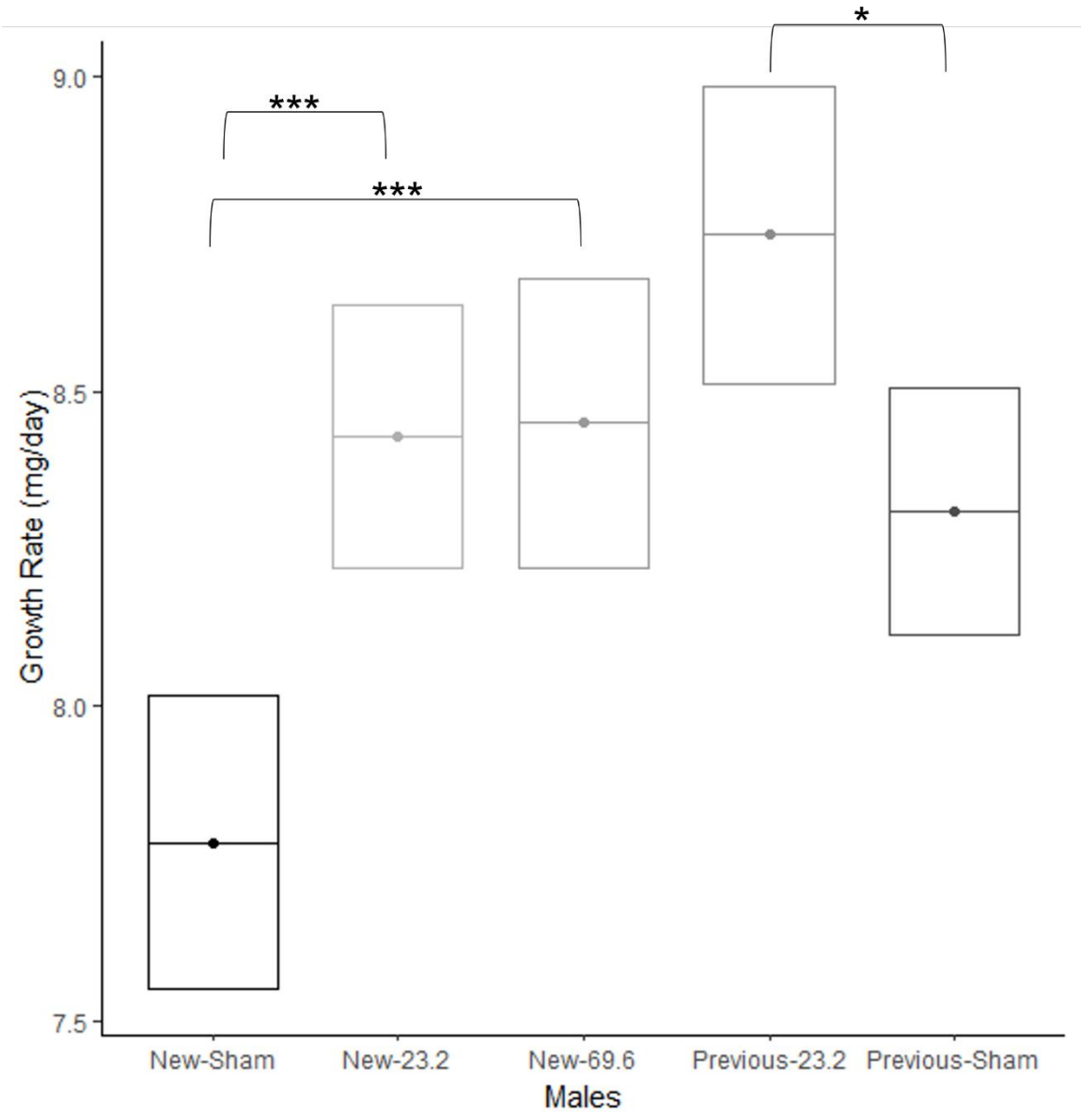

Figure 2.9.8. 95% CI of least square means of growth rate (mg/day) of male crickets. All irradiation groups had significantly higher growth rate when compared to the respective shams. *p < .05, ***p < .001.

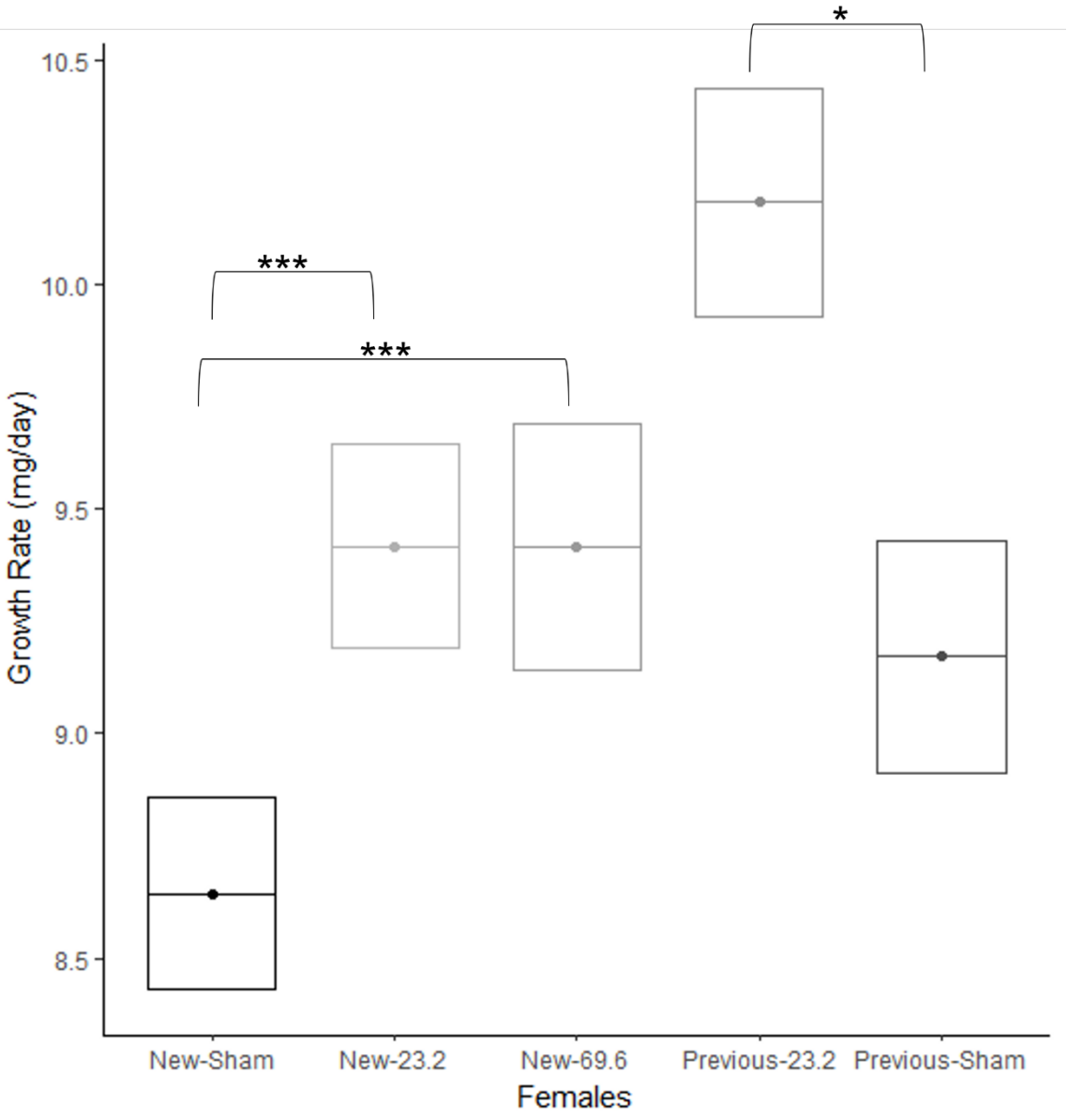

Figure 2.9.9. 95% CI of least square means of growth rate (mg/day) of female crickets. All irradiation groups had significantly higher growth rate when compared to the respective shams. *p < .05, ***p < .001.

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Chapter 3

3. General and radiation-induced bystander effects can be indirectly transmitted via pheromones and biophotons.

3.1. Preface

This chapter is currently in preparation for submission as a journal article. Initially we had planned for this to be two separate papers. However, in analyzing the data it became apparent that indirect mediation of RIBE from either soiled housing materials or biophotons manifested in a similar manner. Furthermore, there appeared a secondary, contradictory effect resulting in maturation suppression in even control populations. As such, we have decided to merge the two into a larger chapter, hoping to elucidate some of the manners in which RIBE can be transferred between individuals who are unable to directly interact to better understand the underlying mechanisms. Two methods of indirect signal transfer were tested, our results demonstrate that both RIBE and general maturation suppression effects can be mediated via soiled housing materials, as well as biophotons. These results highlight the potential widespread ramifications of RIBE, particularly on the environment.

3.2. Introduction

At low doses of ionizing radiation (≤ 0.5 Gy), estimation for radiation exposure risk becomes trickier as innate radioresistance, DNA repair properties, and non-targeted

effects (NTE) dictates the overall response (Ali et al., 2025; Mothersill et al., 2024). Non-targeted effects such as radiation-induced bystander effect (RIBE) can transfer irradiated effects from irradiated individuals to unirradiated individuals (Desouky et al., 2015; Mothersill et al., 2024; Mothersill & Kadhim, 2012). Radiation-induced bystander effect became an area of interest and concern in the 90's, as it suggests wider radiation effect than previously predicted using targeted theories. The field has since uncovered mechanisms of signal transfer mediating RIBE, as well as the impacts of RIBE both within and between individuals in plants, fish, mice, earthworms, and insects (DeVeaux et al., 2006; Li et al., 2023; Mothersill et al., 2006; Rusin et al., 2019; Singh et al., 2011; Smith et al., 2013; Yang et al., 2008). Recently, we have demonstrated interindividual RIBE in crickets using whole-body endpoints, where interactions with irradiated individuals resulted in heightened growth rate at maturation in non-irradiated juveniles due to accelerated maturation and growth (Li et al., 2023).

However, not much is currently known regarding how signals are mediated between individuals. Studies in mice have found that stress signals are able to be transmitted via soiled housing material from irradiated to non-irradiated individuals, impacting genome stability (Glinin et al., 2023; Surinov et al., 2004; Tsyb et al., 2013). Another possible novel avenue for RIBE transmission was recently discovered in UV biophotons. Photons are emitted by all living organisms and have been demonstrated to be a mechanism of cell-cell communication (Cohen & Popp, 1997; Salari et al., 2025). With irradiated cells having the capacity to modulate and alter the quantity of biophotons being released, acting as signals to other cells in the vicinity (Le et al., 2015, 2017). The role of

biophotons in cell-to-cell communication has been demonstrated with work in our lab and others demonstrating its role in RIBE communication using cell lines (Le et al., 2015, 2017; Lyng et al., 2000, 2001; Mothersill et al., 2007).

Our aim for this work is to better understand how these signals are transferred between individuals using whole-body endpoints. Our work confirms the mediation of RIBE via soiled housing materials and biophotons, agreeing with what was discovered previously in mice and cell lines. This suggests that similar RIBE signaling pathways could be conserved between invertebrates and vertebrates. We also suggest that within crickets specifically, there could be other signals at play. We suggest intergenerational signaling, outside of radiation specific signals, may play a role in maturation suppression for *Acheta domesticus*. Overall, our work paints a complex picture of non-eusocial insect development and communication, demonstrating how indirect interactions can mediate RIBE between individuals.

3.3. Methodology

Cricket husbandry:

Long-term breeding colonies are maintained on a 12h/12h light/dark photoperiod at 28 ± 2 °C. *Ad libitum* food (Quicks Country Range Multi-Fowl Grower Rations,17.0% crude protein, 2.5% crude fat, and 4.0% crude fiber) and water (reverse osmosis water in soaked cellulose sponges) were provided. Eggs were collected from our cricket colonies, as described in previous studies (Li et al., 2023). Oviposition medium (Organic Garden Soil, Swiss Farms Products Inc., Maryville, USA) was provided for egg collection, with medium being left in the colony for 24h and subsequently removed. Medium was removed 24h after initial hatching to ensure juveniles were approximately the same age. Only one media was left in the colony at a time, with replicates being subsequent media. Where possible, groups were housed in separate plexiglass enclosures to minimize uncontrolled interactions between groups. Group sizes were chosen in accordance with optimal rearing densities (Mahavidanage et al., 2023).

Experiment 1: Inducement with soiled housing materials

This experiment is set up into two main phases, phase 1 (P1) donors, and phase 2 (P2) receivers of soiled housing materials with the aim of seeing if we can induce RIBE in phase 2 crickets using soiled housing materials from phase 1.

For P1 donor crickets, juvenile crickets were separated into sham control (0 Gy) or irradiated (0.5 Gy) groups (n = 120 per group, 3 replicates per treatment). For P2 receiver crickets, juvenile crickets were separated into sham control (0 Gy), bystander (0 Gy), or irradiated (0.5 Gy). P2 sham received cartons from P1 sham, P2 bystander received

cartons from P1 irradiated, P2 irradiated received new cartons and were used as an outgroup. All groups were housed in separated plexiglass incubators but were housed in the same incubator as their housing material donors. P2 irradiated groups were housed alone. Due to timing of maturation between P1 and P2, P1 crickets were nearing the end of their maturation when the carton was removed and introduced to P2 cricket populations at 14 days post hatch, this meant that on average, housing materials were exposed to P1 crickets for ~26 days before being permanently introduced to P2 (Figure 3.8.1).

Experiment 2: Inducement with UV biophotons

To induce biophoton transfer, irradiated adult crickets were placed in a separate housing box underneath the juvenile housing box. To block out biophotons, UV filter sheets (Edmund Optics, stock #29-426 UV Filter Sheet, deep-dyed polyethylene terephthalate (PET), 0.04 mm thick.) which absorbs UVA, UVB, UVC, and has <10% transmission below 390nm was. The filter sheets were crafted into boxes which surrounding the juvenile housing containers to block transmission.

Juvenile crickets were separated into sham with filter, sham without filter, bystander with filter, and bystander without filter (n = 60 juveniles per group, 2 replicates per treatment, three male and three female adults per group). Adults were introduced at 14 days post hatch and removed at 28 days post hatch (Figure 3.8.2). Sample size of adults chosen as the number of tubes/adults that can be irradiated at once, equal distribution of sex as radiation and RIBE have been demonstrated to be sex specific. Deaths occurred among the groups for the adults and were removed when discovered. At least one male and one

female cricket was present and alive by the end of the exposure period for each group and replicate.

Radiation:

For all experiments, whole-body ionizing radiation was performed at the Taylor Radiobiology Source at McMaster University. All crickets were randomly distributed into groups (10 crickets at a time, snake order, 1 2 3 4, 4 3 2 1, 1 2 3 4, etc.) and brought over to the source at 14 days post hatch. Crickets were irradiated at approximately 0.56 Gy/min for the first set of experiments on housing material, and approximately 0.55 Gy/min for the second set of experiments on biophotons. The difference in dose rate is due to natural decay of the Caesium-137 gamma source between experiments as the distance from the source remained the same.

Data collection:

Containers were checked daily at 4 weeks post hatch, with all containers receiving similar amounts of handling. Crickets began maturing approximately 6 weeks post hatch. Maturation was checked daily at approximately the same time; a cricket is considered matured once wings fully develop. Adult crickets were removed and placed in a separate container to ensure they were not counted twice.

Maturation mass was collected in grams (resolution of 0.001 g ± 0.002 g) but converted to mgs for analysis, maturation time was collected in days, and growth rate was calculated as a function of maturation mass/maturation time and given as mg/day.

Some crickets matured with missing legs or pieces of shed stuck; these are noted in the data files, but no data points were removed.

Statistical analysis:

All statistical analysis were performed in R 4.3.2.

Mixed linear models were used to investigate the effect of treatment and sex, both within and between. Where replications were controlled for as a background effect. 95% confidence intervals of statistical analysis were plotted. Estimated means, significances, and confidence intervals are shown in tables. Significances differences denoted as asterisks on confidence intervals are generated from R automatically, and denote when the confidence interval does not cross zero.

P-values for estimated means sometimes did not align with our confidence intervals, mostly when viewing maturation weight. The discrepancies are minor, and we have opted to describe and discuss our results using the confidence intervals, however we have provided the estimated means P-values alongside the confidence intervals and made the datasets available so that readers can form their own conclusions.

Data Availability:

Datasets and R scripts can be found at https://github.com/Xiaobing9/Indirect-Bystander

3.4. Results

As is the case with *A. domesticus*, males in all our groups, regardless of treatment, took significantly longer time to reach maturation, had lower mass at maturation, and had overall lower average growth rate than their female counterparts.

Housing materials:

Phase 1:

Looking at population level impacts, no significant differences in maturation mass (mg) or growth rate (mg/day) were observed between the irradiated and sham control populations. The irradiated population (n=271, m=122, f=149) matured on average 0.30 [0.006, 0.605] days faster than the sham control (n=294, m=146, f=148) (Figure 3.8.3, Table 3.7.1, 3.7.2, & 3.7.3).

Looking within sexes between populations, we found only significant differences in maturation time between irradiated females versus female sham control (0.38 [0.0183, 0.739] days faster than sham), with everything else non-significant (Figure 3.8.4, Table 3.7.1, 3.7.2, & 3.7.3).

Phase 2:

Time to maturation:

No significant differences were observed between bystander (n=317, m=183, f=134) and sham control (n=322, m=160, f=162) populations. The irradiated outgroup (n=321, m=158, f=163) matured significantly faster than both the sham and bystander populations (1.83 [1.502, 2.168] and 1.92 [1.585, 2.254] days respectively) (Figure 3.8.5a, Table 3.7.1).

Similar results were observed within sexes between the populations. We observed no significant difference between sham and bystander populations in either sex. When comparing to the irradiated outgroup population, both males and females matured significantly faster than their sham (1.76 [1.292, 2.225], and 1.89 [1.415, 2.370] days, respectively] and bystander population (1.93 [1.478, 2.381], and 1.89 [1.387, 2.389] days, respectively) counter parts (Figure 3.8.5b & 3.8.5c, Table 3.7.1).

Mass at maturation

When compared to the sham population, both the bystander and irradiated populations had significantly higher mass at maturation (11.06 [1.375, 20.743], and 10.05 [0.418 19.683] mgs, respectively), with no significant differences between the bystander and irradiated populations (Figure 3.8.6a, Table 3.7.2).

When comparing between sex, there is no longer any significant differences between any of the groups (Figure 3.8.6b & 3.8.4c, Table 3.7.2).

Average growth rate:

When compared to the sham control population, both the bystander and irradiated populations had significantly higher average growth rate at maturation (0.23 [0.041, 0.420], and 0.66 [0.469, 0.846], respectively), the irradiated population was also significantly higher than the bystanders (0.43 [0.238, 0.617]) (Figure 3.8.7a, Table 3.7.3). Looking within sex, sham and bystander of both sexes were not significantly different. However, irradiated males and females had significantly higher growth rate than both sham (0.58 [0.345, 0.818], and 0.74 [0.444, 1.038], respectively) and bystander (0.40 [0.176, 0.633], 0.455 [0.143, 0.766], respectively) (Figure 3.8.7b & 3.8.7c, Table 3.7.3).

Biophotons:

Time to maturation:

Compared to the sham without filter population (n=104, m=46, f=58), we observed no significant differences between them and the bystander without filter (n=113, m=60, f=53) population. Significant differences in time to maturation were observed when comparing the sham without filter population to both the sham with filter (n=108, m=49,

f= 59; 2.00 [1.463, 2.551] days faster than sham without filter) and bystander with filter (n=105, m=55, f=50; 1.16 [0.609, 1.707] days faster than sham without filter) populations (Figure 3.8.8a). When looking at the bystander with filter population, we found that it was also significantly different than both the sham with filter (0.85 [0.306, 1.392] days slower) and bystander without filter (0.87 [0.330, 1.403] days faster) populations (Table 3.7.4).

Comparing within females, only the sham with filter group matured significantly faster than the sham without filter (1.42[0.599,2.228] days) population. Within males when compared to the sham without filter group, all groups (sham with filter, bystander with and without filter) matured significantly faster (2.74[2.054,3.452], 1.69[1.005,2.365], & 0.72[0.047,1.381] days faster, respectively) (Figure 3.8.8b & 3.8.8c). Both bystander groups matured significantly slower than the sham with filter group (1.05[0.399, 0.1736] & 2.02[1.381, 2.694] days slower, with and without filter, respectively). Within bystanders, the population with filter matured significantly slower (0.96[0.336, 1.607] days) than the population without filter (Table 3.7.4).

Mass at maturation:

No significant differences were observed between any of the groups at a population or sex-specific level (Figure 3.8.9, Table 3.7.5).

Average growth rate at maturation:

Compared to the sham without filter population, both the sham and bystander with filter populations had significantly higher growth rates (0.51[0.120, 0.896] & 0.46[0.070, 0.852] mg/day respectively) (Figure 3.8.10a). Compared to the bystander without filter

population, both sham and bystander with filter populations had significantly higher growth rates (0.47[0.088,0.848], & 0.42(0.038,0.803) mg/day, respectively) (Table 3.8.6). No significant differences were observed between the females between any of the groups. Within males, sham with filter had a significantly higher growth rate than sham without a filter (0.56[0.395,1.086] mg/day) (Figure 3.8.10b & 3.8.10c, Table 3.7.6).

3.5. Discussion

The aim of this study was to translate known methods of indirect radiation-induced bystander effect (RIBE) to whole-body endpoints using an insect model. We demonstrate the mediation of RIBE signals through both soiled housing materials and UV biophotons with whole-body endpoints. But we also discovered another interesting phenomenon not related to radiation. Our results show that interindividual communication between crickets does not require direct interactions, and that both pheromones (from soiled housing materials) and biophotons play a major role in maturation suppression of a younger generation even in the absence of ionizing radiation (Figure 3.8.5 & 3.8.8). This agrees with the work done in mice and confirms that in both vertebrates and invertebrates, soiled housing materials play a major role in interindividual communication. We show that soiled housing materials can induce shifts in development to younger individuals. We also confirm that biophotons play a role in indirect interindividual communication, building upon previous work in our lab where we demonstrated the impact of biophoton on cell cultures (Le et al., 2015, 2017). This observed delayed maturation agrees with and expands upon the initial work done by Watler in 1982, who was the first to demonstrate

maturation suppression from direct interactions in *A. domesticus* (Watler, 1982). We demonstrated similar effects using indirect methods, highlighting the importance of these signals in inter-cricket communication, and demonstrating it is not just direct social interactions that can shift development. While the exact mechanisms and reasoning are still unclear 40 years later, we postulate that this is due to sexual fitness and selection, where the older population would want to stratify the generations as much as possible to reduce intergenerational competition. However, more work, particularly in signaling pathways, is required to understand the reasonings evolutionarily.

Looking now at our main goal of investigating RIBE signal mediation, we observe the impacts of RIBE despite the interference of maturation suppression signals. In our soiled housing materials experiment, our results from phase one found no significant difference in average growth rate (mg/day) between the irradiated and sham groups, suggesting that the significant decrease in growth rate observed between phase 2 sham and the irradiated groups must be attributed to the soiled housing materials and the common pheromones that are routinely expressed (Figure 3.8.7a). Furthermore, RIBE has caused the bystander crickets to behave more similarly to the irradiated outgroup than the shams, a transfer of effect from irradiated to unirradiated individuals. Bystander crickets have significantly higher average growth rate than the shams and are beginning to display similar endpoints to the irradiated outgroup, although they are still significantly different, with the irradiated crickets having significantly higher growth rate still. This suggests to us the mediation of RIBE through pheromones within the soiled housing materials, which are causing the bystander group to behave similarly to the irradiated group than the sham

group. Previous work in our lab has demonstrated that ionizing radiation altered hydrocarbon emission in cricket cuticles (Fuciarelli & Rollo, 2021). We therefore suggest that radiation could have changed the composition of expressed hydrocarbons, and that these differing hydrocarbons/pheromones seem to counteract the general maturation suppression effects between older and younger crickets and partially rescue growth rate. Looking closer at our results, we find that this increased growth rate in the P2 bystander group appears to come primarily from an increased mass at maturation with no apparent difference in maturation time. A similar increase in mass was observed in our P2 irradiated group, but it was also accompanied by a significantly faster time to maturation (Figure 3.8.5a & 3.8.6a).

RIBE is also seen in our biophoton experiments to a lesser extent, we see a significant increase in growth rate in our sham and bystander groups with filter, and that our bystander group caught up to the sham group again (3.8.10).

We suggest that older individuals suppress maturation by increasing the younger juvenile's time to reach maturity, and that it does so by impacting their ability to convert food into energy and mass. But this effect is partially rescued by RIBE, and while it may still take longer to reach maturity, they are able to somewhat more effectively convert food into usable energy, leading to a higher mass and average growth rate. Studies have determined 96 mgs as a critical mass for *A. domesticus* during development, with crickets being only a few mgs short of 96 mgs having delayed molts and development (Waltler, 1982). We believe that while RIBE has rescued the ability to convert food to energy, it is not fully restored to baseline, and as a result we observe this increase in maturation time,

but the bystander crickets are able to more effectively use the extra time to gain mass, leading to a higher growth rate compared to the shams.

Looking at sex-specific responses, our results suggests that males and females respond similarly to both pheromones and biophotons, but this might a result of mixed sex pheromones and biophoton emissions. This was unexpected as we previously observed sex-specific RIBE in crickets (Li et al., 2023). It is possible that by only using male or female populations to induce these effects, we could see a skewed sex-specific response from one sex only.

Furthermore, we acknowledge the limitations of indirectly measuring biophotons, and that deaths occurred during the timespan within the irradiated adults. If biophotons signaling is saturable, then it is hard for us to fully understand the picture without measuring and emitting biophotons directly, though our results seem to suggest the range that we used might be adequate, as we see a significant difference as a result of filtering biophotons, though unfortunately not between our unfiltered groups.

We suggest that biophotons and pheromonal cues could act both as general maturation suppression signals, and as an intermediate signal which triggers and releases bystander-eliciting factors when exposed to radiation. We demonstrate the importance of biophotons in interindividual communication, and the role radiation plays in biophoton emission. However, we then also demonstrate that similar effects can be simulated through soiled housing materials, suggesting that there could be various compounding intermediate signals that can induce RIBE as well as suppress maturation, and that it is more complex than previously anticipated.

Our results not only highlight the importances of pheromones and biophotons in invertebrate communication but further confirm the idea of maturation suppression between generations, suggesting that older individuals are able to delay the timing of maturation. We also demonstrate that these emitted signals can be altered by radiation, and that these altered signals mediate RIBE. This has important ramifications for environmental radiation protection and remediation, as we suggest that the current paradigm of environmental radiation protection and consideration fails to account for non-targeted effects of ionizing radiation.

3.6. Acknowledgements

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3.7. Tables

Table 3.7.1. Soiled Housing Average Time to Maturation.

Phase 1 - Time to Maturation	**Sham**	**0.5 Gy**
Sham	(42.5)	0.0466
0.5 Gy	0.30[0.006,0.605]	(42.2)
Phase 1 - Time to Maturation - Female	**Sham**	**0.5 Gy**
Sham	(41.5)	0.0404
0.5 Gy	0.38[0.018,0.739]	(41.2)
Phase 1 - Time to Maturation – Male	**Sham**	**0.5 Gy**
Sham	(43.5)	0.4340
0.5 Gy	0.20[-0.297,0.693]	(43.3)

Phase 2 – Time to Maturation	**Sham**	**Bystander**	**0.5 Gy**
Sham	(44.0)	0.8734	<0.0001
Bystander	-0.08[-0.419,0.250]	(44.1)	<0.0001
0.5 Gy	1.83[1.503,2.168]	1.92[1.585,2.254]	(42.1)
Phase 2 - Time to Maturation - Female	**Sham**	**Bystander**	**0.5 Gy**
Sham	(43.4)	0.9999	<0.0001
Bystander	0.00[-0.498,0.506]	(43.4)	<0.0001
0.5 Gy	1.89[1.415,2.369]	1.89[1.387,2.389]	(41.5)
Phase 2 - Time to Maturation Male	**Sham**	**Bystander**	**0.5 Gy**
Sham	(44.6)	0.7380	<0.0001
Bystander	-0.17(-0.621,0.279]	(44.7)	<0.0001
0.5 Gy	1.76[1.292,2.225]	1.93[1.477,2.381]	(42.8)

Note: Brackets denote average time (days), top right cells denote significance from estimated means, bottom left cells denote confidence intervals [2.5%, 97.5%].

Table 3.7.2. Soiled Housing Material Average Mass at Maturation.

Phase 1 - Mass at Maturation	**Sham**	**0.5 Gy**
Sham	(403)	0.4288
0.5 Gy	3.51[-5.241,12.099]	(399)
Phase 1 - Mass at Maturation - Female	**Sham**	**0.5 Gy**
Sham	(422)	0.1997
0.5 Gy	7.65[-3.951,19.259]	(415)
Phase 1 - Mass at Maturation - Male	**Sham**	**0.5 Gy**
Sham	(383)	0.8472
0.5 Gy	-1.29[-14.304,11.721]	(385)

Phase 2 - Mass at Maturation	**Sham**	**Bystander**	**0.5 Gy**
Sham	(433)	0.0656	0.1025
Bystander	-11.06[-20.743,-1.375]	(445)	0.9773
0.5 Gy	-10.05[-19.683,-0.418]	1.01[-8.685,10.703]	(444)
Phase 2 - Mass at Maturation - Female	**Sham**	**Bystander**	**0.5 Gy**
Sham	(456)	0.2180	0.2256
Bystander	-13.16[-28.571,2.310]	(469)	0.9945
0.5 Gy	-12.37[-27.023,2.344]	0.79[-14.628,16.210]	(468)
Phase 2 - Mass at Maturation - Male	**Sham**	**Bystander**	**0.5 Gy**
Sham	(411)	0.2936	0.4126
Bystander	-9.31(-21.509,2.866]	(420)	0.9827
0.5 Gy	-8.19[-20.852,4.427]	1.11[-11.127,13.345]	(419)

Note: Brackets denote average mass (mg), top right cells denote significance from estimated means, bottom left cells denote confidence intervals [2.5%, 97.5%].

Table 3.7.3. Soiled Housing Material Average Growth Rate at Maturation.

Phase 1 - Growth Rate	**Sham**	**0.5 Gy**
Sham	(9.48)	0.8695
0.5 Gy	0.02[-0.165,0.194]	(9.47)
Phase 1 - Growth Rate - Female	**Sham**	**0.5 Gy**
Sham	(10.2)	0.4393
0.5 Gy	0.10[-0.153,0.350]	(10.1)
Phase 1 - Growth Rate - Male	**Sham**	**0.5 Gy**
Sham	(8.80)	0.5536
0.5 Gy	-0.08[-0.339,0.180]	(8.88)

Phase 2 - Growth Rate	**Sham**	**Bystander**	**0.5 Gy**
Sham	(9.86)	0.0459	<0.0001
Bystander	-0.23[-0.420,-0.041]	(10.09)	<0.0001
0.5 Gy	-0.66[-0.856,-0.469]	-0.43[-0.617,-0.238]	(10.52)
Phase 2 - Growth Rate - Female	**Sham**	**Bystander**	**0.5 Gy**
Sham	(10.5)	0.1690	<0.0001
Bystander	-0.29[-0.598,0.0251]	(10.8)	0.0121
0.5 Gy	-0.74[-1.038,-0.444]	-0.46[-0.766,-0.143]	(11.3)
Phase 2 - Growth Rate - Male	**Sham**	**Bystander**	**0.5 Gy**
Sham	(9.21)	0.2827	<0.0001
Bystander	-0.18[-0.405,0.051]	(9.39)	0.0017
0.5 Gy	-0.58[-0.818,-0.345]	-0.40[0.634,-176]	(9.79)

Note: Brackets denote growth rate (mg/day), top right cells denote significance from estimated means, bottom left cells denote confidence intervals [2.5%, 97.5%].

Table 3.7.4. Average Maturation Time for Biophoton Experiments.

Time to Maturation - Photon	**Sham - Filter**	**Sham + Filter**	**Bystander + Filter**	**Bystander - Filter**
Sham - Filter	(39)	<0.0001	0.0003	0.7189
Sham + Filter	2.00 [1.463,2.551]	(37)	0.0128	<0.0001
Bystander + Filter	1.16[0.609,1.707]	-0.85[-1.392,-0.306]	(37.8)	0.0093
Bystander - Film	0.29[-0.247,0.830]	-1.71[-2.249,-1.182]	-0.87[-1.403,-0.330]	(38.7)
Time to Maturation - Photon Female	**Sham - Filter**	**Sham + Filter**	**Bystander + Filter**	**Bystander - Filter**
Sham - Filter	(38.4)	0.0045	0.3743	0.9991
Sham + Filter	1.42[0.599,2.228]	(37)	0.3602	0.0040
Bystander + Filter	0.70[-0.133,1.569]	-0.72[-1.543,0.1523]	(37.7)	0.3200
Bystander - Filter	-0.06[-0.877, 0.801]	-1.47[-2.287,-0.615]	-0.76[-1.624, 0.113]	(38.4)
Time to Maturation - Photon Male	**Sham - Filter**	**Sham + Filter**	**Bystander + Filter**	**Bystander - Filter**
Sham - Filter	(39.6)	<0.0001	<0.0001	0.1520
Sham + Filter	2.74[2.054,3.452]	(36.9)	0.0134	<0.0001
Bystander + Filter	1.69[1.005,2.365]	-1.05[-0.1736,-0.399]	(37.9)	0.0177
Bystander - Filter	0.72[0.047,1.381]	-2.02[-2.694,-1.381]	-0.96[-1.607,-0.336]	(38.9)

Note: Brackets denote time (days), top right cells denote significance from estimated means, bottom left cells denote confidence intervals [2.5%, 97.5%].

Table 3.7.5. Average Mass at Maturation for Biophoton experiment.

Mass at Maturation - Photon	**Sham - Filter**	**Sham + Filter**	**Bystander + Filter**	**Bystander - Filter**
Sham - Filter	(410)	0.9872	0.9031	0.9929
Sham + Filter	2.69[-13.043,18.420]	(408)	0.7346	0.9999
Bystander + Filter	-5.56[-21.429,10.304]	-8.25[-23.963,7.461]	(416)	0.7645
Bystander - Filter	2.18[-13.413,17.768]	-0.51[-15.943,14.922]	7.74[-7.781,23.261]	(408)
Mass at Maturation - Photon Female	**Sham - Filter**	**Sham + Filter**	**Bystander + Filter**	**Bystander - Filter**
Sham - Filter	(443)	1.0000	0.8437	0.9957
Sham + Filter	-0.02[-23.655,23.529]	(443)	0.8432	0.9956
Bystander + Filter	-10.40[-34.611,14.655]	-10.38[-34.430,14.621]	(453)	0.7321
Bystander - Filter	2.86[-20.860,27.689]	2.88[-20.640,27.653]	13.2601[-11.743,38.565]	(440)
Mass at Maturation - Photon Male	**Sham - Filter**	**Sham + Filter**	**Bystander + Filter**	**Bystander - Filter**
Sham - Filter	(378)	0.9294	0.9994	0.9992
Sham + Filter	6.37[-14.318,26.481]	(372)	0.8763	0.9567
Bystander + Filter	-1.18[-20.908,18.799]	-7.55[-26.656,12.383]	(379)	0.9937
Bystander - Filter	1.31[-17.873,21.073]	-5.06[-23.614,14.651]	2.49[-15.895,21.203]	(377)

Note: Brackets denote average mass (mg), top right cells denote significance from estimated means, bottom left cells denote confidence intervals [2.5%, 97.5%].

Table 3.7.6. Average Growth Rate at Maturation for Biophoton Experiment.

Growth Rate - Photon	**Sham - Filter**	**Sham + Filter**	**Bystander + Filter**	**Bystander - Filter**
Sham - Filter	(10.5)	0.0529	0.0994	0.9970
Sham + Filter	-0.51[-0.896,-0.120]	(11.1)	0.9952	0.0782
Bystander + Filter	-0.46[-0.852,-0.070]	0.05[-0.340,0.434]	(11.0)	0.1399
Bystander - Filter	-0.04[-0.424,0.344]	0.47[0.088,0.848]	0.42(0.038,0.803)	(10.6)
Growth Rate - Photon Female	**Sham - Filter**	**Sham + Filter**	**Bystander + Filter**	**Bystander - Filter**
Sham - Filter	(11.5)	0.3963	0.3908	0.9958
Sham + Filter	-0.46[-1.024,0.110]	(12.0)	0.9998	0.3017
Bystander + Filter	-0.48[-1.074,0.109]	-0.03[-0.615,0.564]	(12.0)	0.2863
Bystander - Filter	0.07[-0.514,0.652]	0.53[-0.054,1.106]	0.55[-0.053,1.0156]	(11.5)
Growth Rate - Photon Male	**Sham - Filter**	**Sham + Filter**	**Bystander + Filter**	**Bystander - Filter**
Sham - Filter	(9.53)	0.1647	0.2899	0.9148
Sham + Filter	-0.56[-1.086,-0.395]	(10.09)	0.9828	0.4174
Bystander + Filter	-0.46[-0.966,0.051]	0.09[-0.397,0.607]	(9.99)	0.6212
Bystander - Filter	-0.17[-0.659,0.340]	0.39[-0.906,0.897]	0.29[-0.177,0.773]	(9.70)

Note: Brackets denote growth rate (mg/day), top right cells denote significance from estimated means, bottom left cells denote confidence intervals [2.5%, 97.5%].

3.8. Figures

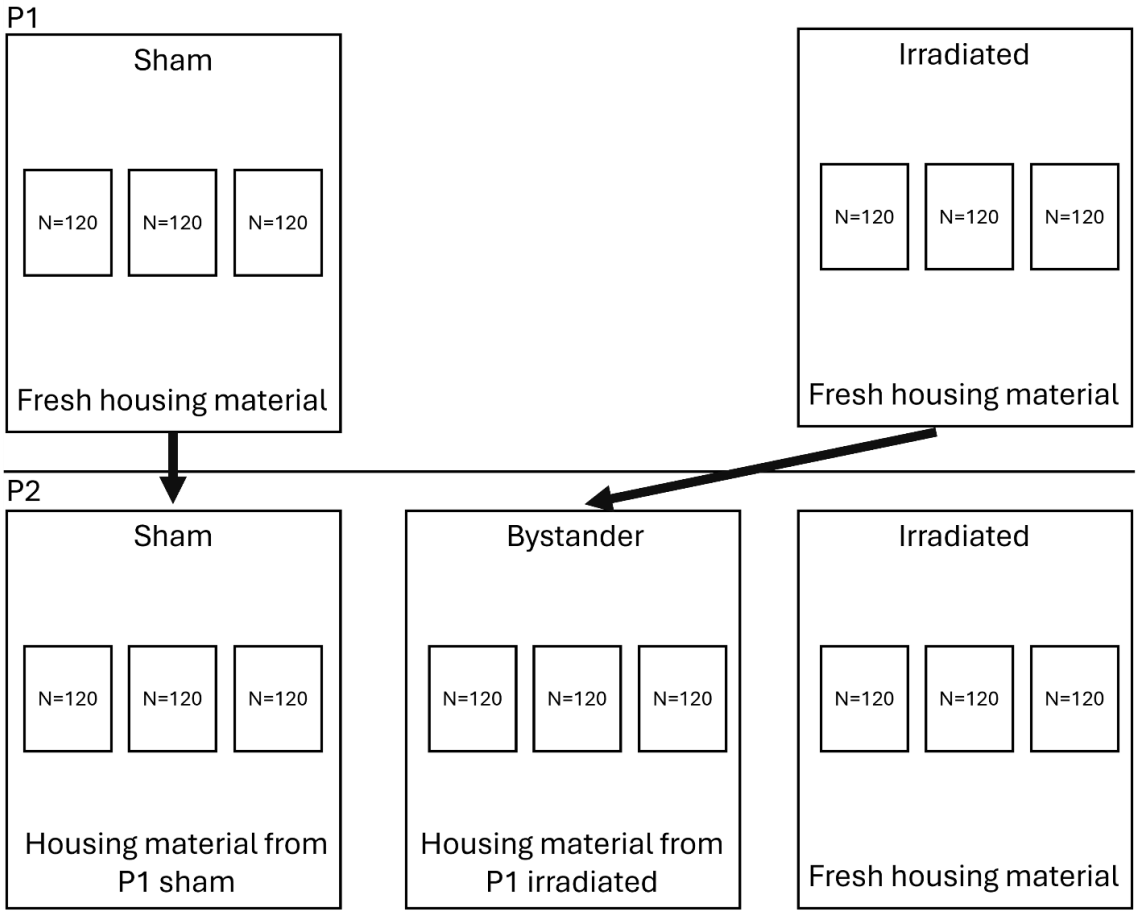

Figure 3.8.1. Experimental Design for Experiment 1, soiled housing materials. In phase 1 (P1), both sham and irradiated (0.5 Gy) individuals received fresh housing materials at 14 days post hatch. Phase 2 (P2) shams received housing materials from P1 sham, while P2 bystander received housing materials from P1 irradiated, and P2 irradiated (0.5 Gy) received fresh housing materials. For each of the triplicates, soiled housing materials were introduced at 14 days post hatch of P2 crickets, which meant that on average the housing materials were in P2 for ~26 days.

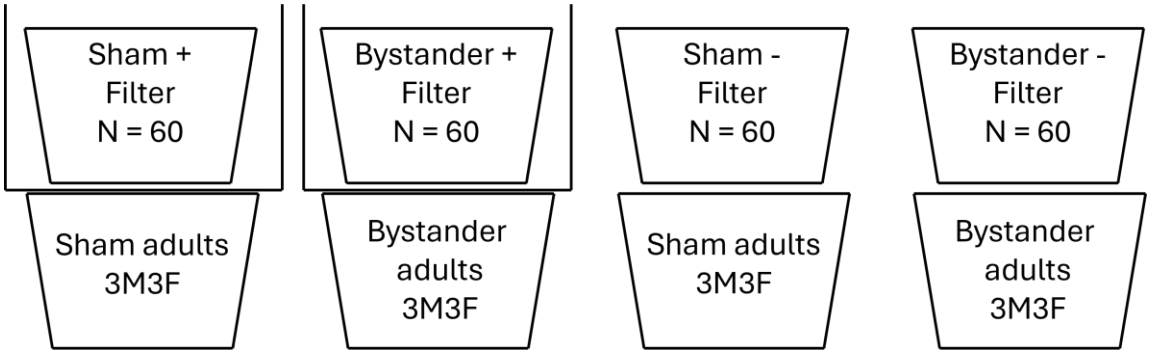

Figure 3.8.2. Experimental Design for Experiment 2, biophotons. Juveniles were housed from 14 days post hatch into containers which were either protected with UV photon absorptive filter or not. Irradiated and sham adults were introduced into containers beneath them at 14 days post hatch until 28 days post hatch, with initial population of 3 males and 3 females per treatment.

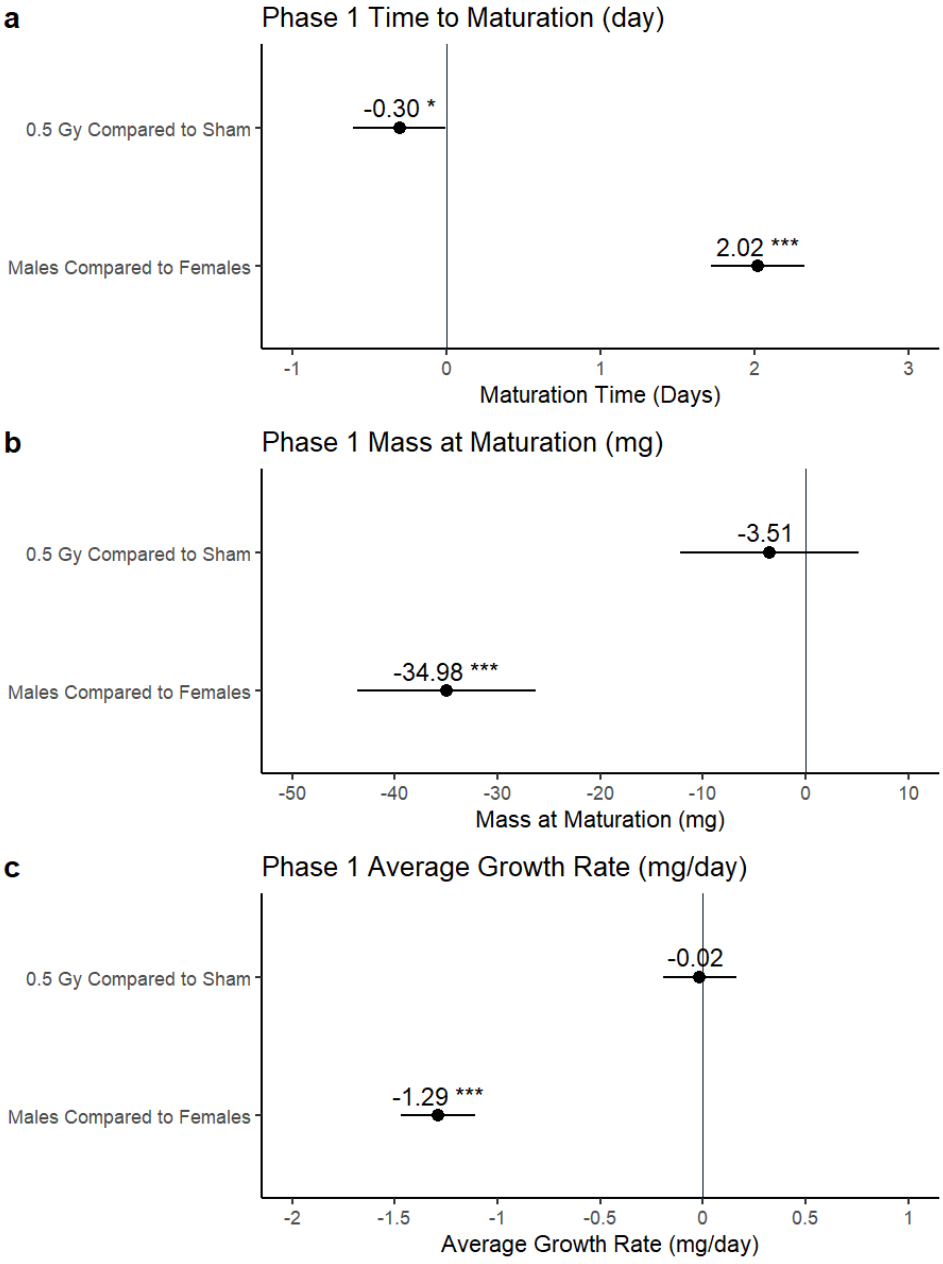

Figure 3.8.3. Maturation Endpoints in Phase 1. a) Average time to reach maturation(day), 0.5 gy irradiated population matured significantly faster than sham controls, overall males took longer to mature in all groups when compared to females. b) Average mass at maturation (mg), no significant differences between irradiated and sham populations, overall males had a lower mass than females at maturation. c) Average growth rate (mg/day), no significant difference between irradiated and sham populations, overall males on average had significantly lower average growth rate than females. n = 271 for 0.5 Gy, 294 for sham, 268 males, 297 females.

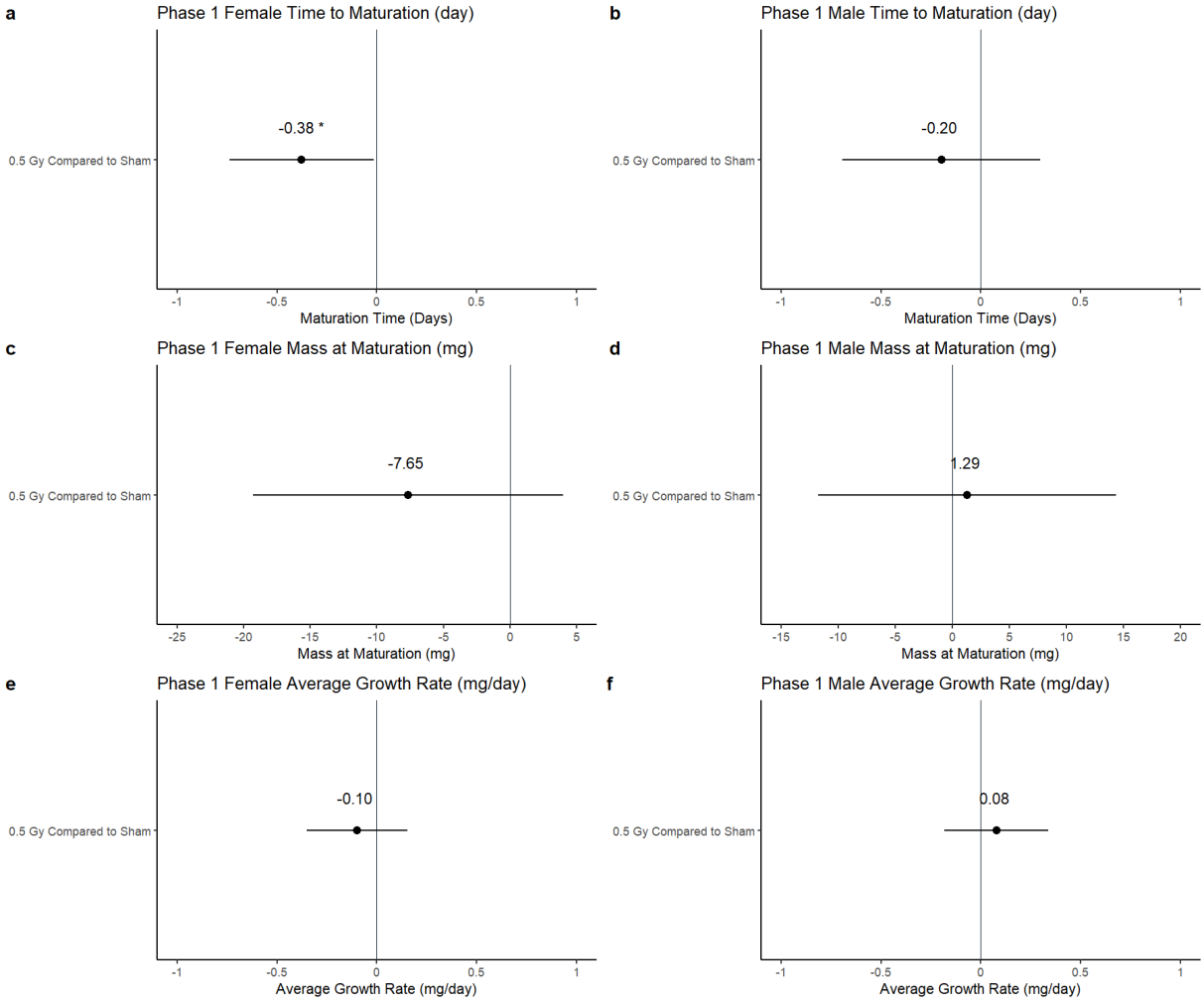

Figure 3.8.4. Sex-Specific Maturation Endpoints in Phase 1 When Comparing 0.5 Gy to Sham. a) 0.5 Gy females matured significantly faster than sham population. b-f) no significant differences observed when comparing within sexes between 0.5 Gy and Sham populations. n = 148, 149 for sham and 0.5 Gy females; n = 146, 122 for sham and 0.5 Gy males

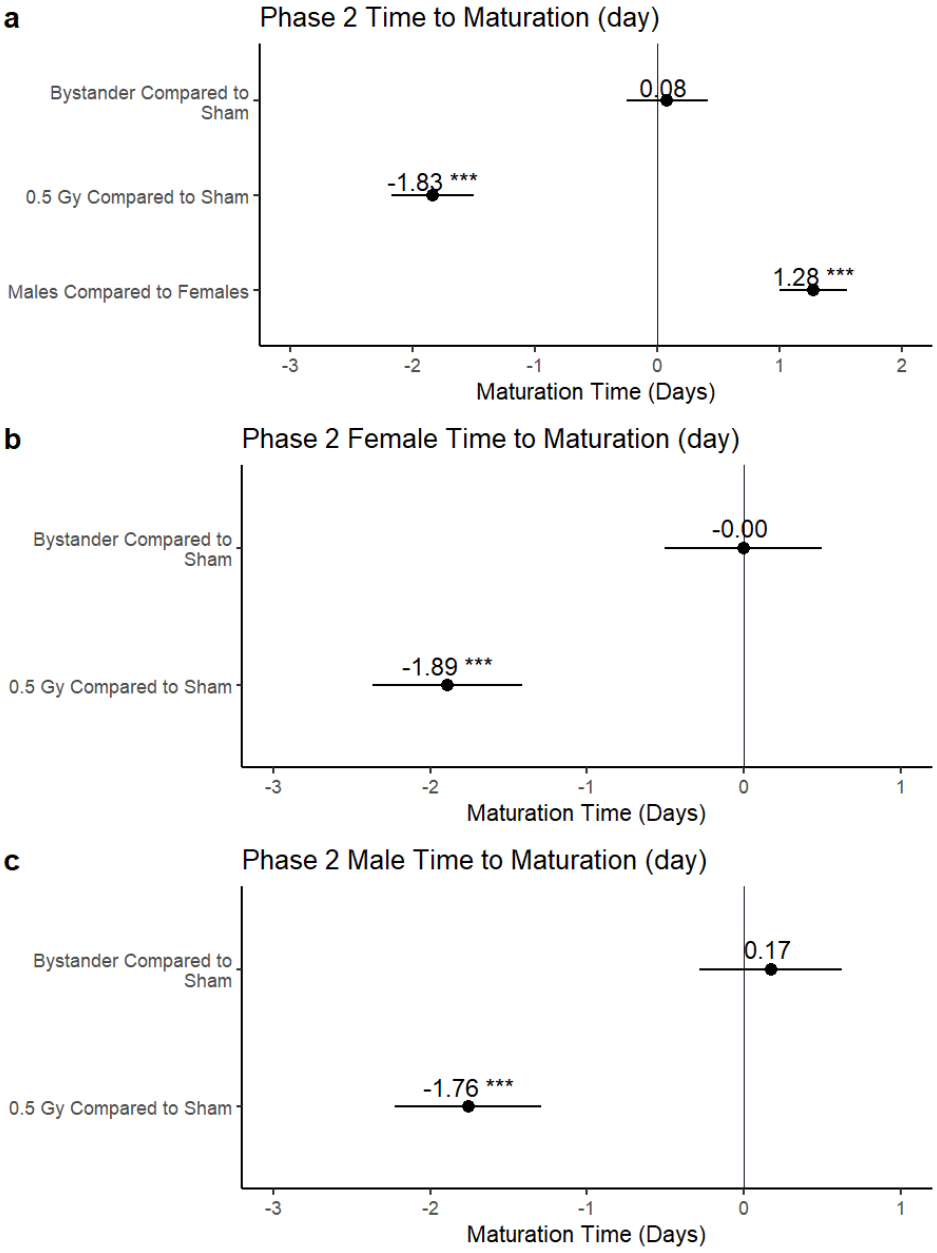

Figure 3.8.5. Phase 2 Time to Reach Maturation. a) average time to reach maturity (day) of the population when compared to Shams, and average time to reach maturity of males when compared to females. 0.5 Gy population reached maturity significantly faster than both sham and bystander groups, who were not significantly different from each other. Males matured significantly later than females. b&c) 0.5 Gy females and males both matured significantly faster than sham and bystander groups within sexes. n = 322 (f = 162, m = 160), 317 (f = 134, m = 183), & 321 (f= 163, m = 158) for sham, bystander, and 0.5 Gy; n = 459, 501 for females, males.

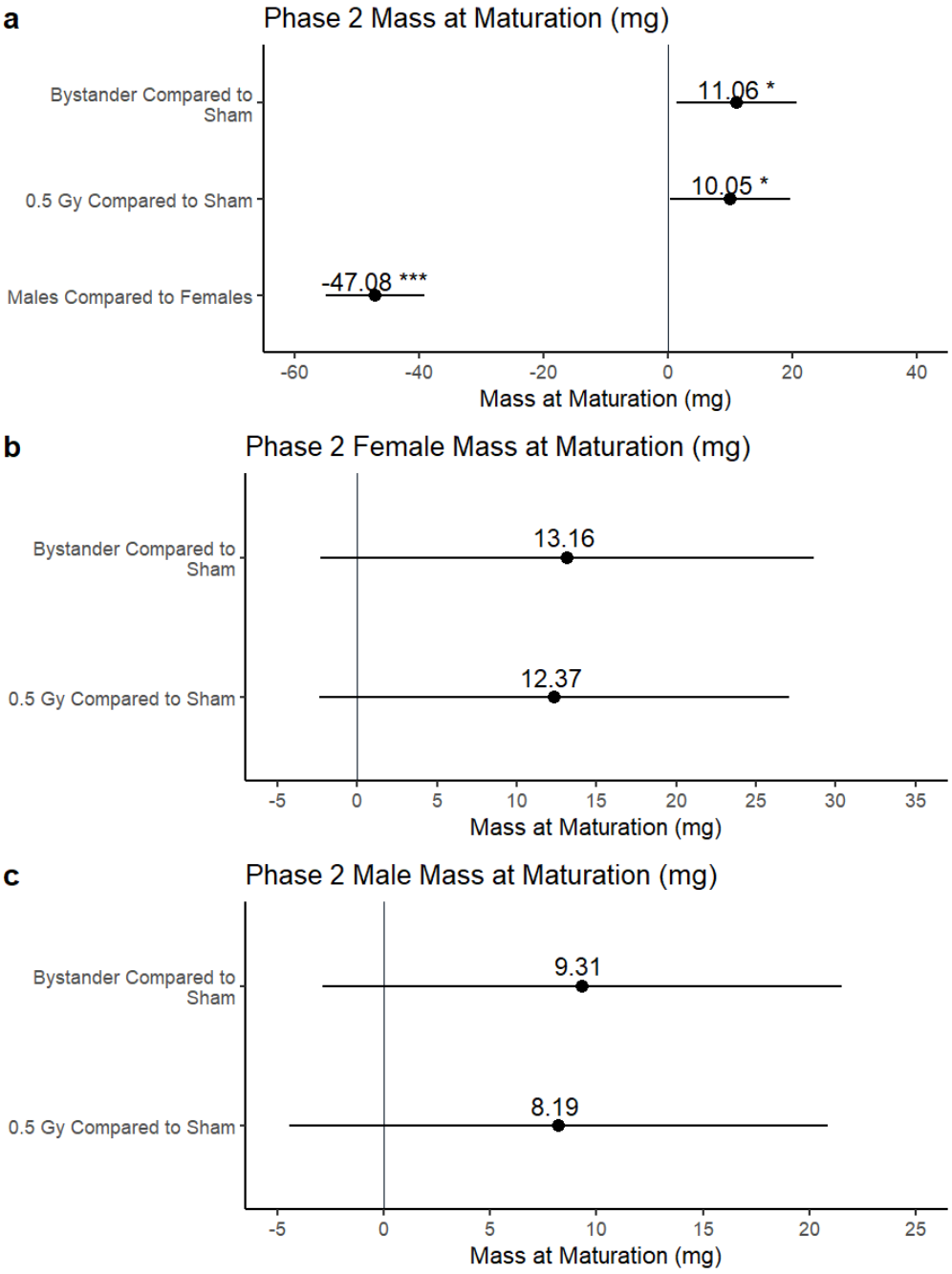

Figure 3.8.6: Phase 2 Mass at Maturation. a) both bystander and 0.5 Gy populations had significantly higher mass at maturation (mg) than sham population, with no significances between them. Males on average within all groups had lower maturation mass than females. b&c) no significant differences in maturation mass between any of populations. n = 322 (f = 162, m = 160), 317 (f = 134, m = 183), & 321 (f= 163, m = 158) for sham, bystander, and 0.5 Gy; n = 459, 501 for females, males.

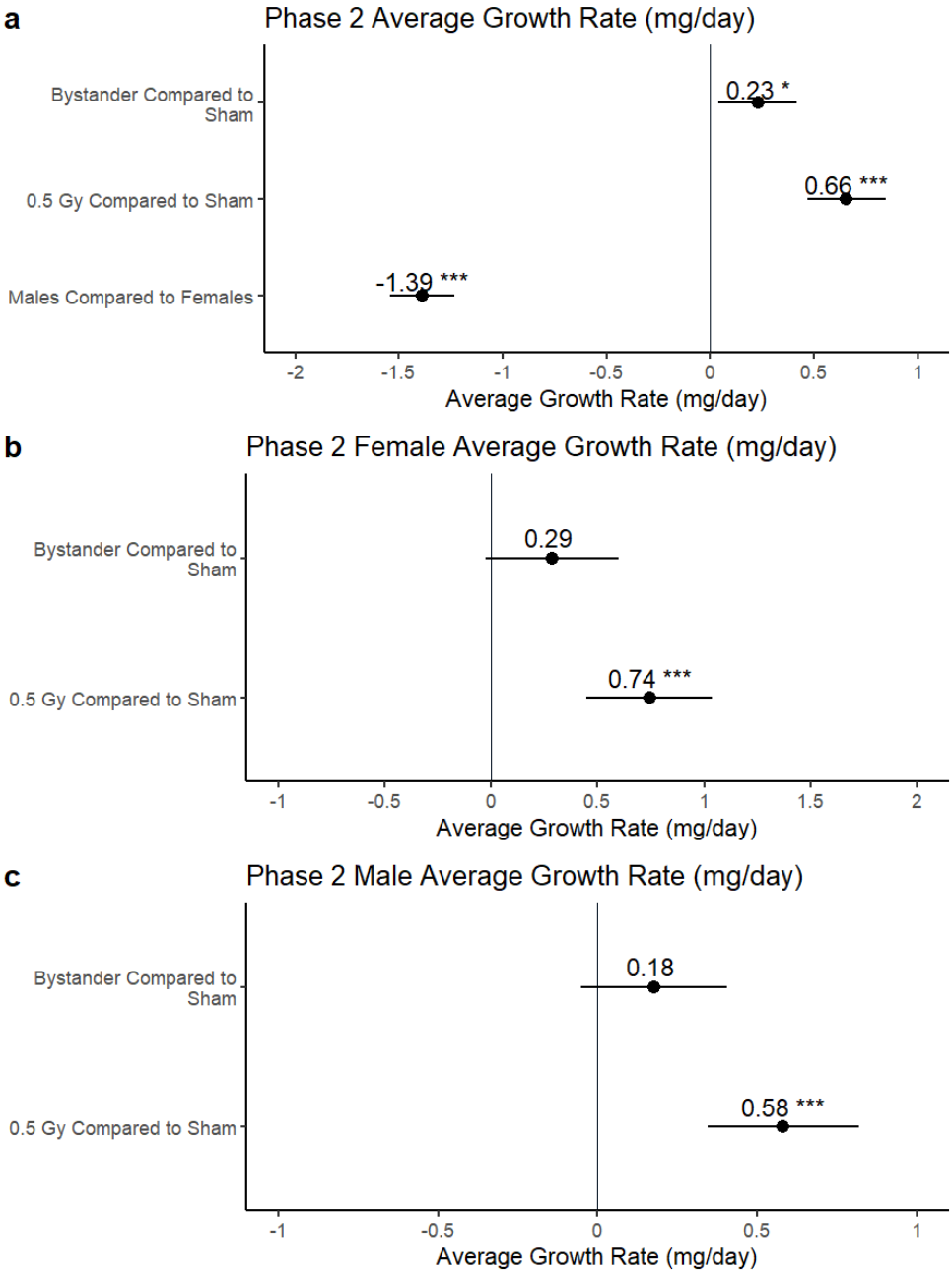

Figure 3.8.7. Phase 2 Average Growth Rate to Maturation. a) both bystander and 0.5 Gy populations had significantly higher average growth rate than sham population, 0.5 Gy also had significantly higher growth rate than bystander population. Males on average between all groups had significantly lower average growth rate than females. b&c) Both females and males in the 0.5 Gy population had significantly higher average growth rate than both bystander and sham populations within sexes, with no significant difference between those two populations. n = 322 (f = 162, m = 160), 317 (f = 134, m = 183), & 321 (f= 163, m = 158) for sham, bystander, and 0.5 Gy; n = 459, 501 for females, males.

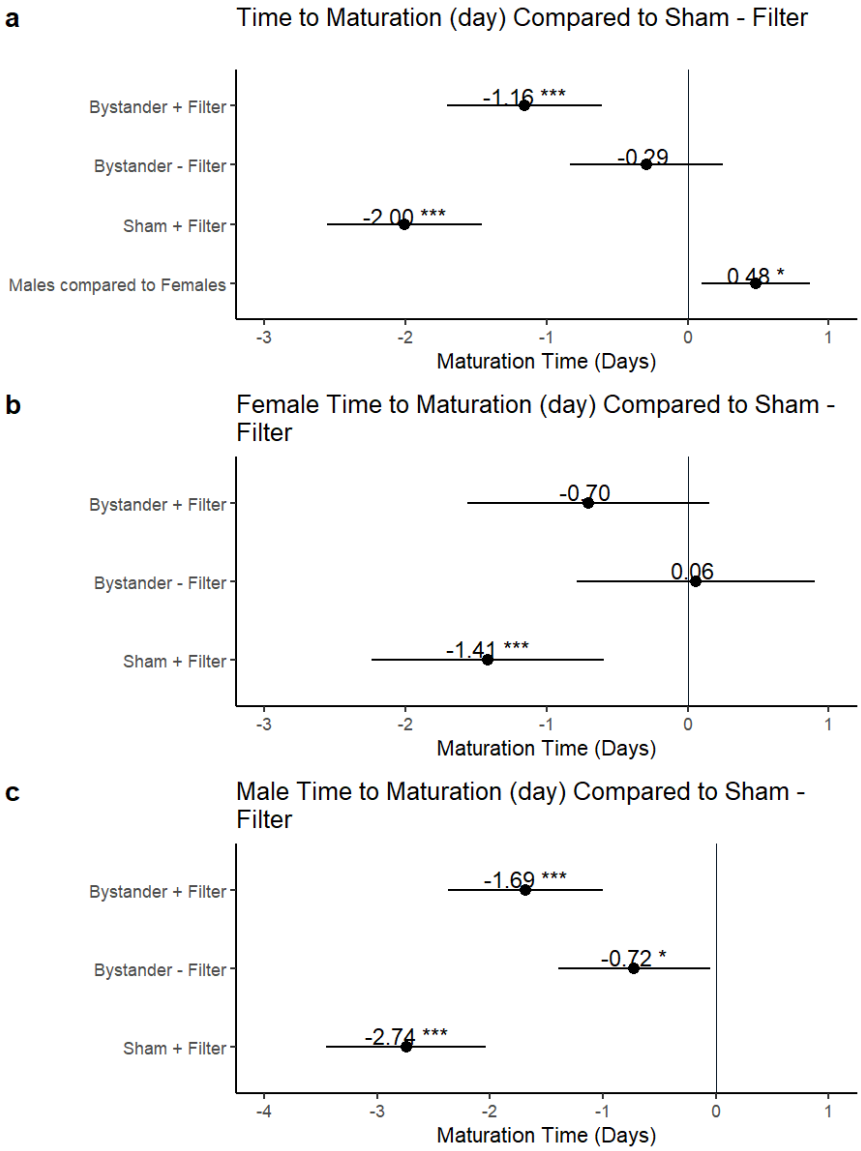

Figure 3.8.8. Average Time to Reach Maturation When Compared to Sham Without Biophoton Filters. a) at a population level, both populations without a biophoton filter matured at similar times, and both population with filter matured significantly faster than the populations without filters. Within the filter groups, sham population matured significantly faster than bystander population. b) Looking specifically at females, sham with filter population matured significantly faster than both populations without filters, with every other population having no significant differences between each other. c) within males, all groups are significantly different from one another, with the sham and bystander without filter males taking the longest to mature, then bystander and sham with filter males maturing faster, respectively. n = 104 (f = 58, m = 46), 113 (f = 53, m = 60), 108 (f = 59, m = 49), 105 (f = 50, m = 55) for sham without filter, bystander without filter, sham with filter, and bystander with filter respectively.

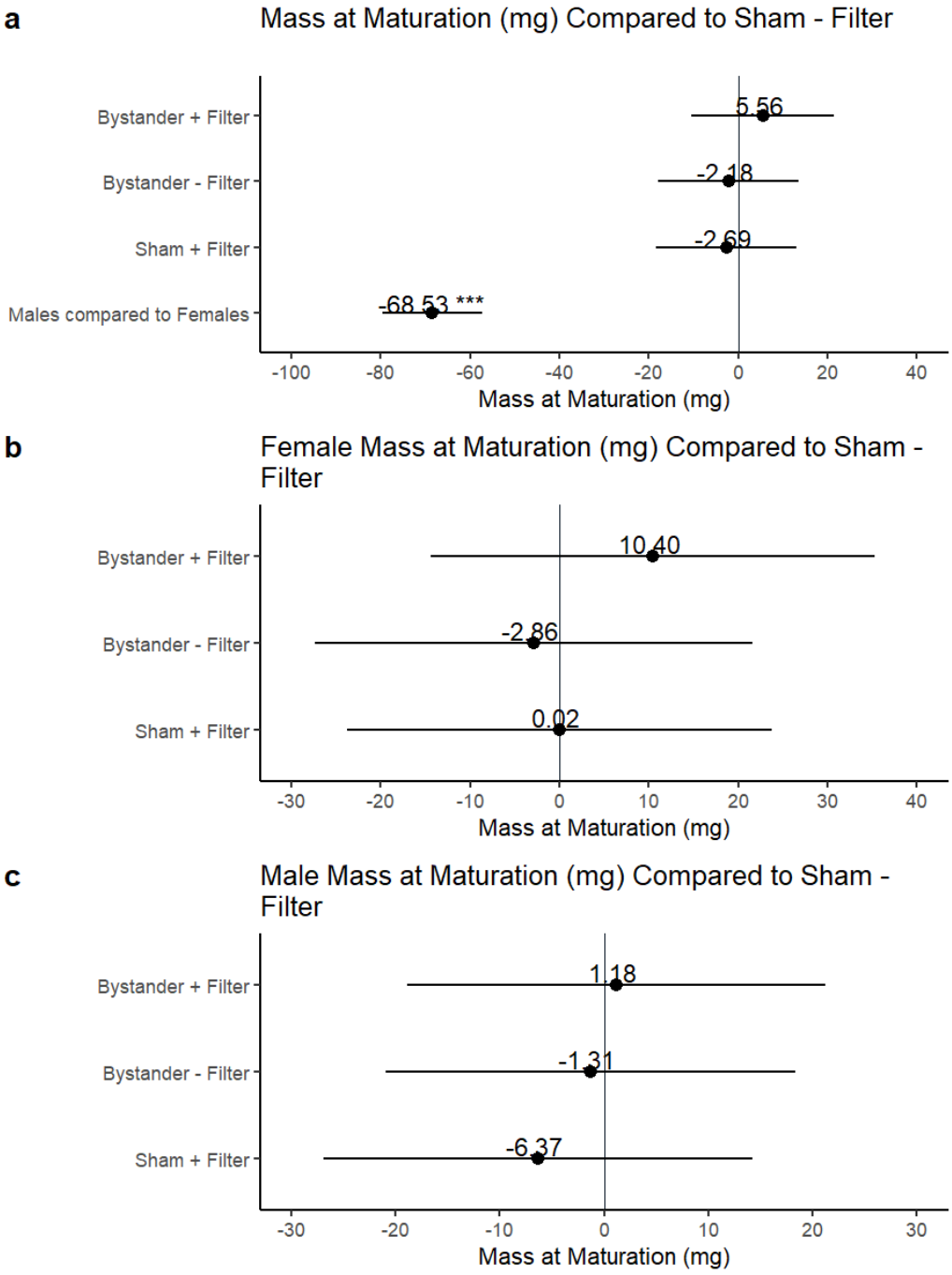

Figure 3.8.9. Average Mass at Maturation When Compared to Sham Without Biophoton Filter. a) no significant differences between any of the populations, males between all populations had significantly lower mass at maturation than females. b&c) no significant differences between groups when comparing within sexes. n = 104 (f = 58, m = 46), 113 (f = 53, m = 60), 108 (f = 59, m = 49), 105 (f = 50, m = 55) for sham without filter, bystander without filter, sham with filter, and bystander with filter respectively.

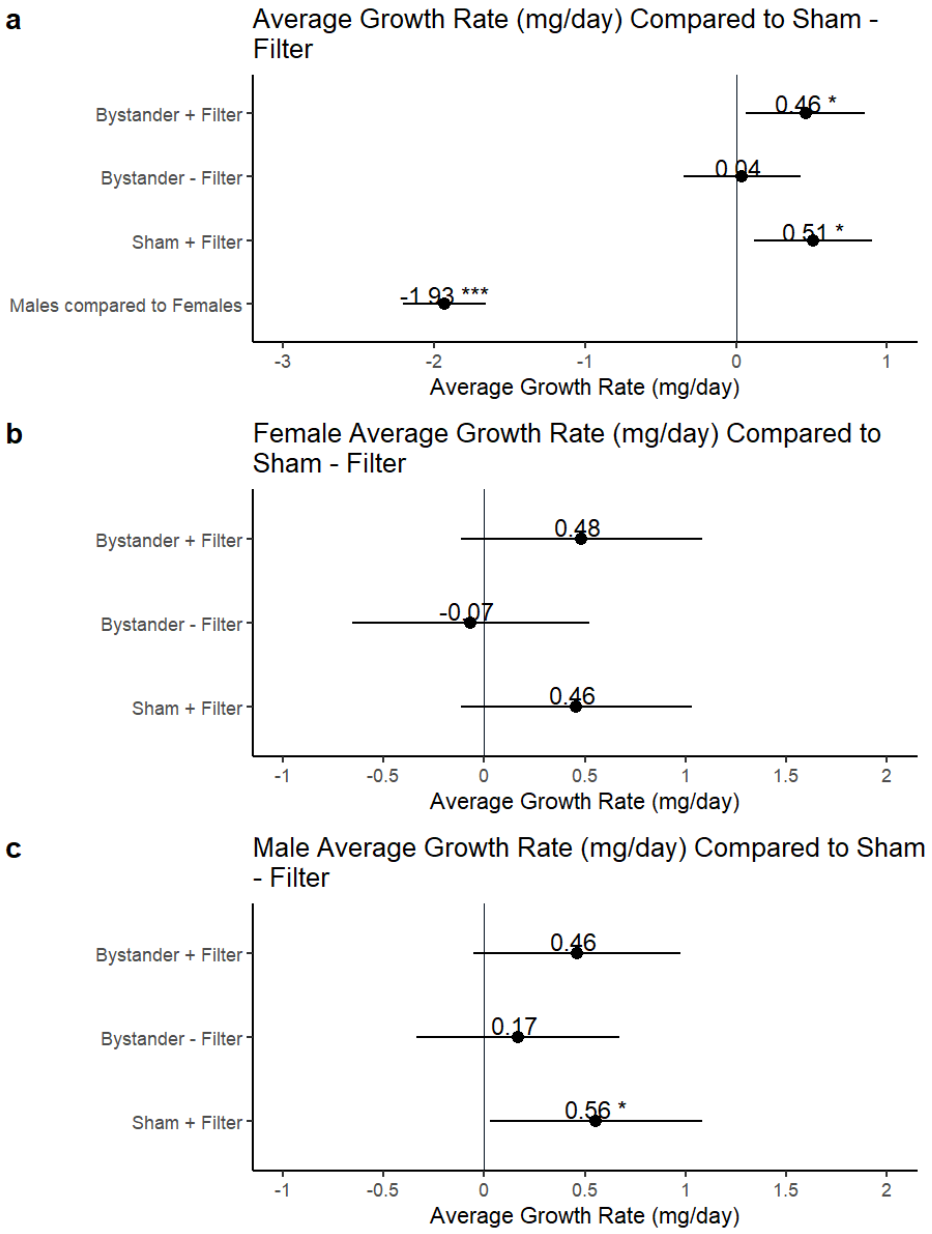

Figure 3.8.10. Average Growth Rate to Maturation When Compared to Sham Without Biophoton Filter. a) Both sham and bystander populations with filter had significantly higher growth rate (mg/day) than sham and bystander without filter populations, who were not significantly from each other. b) no significant differences when comparing between females of all populations. c) Sham with filter males had significantly higher growth rate than sham without filter population, with no significant differences when comparing males of any of the other populations. n = 104 (f = 58, m = 46), 113 (f = 53, m = 60), 108 (f = 59, m = 49), 105 (f = 50, m = 55) for sham without filter, bystander without filter, sham with filter, and bystander with filter respectively.

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Chapter 4

4. Radiation-induced bystander effect in cricket *Acheta domesticus* mediated by developing egg sacks

4.1. Preface

This prepared manuscript aims to understand autogenous signaling of developing cricket eggs and if these signals can mediate radiation-induced bystander effect (RIBE). We found that radiation can cause eggs to mediate RIBE to unirradiated juvenile crickets and suggest that these signals are mediated via disruptions through the fatty acids coated on the exterior of the eggs. This chapter discusses the intricacies of signalling in insect eggs and suggests various options for how RIBE signals are being mediated from egg to developing juveniles.

4.2. Introduction

Eggs are viewed by many as an easy source of energy as they contain high levels of proteins and fats and are unable to defend themselves (Pal & Molnár, 2021). For some, lots of energy and time are invested into taking care of and protecting their few eggs to ensure safe hatching and the passing of their genetic material (Deas & Hunter, 2014). For others, the strategy is to lay many eggs and hope that enough hatch and survive with minimal parental support (Deas & Hunter, 2014). This is often the case with insects, where females can often lay many clutches with different partners over a single breeding

season (Yao et al., 2009). This isn't to say that they do not offer any protection for their eggs, many orders of insects are known to provide a physical or chemical barrier for the eggs, laying them underground, coating them with fecal matter, hairs, scales, or secretions to protect against predation and parasitization (Yao et al., 2009).

For many orthopteras who lay their eggs in the soil or vegetation, their main concerns of predation are from various ground beetles and other orthoptera, as well as parasitization from flies and wasps. House crickets (*Acheta domestics*) have evolved to have a chemical barrier of fatty acids on the outside of the eggs, disguising them as dead conspecifics of their potential predators to avoiding predation (Chen et al., 2024).

Ionizing radiation is often considered a physical stressor, and in *A. domesticus*, ionizing radiation has been shown to cause shifts in concentrations of the hydrocarbons secreted from adult male cuticles, potentially altering secreted pheromones (Fuciarelli & Rollo, 2021). This shift might be caused by lipid peroxidation, where increased reactive oxygen species (ROS) production leads to the degradation of lipids via increased oxidative stress (Ayala et al., 2014; Zheng et al., 2023).

Radiation-induced bystander effect (RIBE) has been demonstrated in house crickets, where interactions with irradiated crickets will cause shifts in maturation time and average growth rate to maturation (Li et al., 2023). Previous work from our lab has also demonstrated the ability of chemical cues (pheromones) to indirectly induce RIBE, a non-targeted effect of ionizing radiation causing unirradiated individuals to alter their development as if they were irradiated (Chapter 3).

We hypothesize then that ionizing radiation can alter the concentration of fatty acids found on the eggs via lipid peroxidation, altering detectable chemical cues that mediates RIBE in unirradiated juveniles.

Our results suggest that irradiated cricket eggs can communicate and mediate RIBE to unirradiated juveniles, resulting in an overall lower average growth rate caused by delayed maturation. This has significant ramifications for populations surrounding contaminated areas exposed to ionizing radiation. Our results suggest that when tracking the effects of radiation on the environment, the migratory patterns and distance of exposed individuals should be considered. As previous work shows that signals altered by radiation are persistent and can cause RIBE well into adulthood, potentially affecting multiple generations of unirradiated individuals and causing shifts in their development (Li et al., 2023).

4.3. Methodology

Cricket husbandry:

Fertilized eggs were collected from our cricket colonies, as described by previous studies (Li et al., 2023). In brief, long-term breeding colonies are maintained with the lab on a 12h/12h light/dark photoperiod at 28 ± 2 °C. *Ad libitum* food (Quicks Country Range Multi-Fowl Grower Rations,17.0% crude protein, 2.5% crude fat, and 4.0% crude fiber) and water (reverse osmosis water in soaked cellulose sponges) were provided. Oviposition medium (Organic Garden Soil, Swiss Farms Products Inc., Maryville, USA) was provided for egg collection, with medium being left in the colony for 24h and

subsequently removed. Medium was removed 24h after initial hatching to ensure juveniles were approximately the same age. Only one media was left in the colony at a time, with replicates being subsequent media from the next days. Where possible, groups were housed in separate plexiglass enclosures to minimize unwanted exposures.

Egg and Crickets:

Fertilized eggs for the experiment were collected in a similar manner from the same parent colony. Except eggs were collected in a large petri dish with moist medical gauze as media instead of soil. Eggs were then individually separated and transplanted into either sham control or bystander group through smaller petri dishes with gauze, the eggs were placed in the middle layers of gauze to maintain moisture. Eggs were separated from initial petri dish to water to separate, and then into the gauze.

Initially 115 crickets and eggs per treatment, replicates for all groups, both eggs and crickets were sorted randomly through snake case sorting, 10 at a time.

There is also a just medical gauze outgroup which contained no eggs but went through the exact same procedures as all other groups.

Radiation:

For all experiments, whole-body ionizing radiation was performed at the Taylor Radiobiology Source at McMaster University. Eggs were irradiated when they were three days old, and juveniles were 14 days old. The eggs were irradiated with the petri dish lid off for a total of 0.5 Gy @ 0.544 Gy/min, or 56 seconds. All eggs were brought to the source, eggs were introduced within an hour of irradiation to juveniles, for the first day the petri dish lid was left on, the lid was then removed for the subsequent four days until

the eggs were removed and set aside for monitoring of hatching. Eggs in total was introduced to the juveniles for five days, from when they were three days old to eight days old.

Egg Hatching:

Once removed from the juveniles, petri dish lids were returned, with the whole plate being placed in an empty container to incubate. After 12-13 days, approximately 6 juveniles hatched from each of the petri dishes (only 1 group had 8 juveniles hatch, while the rest 6), suggesting similar survivorship between irradiated and sham groups (Table 4.7.1).

Data collection:

Containers were checked daily at 4 weeks post hatch, with all containers receiving similar amounts of handling. Crickets began maturing approximately 6 weeks post hatch. Maturation was checked daily at approximately the same time; a cricket is considered matured once wings fully develop. Adult crickets were removed and placed in a separate container.

Maturation mass was collected in grams (resolution of 0.001 g ± 0.002 g) but converted to mgs for analysis, maturation time was collected in days, and growth rate was calculated as a function of maturation mass/maturation time and given as mg/day.

Some crickets matured with missing legs or pieces of shed stuck; these are noted in the data files, but no data points were removed.

Statistical analysis:

All statistical analysis were performed in R 4.3.2.

Mixed linear models were used to investigate the effect of treatment and sex, both within and between. Where replications were controlled for as a background effect. 95% confidence intervals of statistical analysis were plotted. Estimated means significances, and confidence intervals are shown in tables. Confidence intervals are used to interpret results, but the estimated means p-values are provided as reference. Significances differences denoted as asterisks on confidence intervals are generated from R automatically and denote when the confidence interval does not cross zero.

Data availability:

Datasets and R scripts are available at https://github.com/Xiaobing9/EggRIBE

4.4. Results

As is the case within *A. domesticus*, males are on average significantly smaller, take longer to mature, and therefore have a lower average growth rate at maturation than females.

Time to Maturation:

Looking between the gauze outgroup (n = 222, m = 117, f = 105) and sham control (n = 205, m = 114, f = 91) treatments, there are no significant differences in time to reach maturation (days) at a population level, or within sexes. When comparing to the bystander population (n = 213, m = 104, f = 109), both gauze and sham matured significantly faster (1.09[0.60, 1.59] & 1.03 [0.54, 1.52] days, respectively) (Figure 4.8.1a, Table 4.7.2). Similar results were observed in females, where both sham and gauze females matured

significantly faster (1.09[0.34,1.84] & 1.60[0.88,2.32]) days, respectively) (Figure 4.8.1b). However, in males, bystander and gauze males were not significantly different, and only the sham males matured significantly faster (1.08[0.43,1.75] days) than bystander (Figure 4.8.1c).

Mass at Maturation:

No significant differences were observed in maturation mass (mg) between any of the population or within sexes (Figure 4.8.2).

Average Growth Rate at Maturation:

The bystander population had significantly lower average growth rate compared to the sham population (0.34 [0.13,0.56] mg/day less) (Figure 4.8.3a). No significant differences in average growth rate (mg/day) were seen the gauze versus the sham or bystander populations (Table 3.7.4). No significant differences were observed between the any of the males between populations. Bystander females have significantly lower growth rate than sham females (0.46[0.11,0.82] mg/day less), with no other significances between females (Figure 4.8.3c).

4.5. Discussion

When comparing between our sham control with unirradiated cricket eggs and our negative control with no cricket eggs, we observed no significant differences in any of our endpoints, suggesting that the difference observed between them and our bystander group (irradiated eggs) must be caused by ionizing radiation (Figures 4.8.1-4.8.3). Our results suggest that ionizing radiation causes cricket eggs to produce signals that can mediate

radiation-induced bystander effect (RIBE) in unirradiated juvenile crickets, delaying their maturation and reducing their average daily growth rate compared to those not exposed to irradiated cricket eggs (Figures 4.8.1 & 4.8.3).

Insect eggs are known to autogenously produce defensive chemicals to protect against predation and parasitization (Eisner et al., 2000; Hilker, 2003). In house crickets (*A. domesticus*), it was recently discovered that surface of cricket eggs is covered in various fatty acids such as lauric, myristic, palmitoleic, palmitic, linoleic, oleic, and stearic acids (Chen et al., 2024). These fatty acids act as a defense mechanism by signalling to and fooling potential predators into believing that they are dead conspecifics, evading predation. Particularly of interest to us is the presence of linoleic and oleic acids, which are known as necromone, or chemicals emitted upon death, of multiple predators of crickets as well as house crickets themselves (Aksenov & David Rollo, 2017; Yao et al., 2009).

It is currently unclear if *A. domesticus* cricket eggs are continuously generating fatty acids, or if it is coated by the mother as they are being laid. While the eggs here were washed briefly with water, it should not have removed a significant amount of the fatty acids from the eggs. And as we observed almost identical hatching rates within the two treatment groups, we do not believe that radiation caused higher mortality within the eggs, resulting in higher levels of necromones that negatively impacted development (Table 4.7.1).

The presence of fatty acids and other signals in unirradiated eggs does not appear to impact development of juvenile crickets (Figures 4.8.1-4.8.3). Previous research in our

lab has shown that ionizing radiation can alter hydrocarbon concentrations on the cuticle of male crickets (Fuciarelli & Rollo, 2021). We believe that ionizing radiation is interacting with the fatty acids and generating clastogenic-like factors on the surface of the eggs. Clastogenic factors are products of lipid peroxidation and oxidative stress found within the plasma of irradiated individuals and can induce genomic instability when introduced to unirradiated cells (Emerit, 1994; Lyng & Azzam, 2024). We say clastogenic-like in this case as these were not factors found within the plasma, but factors on the surface of the eggs which caused shifts in the juveniles who interacted with the eggs and might have absorbed these factors.

Oxidative stress and reactive oxygen species (ROS) increase at a dose-dependent manner with ionizing radiation and are known to damage DNA at high concentrations (Han et al., 2014). Radiation could be generating higher levels of ROS within the eggs, increasing DNA damage of the developing embryos via both indirect ionization and lipid peroxidation, which breaks down fatty acids into peroxides and hydroperoxides (Ayala et al., 2014). Lipid peroxidation of linoleic acid has been shown to increase DNA damage (de Kok et al., 1994). Linoleic acid is known to be found on the surface of *A. domesticus* eggs and could be inducing DNA damage to the surrounding developing juveniles. Regardless of the mechanism of transfer, our results do not suggest that there were any significant differences in mass between any of the groups (Figure 4.8.2), but it does show a significantly reduced average growth rate to maturation and delay in maturation in our treatment group (Figure 4.8.3). We therefore suggest that this delay in maturation is caused either by the crickets eating less due to the presence of these signals, or that the

signals are reducing the efficiency of the crickets to convert food into usable energy and mass, delaying the time it takes to reach their critical molting mass. We cannot say what pathways are being regulated to potentially affect maturation and growth from our experiment, and further experiments will be required to fully understand the impact of ionizing radiation on cricket eggs and of RIBE on cricket development.

Future work could involve analyzing the composition of chemicals found on the outer layer of the eggs and how ionizing radiation could affect them over time and seeing if similar effects could be induced by mimicking the chemical composition of irradiated eggs. Furthermore, we need to identify how these signals are being mediated between eggs and juveniles, as the cricket eggs were in layers of moist medical gauze, juveniles were able to freely interact with the eggs and gauze, drinking water from the gauze. By identifying if these signals are mediated through air or water, it would allow us a better understanding of RIBE transfer between individuals.

Overall, our results here contribute to the growing pool of both insect development and RIBE research, particularly in demonstrating that ionizing radiation can alter the autogenously generated defence chemicals found on the exterior of eggs, resulting in RIBE and delayed development of juveniles. This has important ramifications for environmental radiation protection, and more work is required to fully comprehend the extend of ionizing radiation on altering chemical concentrations, and if an acute dose of ionizing radiation to developing eggs can cause persistent bystander signals well into adulthood of these developing eggs.

4.6. Acknowledgments

The authors would like to humbly thank Dr. George Samuel Long for his aid with the statistical analysis. As well as Dr. Tamara Fuciarelli for her help in editing this manuscript.

4.7. Tables

Table 4.7.1. Time to hatch (days) and number of hatchlings from each replicate and treatment group.

Group	Time to Hatch (days)	Hatchlings
Sham replicate 1	13	6
Sham replicate 2	13	8
Bystander replicate 1	12	6
Bystander replicate 2	13	6

Notes: Initially 115 eggs were seeded per group. The bystander group received 0.5 Gys of radiation while the shams received 0 Gys of radiation. The time to hatch was measured by when the first hatchling was spotted, and all recorded hatchlings were observed within 48 hours of the first hatchling.

Table 4.7.2. Average Time to Maturation (days).

Average Time to Maturation	**Sham**	**Bystander**	**Gauze**
Sham	(43.5)	<0.0001	0.9654
Bystander	-1.09[-1.59,-0.60]	(44.6)	0.0001
Gauze	-0.06[-0.56,0.43]	1.03[0.54,1.52]	(43.6)
Average Female Time to Maturation	**Sham**	**Bystander**	**Gauze**
Sham	(43.3)	0.0132	0.3746
Bystander	-1.09[-1.84,-0.34]	(44.4)	<0.0001
Gauze	0.52[-0.24,1.27]	1.60[0.88,2.32]	(42.8)
Average Male Time to Maturation	**Sham**	**Bystander**	**Gauze**
Sham	(43.7)	0.0044	0.1729
Bystander	-1.08[-1.75,-0.43]	(44.8)	0.3088
Gauze	-0.59[-1.24,0.05]	0.49[-0.16,1.15]	(44.3)

Notes: Brackets denote average time (days), top right are estimated means p-values, bottom left are 97.5% confidence intervals. Gauze: n = 222, m = 117, f = 105; Sham: n = 205, m = 114, f = 91; Bystander: n = 213, m = 104, f = 109.

Table 4.7.3. Average Mass at Maturation (mg).

Average Mass at Maturation	**Sham**	**Bystander**	**Gauze**
Sham	(381)	0.5571	0.434
Bystander	5.61[-4.93,16.37]	(376)	0.9802
Gauze	6.63[-3.82,17.23]	1.01[-9.44,11.40]	(375)
Average Female Mass at Maturation	**Sham**	**Bystander**	**Gauze**
Sham	(407)	0.4768	0.2816
Bystander	9.89[-6.76,26.53]	(397)	0.9203
Gauze	13.07[-3.72,29.86]	3.18[-12.85,19.20]	(394)
Average Male Mass at Maturation	**Sham**	**Bystander**	**Gauze**
Sham	(356)	0.9641	0.9888
Bystander	1.79[-11.61,15.57]	(354)	0.9921
Gauze	0.96[-12.07,14.29]	-0.83[-14.35,12.61]	(355)

Notes: Brackets denote average mass (mg), top right are estimated means p-values, bottom left are 97.5% confidence intervals. Gauze: n = 222, m = 117, f = 105; Sham: n = 205, m = 114, f = 91; Bystander: n = 213, m = 104, f = 109.

Table 4.7.4. Average Growth Rate to Maturation (mg/day).

Growth Rate	**Sham**	**Bystander**	**Gauze**
Sham	(8.77)	0.0063	0.3411
Bystander	0.34[0.13,0.56]	(8.42)	0.1977
Gauze	0.15[-0.06,0.37]	-0.19[-0.40,0.03]	(8.61)
Female Growth Rate	**Sham**	**Bystander**	**Gauze**
Sham	(9.41)	0.0276	0.5233
Bystander	0.46[0.11,0.82]	(8.94)	0.2714
Gauze	0.20[-0.16,0.55]	-0.27[-0.61,0.07]	(9.21)
Male Growth Rate	**Sham**	**Bystander**	**Gauze**
Sham	(8.14)	0.2184	0.6323
Bystander	0.23[-0.04,0.50]	(7.91)	0.7091
Gauze	0.12[-0.14,0.39]	-0.11[-0.38,0.16]	(8.02)

Notes: Brackets denote average growth rate (mg/day), top right are estimated means p-values, bottom left are 97.5% confidence intervals. Gauze: n = 222, m = 117, f = 105; Sham: n = 205, m = 114, f = 91; Bystander: n = 213, m = 104, f = 109.

4.8. Figures

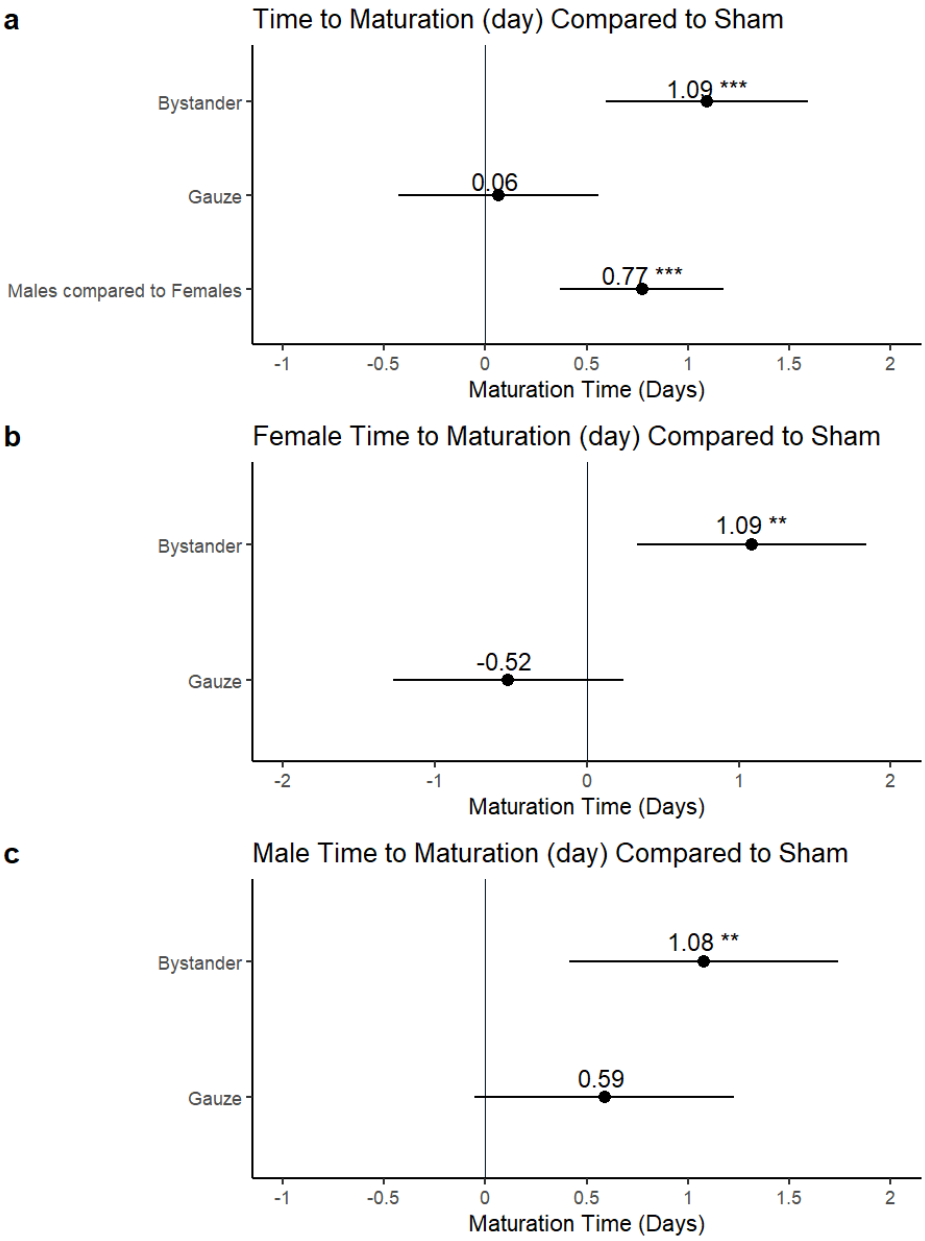

Figure 4.8.1. Average Time to Maturation (days). a) Bystander population matured significantly slower than both the sham and gauze population, who were not significantly different. b) Bystander females matured significantly slower than both the sham and gauze females, who were not significantly different. c) Bystander males matured significantly slower than the sham males, the gauze males were not significantly different than any other group. Gauze: n = 222, m = 117, f = 105; Sham: n = 205, m = 114, f = 91; Bystander: n = 213, m = 104, f = 109.

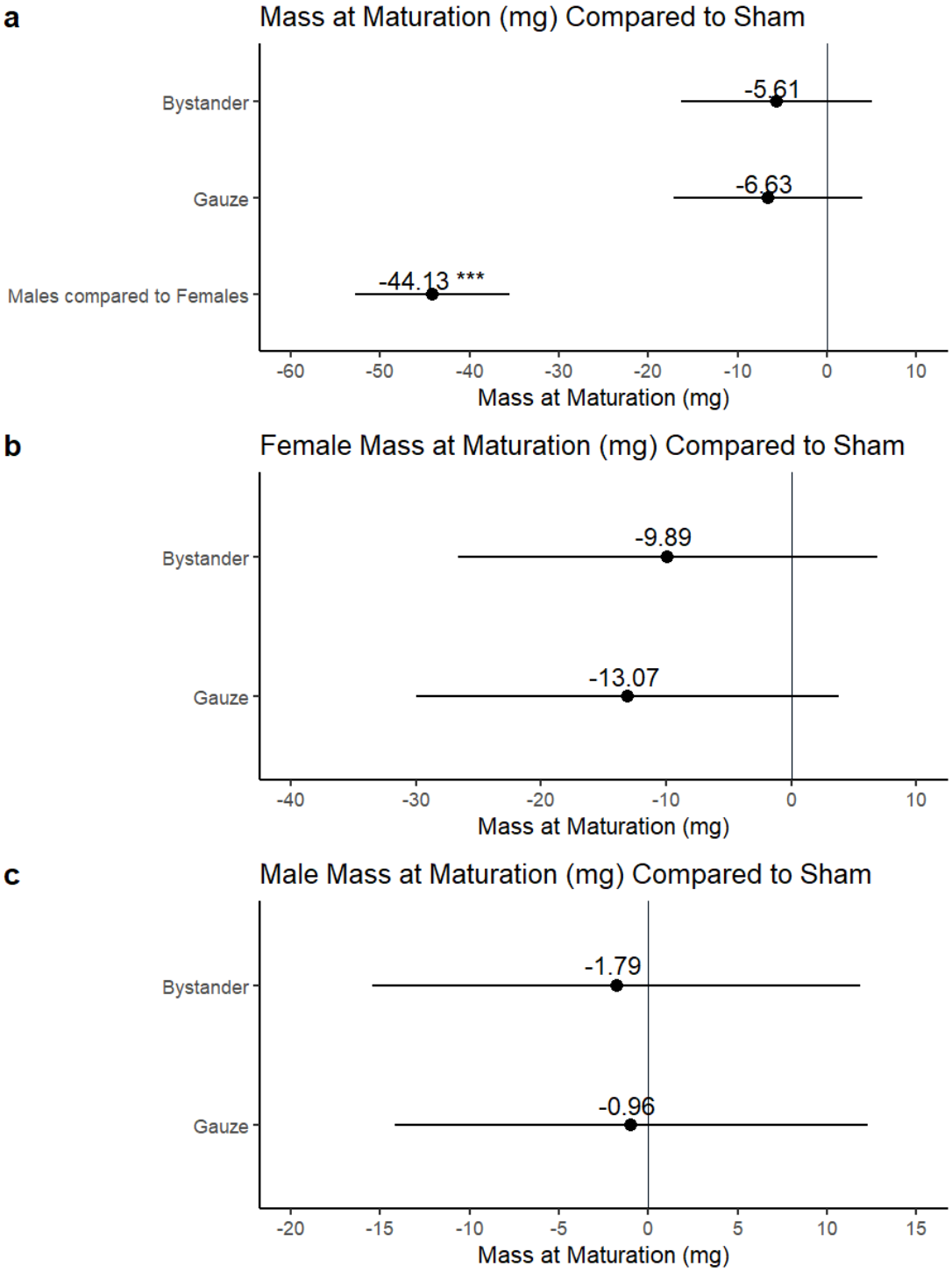

Figure 4.8.2. Average Mass at Maturation (mg). No significant differences were observed between any of the populations or within sexes. Gauze: n = 222, m = 117, f = 105; Sham: n = 205, m = 114, f = 91; Bystander: n = 213, m = 104, f = 109.

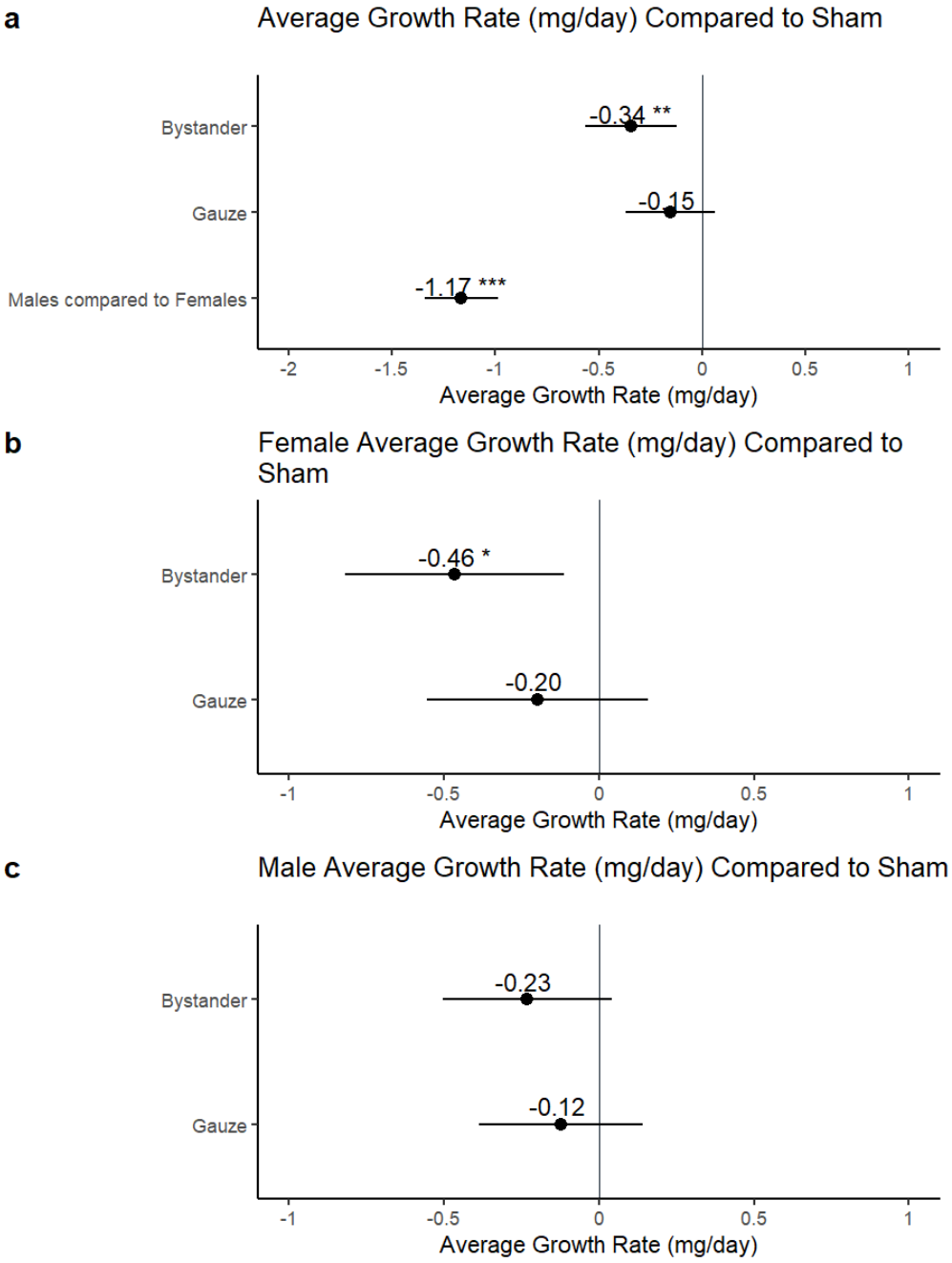

Figure 4.8.3. Average Growth Rate (mg/day) to Maturation. a) Bystander population had significantly lower average growth rate than the sham population. No significant differences between the gauze and any other group. b) Bystander females had significantly lower average growth rate than the sham females. No significant differences between the gauze females and any other group. c) no significant differences between any of the males of any populations. Gauze: n = 222, m = 117, f = 105; Sham: n = 205, m = 114, f = 91; Bystander: n = 213, m = 104, f = 109.

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5. Conclusion

My hope is that after reading this work, you have come away with a newly founded respect for radiation and radiation-induced bystander effect (RIBE). The major takeaway is that it is important to acknowledge the potentials dangers and to employ a holistic approach as the world embraces radiation and nuclear technology. Readers are of course suggested to form their own conclusions and opinions, but I will in brief summarize what I believe are the major findings of this work, discuss their implications, and suggest potential future directions should others find this work intriguing and wish to continue.

In chapter 2, using both direct and indirect comparison methods, we demonstrated the presence and effect of whole-body RIBE on crickets. We saw shifts in development, with those affected by RIBE having significantly higher average growth rate at adulthood, largely caused by an accelerated time to reach maturation. Interestingly, this acceleration did not come at a cost of mass, as the individuals did not significantly differ, and most times we even saw trends towards higher mass at the time of maturation. This was a surprising result, given that all populations had access to unlimited resources and were not limited by density. It appears that the impacted bystander crickets were either eating more or were more efficient at converting food to mass. But either way, the results were clear, bystander crickets matured faster with no cost to mass and overall gained more mass per day on average than those who did not interact with irradiated crickets.

There are three secondary conclusions within chapter 2 that are highly interesting and suggests that what we demonstrated is in fact RIBE. The first being saturability, RIBE is saturable at a low dose and increased dosages beyond that will not have any increased

effects. Our results show that even when we triple the total dose, the response of bystander crickets did not shift significantly. Secondly, we demonstrated the persistence of RIBE throughout development, and how a single acute dose of ionizing radiation early on in development will continue to generate RIBE signals well into adulthood, inducing bystander effect just as effectively as newly irradiated adults. Lastly, we observe some sex-specific RIBE, males and females appear to respond similarly to RIBE, but when the ceiling of development for one of the two metrics (maturation time or mass) is reached, the other one then begins to be affected, resulting in similar shifts to growth rate. We see this when some of the females appeared to not be able to mature any faster, but instead used that time to gain more mass, resulting in the same increase in growth rate, but now driven by a different end point. This is fascinating and validates in part our theory on efficiency of energy conversion, as now in the same amount of time, these females are either eating more food or are more efficiently converting the food to mass.

RIBE research looking within individuals or using cell cultures oftentimes report a negative response by the bystander cells or individual, so why is it we observe a positive relationship on a whole-body level between individuals? The simplest answer is that we cannot say that we observed a positive relationship, we observed what we believe to be a positive relationship between the endpoints that we measured, it is possible that being bigger or maturing faster is detrimental in nature, and there is a reason why in our control populations, even with unlimited food, they did not grow as fast as they could, as suggested by the bystander population. It is likely that there are trade-offs that we are not

aware of, and more work should be conducted investigating fecundity and sexual fitness of these bystander crickets, or their general stress response and plasticity.

Chapter 3 investigated some of the mechanisms behind RIBE and the signal mediated transfer of RIBE. As addressed in the general introduction, there are a few mechanisms in which RIBE is believed to be propagated through, and while we did not explore the mechanisms directly, the work still shows that RIBE is able to be induced via these secondary signals. Crickets are known to be sensitive to their own species pheromones, and work from our lab previously have demonstrated that irradiated males secrete different concentrations of hydrocarbons. The interesting part of our results is that similar to higher vertebrates (mice), crickets also respond to soiled housing materials, demonstrating RIBE. Suggesting potentially conserved pathways between vertebrates and invertebrates. The most important part of this chapter, in my humble opinion, are the biophoton results. To demonstrate that crickets are able to communicate stress through biophotons, and that these effects can be mitigated by filtering out the biophotons are quite exciting. It has been speculated that biophotons play a major role in signaling and communication, with previous work in our lab showing a similar experiment but between cell cultures, but never has it been shown between individuals to such an extent both with radiation, and in general. This opens an entirely new avenue for signal transfer between individuals of not just ionizing stress, but potentially various other stressors.

The RIBE observed in chapter 3 are more diminished than in chapter 2, and we also see a second, contradicting effect of maturation suppression. This was not observed when the crickets were directly cohabitated, perhaps in closer proximity with direct interactions,

RIBE signals were strong enough to outcompete any of the maturation suppression effects.

This effect was found even in our non bystander groups of delayed maturation after interacting with soiled housing materials or biophotons of non-irradiated crickets, which was absent in our control without any housing material or biophotons. We see the recouperation of this effect some what with RIBE, suggesting that they are counteracting each other. More work is required to untangle the two results to fully comprehend the effects. However, we are still able to elucidate RIBE and see a trends where the bystander populations are developing differently than the sham populations. In our soiled housing materials experiment, we saw the bystander population's average growth rate increase like our irradiated outgroup. In the biophotons, it is a bit less obvious, but we observed a difference between the groups with UV filters and saw that there was a difference caused by difference in biophoton expression levels.

Chapter 4 is more exploratory as we investigated the extend of signaling by using developing eggs to see if they are able to mediate RIBE to unirradiated juveniles. The results were extremely fascinating and suggests that developing cricket eggs are emitting signals that can be modified through radiation, and that those modified signals can induce RIBE in already hatched juveniles. We suggest that RIBE is being mediated through the fatty acids found on the surface of the eggs, and that radiation is either actively modifying the preexisting fatty acids, or the eggs are emitting different fatty acids.

It is however prudent to mention that the works explored here are not without limitations. Firstly, all work was conducted on a lab colony of house crickets who are living in near

optimal conditions with *ad libitum* high quality food and water. While it serves as valuable research and contributes to the field, only if field data were to be collected and compared, could it properly serve as a valuable reference and resource for environmental radiation protection. Secondly invertebrates are extremely diverse, and as such this work cannot speak for all invertebrates, indeed it could not speak even for all types of crickets. Thirdly, astute readers will have no doubt realized that the data presented here is only to maturation, and while the crickets are kept for a period of time after data collection to ensure there's no sudden die offs, there is no survival or sexual fitness data present. Radiation is known to alter sexual fitness, survival, and longevity, and our data cannot capture the overall fitness of the population. However, in an environment devoid of predators and major external stressors, we believe that the survival and longevity data would also be biased. And for my own sanity of coming in daily for months on end, I chose to not collect such data.

I believe that this work acts as a strong base for future research, as the impacts of RIBE has now been properly demonstrated in a whole-body system. It will ideally open the possibility for researchers to take a risk and attempt some longer-term experiments. Overall, the works found within this thesis demonstrates the impact of radiation-induced bystander effects not just on crickets, but on the potential widespread impacts on an ecosystem and the environment. We need to consider the range of irradiated individuals when investigating ramifications of radiation on ecosystems and cannot stop at only the direct targeted effects on directly impacts areas.

6. Appendix A: Radiation induces stress and transgenerational impacts in the cricket, *Acheta domesticus*

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6.1. Preface

This is the first scientific peer-reviewed article that I ever published, and as such even though it does not fit in with the rest of the thesis, I felt compelled to at least include it in the appendices. This long-term project begun at the start of my graduate career in September 2019, I need not mention the devastation and tragedies caused by COVID-19 which followed soon after. What was originally a multi-year project was forced to be concluded in just 6 months. Nevertheless, this paper demonstrates how early-life exposure to high levels of ionizing radiation caused lower growth rate and maturation mass and found sex-specific responses in unirradiated offspring of irradiated parents, particularly in male offspring, resulting in a faster maturation time at a cost to its mass. The results show that a single early-life exposure to ionizing radiation can alter male offspring development through accelerated maturation and reduced maturation mass. This paper was fortunate enough to be published as a part of a special issue covering environmental radiobiology within the Internation Journal of Radiation Biology.

6.2. Introduction

Ionizing radiation is ideal for applying precise stress on large number of individuals because the dose-rate can be precisely controlled across all irradiation samples. With the distance between individuals and the source determining the dose-rate for the exposure (Crofton et al. 1996). Crickets are an ideal research subject as their short lifespan and small size, allows rapid generation of large populations for controlled dose-response experiments as well as studying trans-generational impacts. Furthermore, juvenile

crickets undergo multiple molts and maturation is easily identified by development of wings.

Exposure to ionizing radiation has a wide range of impacts on development and behavior of organisms. Radiation impacts on organisms can be divided into targeted and non-targeted effects. Targeted or direct effects include damage to DNA and cellular features. DNA damage can also result in loss of cell integrity and ultimately cell death. Besides direct impacts, radiation also hydrolyzes water to produce reactive oxygen species (ROS) that can attack DNA and other cellular features to induce cellular damage, and death at high levels (Desouky et al. 2015).

The linear no-threshold (LNT) model is commonly applied to estimate targeted and non-targeted impacts of ionizing radiation (Christensen et al. 2014). The LNT model estimates that radiation damage is proportional to dose and dose-rate. The LNT model predicts that even if exposure is insufficient to inflict significant direct damage, generation of free radicals can damage DNA (Christensen et al. 2014; Desouky et al. 2015).

The 'Hormetic' model of radiation stress holds that low-level radiation can have positive effects on physiology, development and behavior, even though higher doses can cause damage (Luckey 1991). Hormetic and damaging doses vary among species, but invertebrates are generally more radio-resistant compared to vertebrates (Paithankar et al. 2017; Timbadiya et al. 2018). Insect resistance to irradiation compared to vertebrates likely reflects that most adult tissues in insects are post-mitotic (proliferating mitotic cells are more vulnerable) (Bhatnagar et al. 1965).

Regardless, gonads of mature insects are mitotic and ionizing radiation can induce sterility. In the house fly, *Musca domestic L.*, exposure of pupae to high levels of ionizing radiation induces gender-specific responses (Bhatnagar et al. 1965). In *A. domesticus*, female hormetic doses are between 0.5 − 2 Gy of radiation (dose-rate = 0.25 Gy/min) (Shephard et al. 2018).

Previous research of stress in invertebrates looked primarily at post-fertilization prenatal stress. In the water fleas, *Daphnia pulex* and *D. longispina*, pregnant females exposed to predators cues, produced offspring expressing defensive phenotypes (i.e. longer tail spines, wider bodies, and expression of neck teeth) (Imai et al. 2009; Sperfeld et al. 2020). In the cuttlefish Sepia officinalis, prenatal maternal stress altered offspring behavior (O'Brien et al. 2017). In humans, offspring of parents with early-life dietary stress can express a similar obesity syndrome as their parent, even if they never experienced the stress (Franklin et al. 2010; Curley et al. 2011; Skinner 2014). Such trans-generational transfer of stress responses suggests a heritable, potentially epigenetic mechanism.

Research on trans-generational stress typically focuses on maternal stress. Working with insects that lack parental care and that were exposed to radiation stress (both parents) allows exploration of trans-generational stress response without parental care. In this case, stress associated with parents can still impact sperm or eggs, and thus, offspring. Here, we examine trans-generational consequences of a single, early life exposure to ionizing radiation on maturation size and growth rate from parent to offspring. Specifically, we exposed groups of juvenile house crickets (*Acheta domesticus*) to a

moderate dose (13.92 Gy @ 0.58 Gy/min) at 14 days post-hatch and examined the impacts on maturation mass and growth rate within and across generations.

6.3. Methods

Study animals and experimental groups

Breeding colony

Common house crickets (*Acheta domesticus*) were initially acquired from a local pet store, and a long-term breeding colony was established. Crickets were maintained in a large acrylic terrarium (93 × 64.2 × 46.6 cm) with a constant temperature at 29 °C ± 2 °C using 60 volt UV heat lamps, and a photoperiod of 12 h light/12 h dark photoperiod generated with overhead LED strip lights and florescent lamps. Egg carton shelters were provided, distilled water was available in soaked cellulose sponges, and food was *ad libitum* access to Quicks Country Range Multi-Fowl Grower Rations (17.0% crude protein, 2.5% crude fat, and 4.0% crude fiber). The enclosure was insulated with 1.5 cm thick Durofoam insulation, fans provided air circulation from top of enclosure.

Experimental groups

When most crickets have reached sexual maturity (expressed wings), the breeding colony was provided with oviposition mediums in small plastic containers (7 × 7 × 7 cm) (Organic Garden Soil, Swiss Farms Products Inc., Marysville, USA). One container of oviposition medium was left in the colony to collect eggs for 48 h and attracted numerous females for oviposition. Eggs were incubated at 29 °C ± 2 °C and they hatched in

approximately 11–13 days. Several replicates of hatchlings were subsequently reared to maturity to ensure quality of experimental crickets.

Juveniles were removed from soil 24 h post-hatch to ensure individuals were of the same age. Juvenile *A domesticus* were collected on day 14 (4th instar) after hatching and randomly assigned to one of the 3 experimental treatments.

The groups ($n \approx 200$ per group) consisted of the negative sham control group (sham control), who had no exposure to radiation in both generations but were brought over to the Taylor Source and exposed to the stress of handling and travel to control for external stress factors. Irradiated Parents and Offspring (IPO) group, where both the parents and offspring were independently irradiated at 13.92 Gy at 14 days of age. And Irradiated Parents and Non-Irradiated Offspring (IPNIO) group, where only the parents were irradiated at 13.92 Gy at 14 days old, the offspring were not irradiated, but were brought the Taylor Source (Figure 6.8.1).

Irradiation

Crickets were irradiated at the Taylor Radiobiology Source (caesium-137) at McMaster University (dose rate = 0.58 Gy/min) totaling 13.92 Gy 14 days post hatch. The 13.92 Gy dose was chosen as prior testing found this dose to have negative impacts on growth. Housing terrariums were brought to the Taylor Source in insulating Styrofoam boxes, where crickets were sorted and temporarily housed in cylindrical plastic vials (9.5 cm height x 2.5 cm width) with approximately 30–35 crickets per vial for the duration of the exposure. Crickets were irradiated one group at a time (six vials per group ~190–200 crickets). Replicates were performed to obtain a total of ~400 crickets per group. Vials

were evenly spaced around another cylindrical vial to ensure even radiation exposure. Following irradiation, crickets were housed in plastic containers (30 × 19 x 12 cm, n ≈ 200 per container, 2 containers per treatment group) and housed in same environment as the colony.

Maturation age and growth rate

Experimental groups were monitored daily for sexual maturity (adult molt) as indicated by expression of wings. Newly mature male and female crickets were immediately weighted with an Accuris analytical balance (readability of 0.001 g ± 0.002 g) and separated into group-dependent adult containers. Containers were monitored several times daily to ensure newly matured crickets were not missed.

Reproductive effort and next generation

After all crickets matured in experimental groups, oviposition containers were placed into the housing containers for 24 h as an oviposition medium to collect viable eggs for the next generation, this was done twice within 2 days (2 oviposition mediums per container, 4 total per treatment).

Two weeks post-hatch ~200 juvenile *A domesticus* were randomly selected from there, respectively, groups while the rest were culled. All three groups of crickets (14 days old) were taken to The Taylor Source but only F1 IPO were exposed to 13.92 Gy of radiation. Groups were monitored daily for sexual maturity (adult molt) and were recorded as described above. Growth rate was calculated by dividing the mass at maturation in mg over days from hatch to maturation. Sample sizes and average time to maturation for all three groups across generations are presented in Table 1.

Statistical analysis

No significant difference was observed between the two containers of each treatment, and all data moving forward are combined data for an approximate sample size of 400 per group (Table 6.7.1).

Maturation weight and growth rate were analyzed using one-way ANOVA and Dunnett's multiple comparisons test to detect difference between experimental groups and control. Unpaired t tests were performed to compare differences between parents and offspring. All statistical analyses were carried out with GraphPad Prism 8.4.3.

Figures are presented as percentage differences from the sham controls. The zero-difference line signifies the average maturation mass or growth rate for the sham controls for that generation or sex. Maturation weights (mg) for all groups are presented as mean percentage differences from sham controls ± SEM. Growth rates (mg/day) were calculated as mean maturation mass (mg) over maturation time (days) and presented as mean percentage differences from sham controls ± SEM. Significances were calculated using empirical data before transforming into percentages.

6.4. Results

F_0 radiation impact on growth rate and mature mass

We examined developmental responses of crickets to damaging levels of ionizing radiation. At 13.92 Gy (0.58 Gy/min), radiation exposure reduced maturation mass (mg) and growth rates mg/day compared to non-irradiated controls.

Significant differences in maturation mass between F_0 sham, IPO, and IPNIO males were observed among F_0 males F (3, 485) = 48.43, $p < .0001$ across the three experimental groups (Figure 6.8.2). A follow-up Dunnett's multiple comparison test showed a significant decrease ($p < .0001$) in mean maturation mass of both male IPO and IPNIO compared to the sham controls. Male IPO and IPNIO crickets weighted 11.35% (334.6 mg) and 13.25% (327.5 mg) less at maturation, respectively, than male F_0 sham controls (377.5 mg).

Comparing growth rate between F_0 sham, IPO, and IPNIO males found a significant difference among the F_0 males F (3, 485) = 81.86, $p < 0.0001$ compared across the three groups (Fig 6.8.4). A Dunnett's multiple comparison test detected a significant decrease ($p < 0.0001$) in growth rate in both male IPO and IPNIO groups independently compared to the sham control. Male IPO and IPNIO crickets grew on average 12.07% (6.968 mg/day) and 16.04% (6.653 mg/day) less than the sham controls (7.924 mg/day).

Significant differences in maturation mass between F_0 sham, IPO, and IPNIO males was observed among F_0 females F (3,468) = 35.09, $p < 0.0001$ among the three groups (Figure 6.8.3). Follow up Dunnett's multiple comparison test found that mean maturation mass of female IPO and IPNIO crickets were significantly lighter ($P < 0.0001$ for both groups, independently) than female F_0 sham crickets. Female F_0 IPO and IPNIO crickets weighed 7.773% (380mg) and 13.57% (356.1 mg) lighter than the female F_0 sham controls (412mg) respectively.

Comparing growth rate between F_0 sham, IPO, and IPNIO females found a significant difference among the F_0 females F (3,468) = 54.54, $p < 0.0001$ compared among the three

groups (Figure 6.8.5). Dunnett's tests further showed that there is a significant decrease ($p < 0.0001$) of growth rate of female IPO and IPNIO crickets compared to the sham control. Female IPO and IPNIO crickets grew on average 9.79% (8.064 mg/day) and 15.54% (7.550 mg/day) less than the sham controls (8.939 mg/day).

Trans-generational impacts of ionizing radiation between F_0 and F_1

One-way ANOVAs demonstrated significant differences in mean maturation mass in F_1 generation among the three groups of both male (F (3,617) = 59.34, $p < .0001$) and female (F (3,445) = 23.81, $p < .0001$) crickets compared within their respective sex. T tests were performed within the IPO group where offspring were re-exposed to radiation and found no significant difference in mean body weight of both sexes when comparing between parents and offspring (Figures 6.8.2 and 6.8.3).

A Dunnett's multiple comparison test comparing among F_1 groups found mean maturation mass of both male and female IPO crickets are significantly ($p < .0001$) lighter than that of their sham counterparts (14.59% and 12.21%, respectively).

Non-Irradiated offspring maturation mass and growth rate

T tests were performed in the IPNIO group between parents and offspring where the offspring were not re-exposed to radiation. A significant difference ($p < .0001$) in male and female mean maturation size was observed when comparing between F_0 and F_1 generations (16.27% and 17.91% increase, respectively). A Dunnett's multiple comparison test between F_1 IPNIO and sham controls offspring found IPNIO males weighed significantly less ($p = .0012$) at maturation (4.658%) compared to the sham

controls while there was no significant difference in mean maturation mass between female offspring.

T tests were performed between parents and offspring of the experimental groups and found no significant difference in average growth rate of F_0 and F_1 IPO males and females. T tests between F_0 and F_1 IPNIO crickets found a significant ($p < .0001$) difference in average growth rate of both males and females (26.8% and 27.1%, respectively).

Significant differences were observed in average growth rate in F_1 generation groups of both male (F (3,617) = 113.8, $p < .0001$) and female (F (3,445) = 52.65, $p < .0001$) crickets compared to their respective groups.

Dunnett's multiple comparison tests were performed and a significant difference ($p < .0001$) in average growth rate was observed in male and female F_1 IPO crickets compared to F_1 sham control crickets (16.8% and 13.64% decrease). No differences in average growth rate were observed in neither male nor female F_1 IPNIO crickets compared to F_1 sham controls.

Average time to maturation

Significantly lower mean maturation time in male and female IPNIO F_1 crickets compared to sham were observed F (3, 552) = 12.11, $p < .0001$ and F (3, 445) = 13.23, $p < .0001$, respectively (Table 6.7.1).

6.5. Discussion

High levels of radiation are linked to free radical generation, oxidative damage, and increased mortality. In insects, most of the focus is on sterilization and lethal dosages related to agricultural pest species (Paithankar et al. 2017; Timbadiya et al. 2018). Trans-generational impacts of acute damaging doses, or non-sterilization doses of radiation are poorly understood. House crickets (Acheta domesticus) prove to be an excellent model to study non-lethal, non-sterilization levels of ionizing radiation.

A key objective of this study was to determine whether developmental shifts caused by radiation stress were heritable. A secondary objective was to understand impacts of early-life radiation stress on development. Here we characterized responses from A domesticus associated with radiation stress for both irradiated parents and their irradiated or non-irradiated offspring. Impacts of ionizing radiation was assessed in terms of maturation mass and growth rate as key measures of development.

We found that early-life radiation stress induces heritable detrimental impacts on maturation mass without impacting survival to reproductive maturity. Developmental responses were also observed in crickets irradiated at 14 Gy (0.58 Gy/min). Results showed that a single early-life exposure to radiation in the first generation will reduce maturation mass of both the parents and their non-irradiated male offspring. These results are consistent with current models of stress inheritance (Soubry et al. 2014). We found that at reproductive maturity, crickets exposed to ionizing radiation had lower growth rate and maturation size compared to non-irradiated sham controls (Fig. 6.8.2-5). These results

are consistent with other radiation studies finding that early life/prenatal exposure to radiation stunted growth (Rugh et al. 1964).

Despite not being exposed to radiation, similar stress responses to their parents were observed in non-irradiated male offspring of irradiated parents. Non-irradiated male offspring of irradiated parents exhibited a similar decrease in maturation mass to their irradiated parents. Male second-generation IPNIO crickets weighed significantly less than male sham control crickets ($p = 0.0012$, 4.65%, Fig 2) while female offspring had no significant difference in maturation weight or growth rate (Fig 6.8.3, 6.8.5).

Our results are consistent with other studies examining offspring epigenetic profiles and health status (Pembrey et al. 2006). Paternal exposure to chemicals also causes defects in gametes and development in offspring, and this effect is more pronounced in male offspring. This has been correlated with altered DNA methylation patterns in germ lines (Filkowski et al. 2010; Anway et al 2006). In humans, radiation treatment for childhood cancers has been linked to elevated sperm DNA fragmentation in adulthood (Romerius et al. 2010). Our results are consistent with human research in suggesting a sex-linked epigenetic mechanism (Guerrero-Bosagna et al. 2012; Cordier 2008). We propose that a similar phenomenon likely occurred in our experiment, where early-life irradiation fragmented gamete DNA that extended into adulthood. This then can pass down via altered methylation patterns to offspring.

While there was a significant impact on maturation mass of nonirradiated male offspring, there was no significant difference in growth rate of these offspring. Our results instead showed a significantly faster time to maturation (at lower maturation mass) in F1 male

and female offspring compared to sham controls (Table 6.7.1). This might suggest beneficial parental shifts in epigenetic markers that cause offspring to develop faster in the presence of ionizing radiation. Faster maturation and decreased maturation mass suggest a tradeoff mechanism, where organisms under stress mature faster as a defense mechanism. Alternatively, slowly growth may impact maturation.

In water fleas, offspring developing in waters with predator cues express longer tail spines and other defensive features not found in predator-free waters (Sperfeld et al. 2020; Imai et al. 2009). Perhaps a more general mechanism responds to stress, altering development to effect earlier maturation if growth rates (or even egg development) are suppressed.

Trans-generational impacts of ionizing radiation on maturation mass and growth rate may involve several mechanisms that could generalize across broad phylogenies. One of the main effects of ionizing radiation on organisms is a generalized stress response that negatively affects maturation size and growth rate. On the one hand, stress could simply disrupt normal functions resulting in reduced growth and altered maturation. Alternatively, mechanisms associated with stress resistance can alter heritable methylation and epigenetic markers that can impact both the adult and subsequent offspring. This calls for understanding of the underlying physiological mechanisms inducing heritable epigenetic changes.

This work adds to a growing body of literature on trans-generational and paternal inheritance in both invertebrate and vertebrate systems. Due to their relatively small body size and short generational times, insects are an excellent model for examining trans-

generational impacts of ionizing radiation. Understanding stress responses to radiation has important practical applications for nuclear power, exploration of space, medical applications, and possible accidents.

Understanding trans-generational consequences of stress is particularly relevant today, given the increased range of novel stressors from anthropogenic sources. Radiation is an excellent stressor for research given the ability to apply highly accurate doses, the wealth of scientific research and the range of topics and applications (energy generation, medicine, space travel, environmental pollution). Our results emphasize that parental and early-life exposure to ionizing radiation impacts development and can be heritable across generations.

6.6. Acknowledgements

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6.7. Tables

Table 6.7.1. Average Time to Sexual Maturation (days) and Survival to Sexual Maturation (n). Containers were monitored daily multiples times a day, all recently matured crickets were weighted immediately, growth rate was calculated using maturation weight and time. Comparing average time to maturation, every group was maturing significantly or trending towards maturing significantly later than the sham controls, except for the IPNIO males and females, who matured significantly faster when compared to the shams.

F0 Males	Survival to Maturation (n)	Average Maturation Time (days)	F1 Males	Survival to Maturation (n)	Average Maturation Time (days)
Sham Control	173	47.71±3.677	Sham Control	223	47.92±2.608
Irradiated Parents & Offspring	170	48±2.955	Positive Control	194	49.38±3.589
Irradiated Parents & Non-Irradiated Offspring	142	49.30±4.194	Non-Irradiated Offspring	200	45.30±3.483
F0 Females	Survival to Maturation (n)	Average Maturation Time (days)	F1 Females	Survival to Maturation (n)	Average Maturation Time (days)
Sham Control	150	46.19±3.928	Sham Control	159	46.44±2.592
Irradiated Parents & Offspring	175	47.13±3.037	Positive Control	151	47.41±3.116
Irradiated Parents & Non-Irradiated Offspring	143	47.38±4.185	Non-Irradiated Offspring	135	43.84±3.891

6.8. Figures

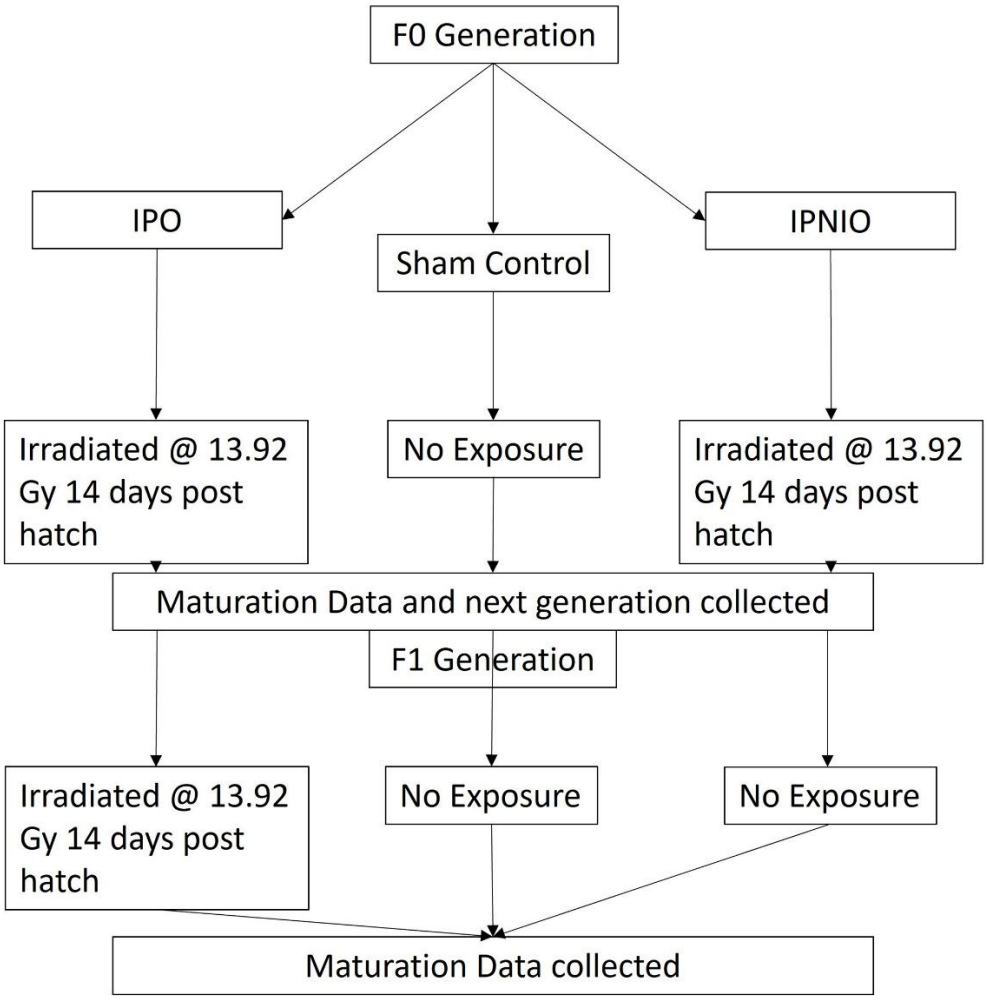

Figure 6.8.1. Experimental design of experiment. Eggs were collected in ovipositing mediums placed inside the main breeding colony and reared until hatch. 14 days post hatch crickets were randomly assigned one of three groups, sham control, irradiated parents and offspring (IPO), or Irradiated Parents and Non-Irradiated Offspring (IPNIO). All three F0 groups were kept separately for the duration of the experiment. At 14 days post hatch, all three groups were transported to the Taylor Source, but only IPO and IPNIO were exposed to radiation (13.92 Gy). F0 Maturation data and F1 eggs were then collected from the three F0 groups. 14 days after the F1 groups hatched, all three groups were transported to the Taylor Source, but only IPO was exposed to radiation (13.92 Gy). Maturation data was collected from the F1 groups

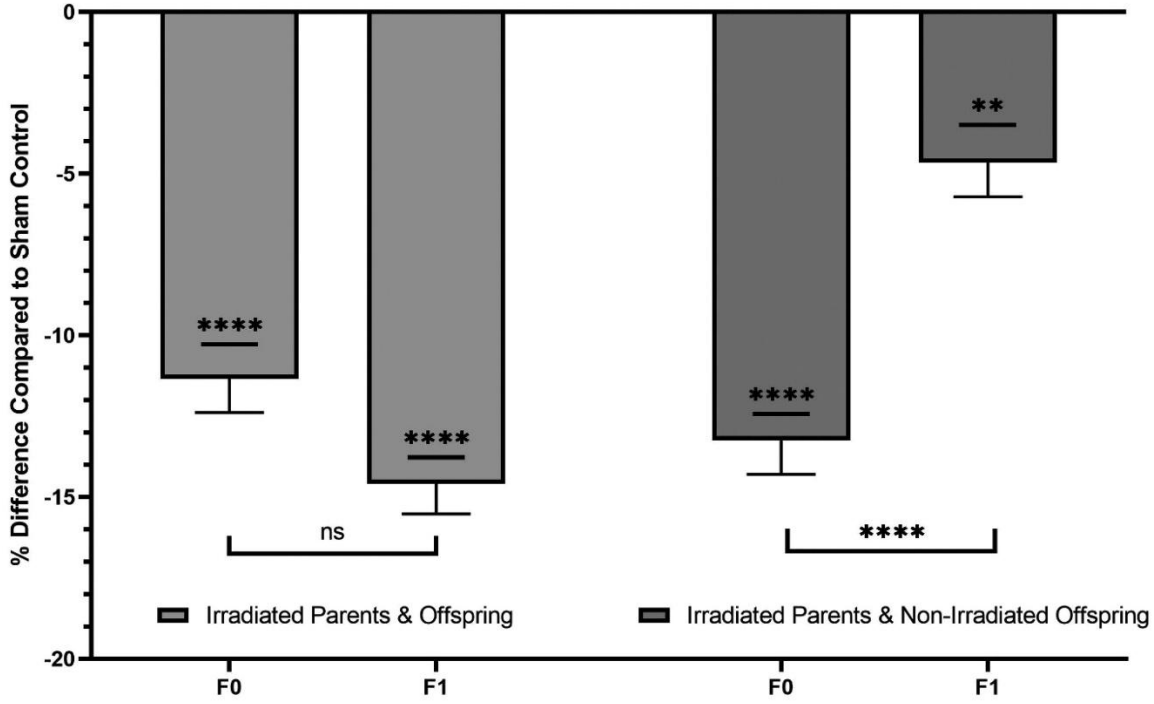

Figure 6.8.2. Effects of early life ionizing radiation on mean maturation mass (mg) ± SEM of F0 and F1 male *Acheta domesticus* Compared to Sham Controls. Percent difference in mean maturation mass of Irradiated Parents & Offspring (IPO) and Irradiated Parents & Non-Irradiated Offspring (IPNIO) compared to sham control. Sham crickets had no exposure to radiation but were exposed to same environmental stressors of being handled and traveling to the source. F0 and F1 Crickets in the IPO group were exposed to 13.92 Gy of ionizing radiation (dose rate = 0.58 Gy/min) at 14 d old. F0 crickets in IPNIO group were exposed to 13.92 Gy of radiation at 14 day old, F1 crickets were exposed to the same environmental stressors as the other groups but were not exposed to radiation. Radiation significantly decreased mean maturation mass in crickets exposed to 13.92 Gy of radiation. F1 IPNIO crickets exhibits significant difference both from the parents and the sham control, suggesting a transgenerational stress response. Dunnett's multiple comparison test indicating significant differences between IPO and IPNIO compared to sham, and significant differences within and between groups. *p < .05, **p < .01, ***p < .001, ****p < .0001.

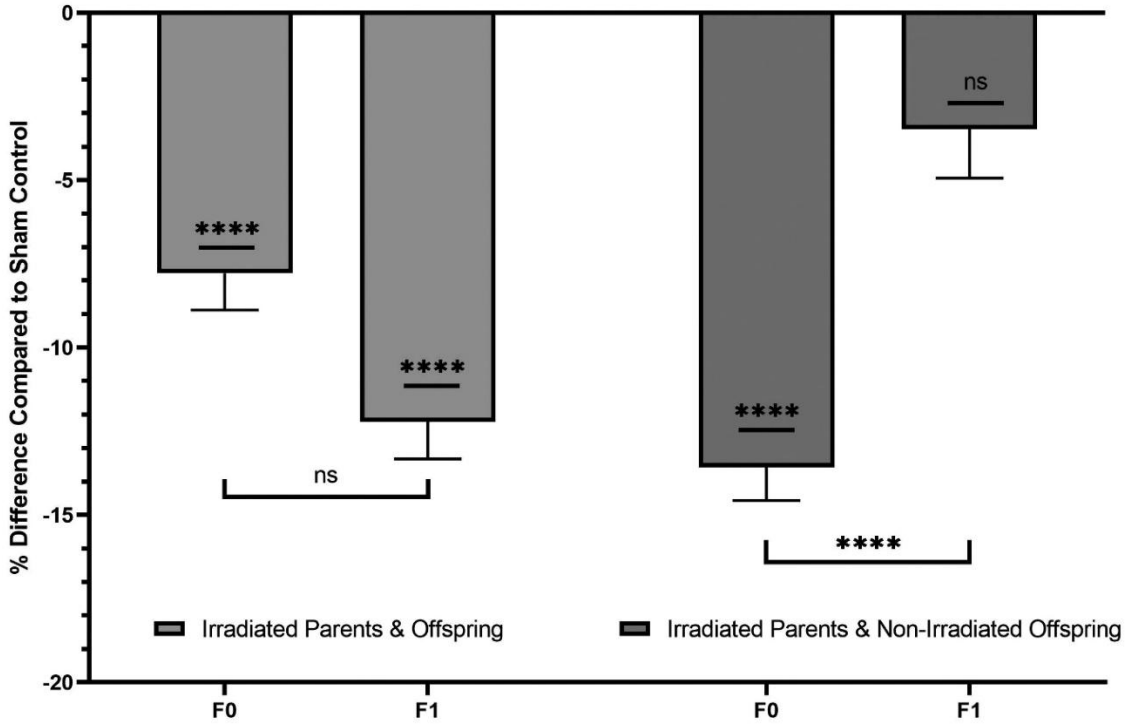

Figure 6.8.3. Effects of early life ionizing radiation on mean maturation mass (mg) ± SEM of F0 and F1 Female *Acheta domesticus* Compared to Sham Controls. Percent difference in mean maturation size of Irradiated Parents & Offspring (IPO) and Irradiated Parents & Non-Irradiated Offspring (IPNIO) compared to sham control. Sham crickets had no exposure to radiation but were exposed to same environmental stressors of being handled and traveling to the source. F0 and F1 Crickets in the IPO group were exposed to 13.92 Gy of ionizing radiation (dose rate = 0.58 Gy/min) at 14 d old. F0 crickets in IPNIO group were exposed to 13.92 Gy of radiation at 14 day old, F1 crickets were exposed to the same environmental stressors as the other groups but were not exposed to radiation. Radiation significantly decreased mean maturation mass in crickets exposed to 13.92 Gy of radiation. F1 IPNIO crickets exhibits significant difference from the parents but no significant difference from the sham control. Dunnett's multiple comparison test indicating significant differences between IPO and IPNIO compared to sham, and significant differences within and between groups. *$p < .05$, **$p < .01$, ***$p < .001$, ****$p < .0001$.

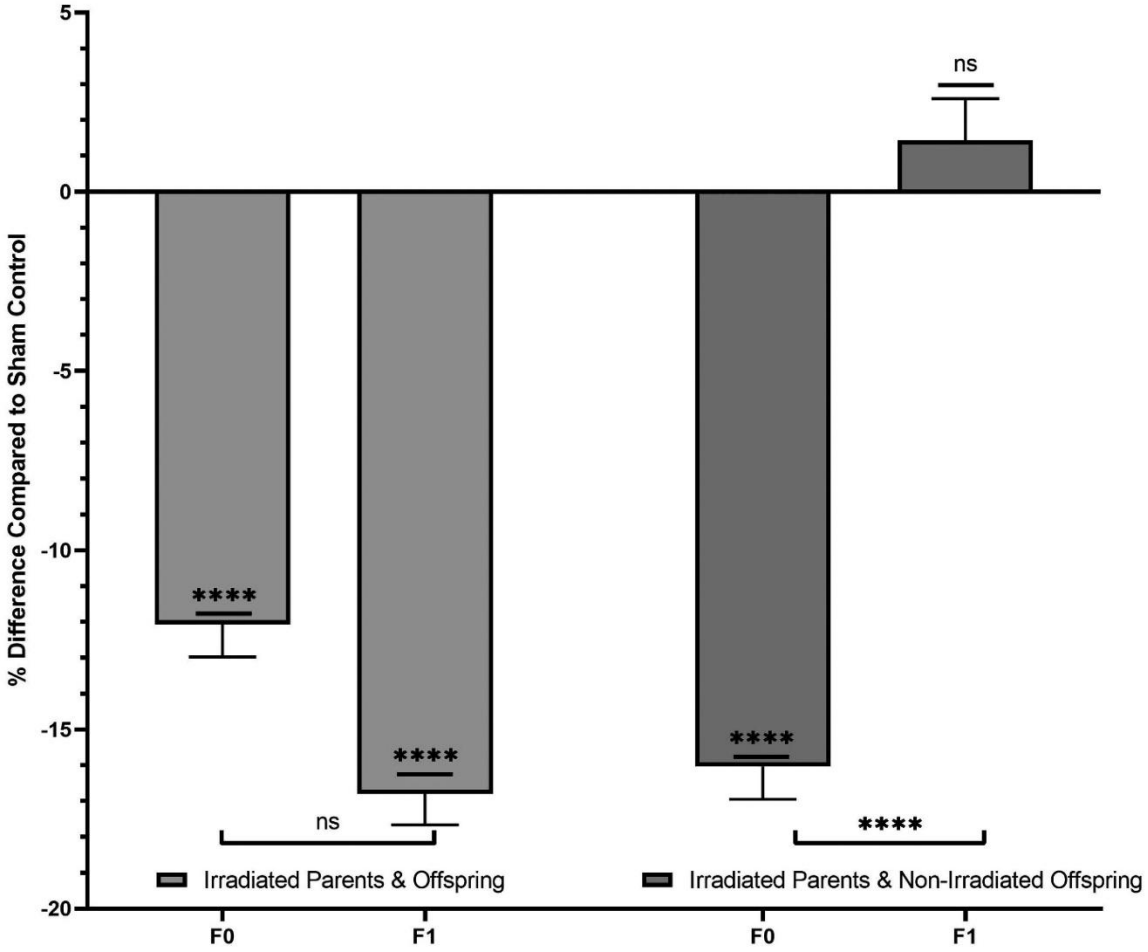

Figure 6.8.4. Effects of Early Life Ionizing Radiation on Average Growth Rate (mg/day) ± SEM of F0 and F1 Male *Acheta domesticus* Compared to Sham Controls. Percent difference in average growth rate of Irradiated Parents & Offspring (IPO) and Irradiated Parents & Non-Irradiated Offspring (IPNIO) compared to sham control. Sham crickets had no exposure to radiation but were exposed to same environmental stressors of being handled and traveling to the source. F0 and F1 Crickets in the IPO group were exposed to 13.92 Gy of ionizing radiation (dose rate = 0.58 Gy/min) at 14 d old. F0 crickets in IPNIO group were exposed to 13.92 Gy of radiation at 14 day old, F1 crickets were exposed to the same environmental stressors as the other groups but were not exposed to radiation. Radiation significantly decreased mean growth rate in crickets exposed to 13.92 Gy of radiation. F1 IPNIO crickets exhibits significant difference from the parents but no significant difference from the sham control. Dunnett's multiple comparison test indicating significant differences between IPO and IPNIO compared to sham, and significant differences within and between groups. *p < .05, **p < .01, ***p < .001, ****p < .0001.

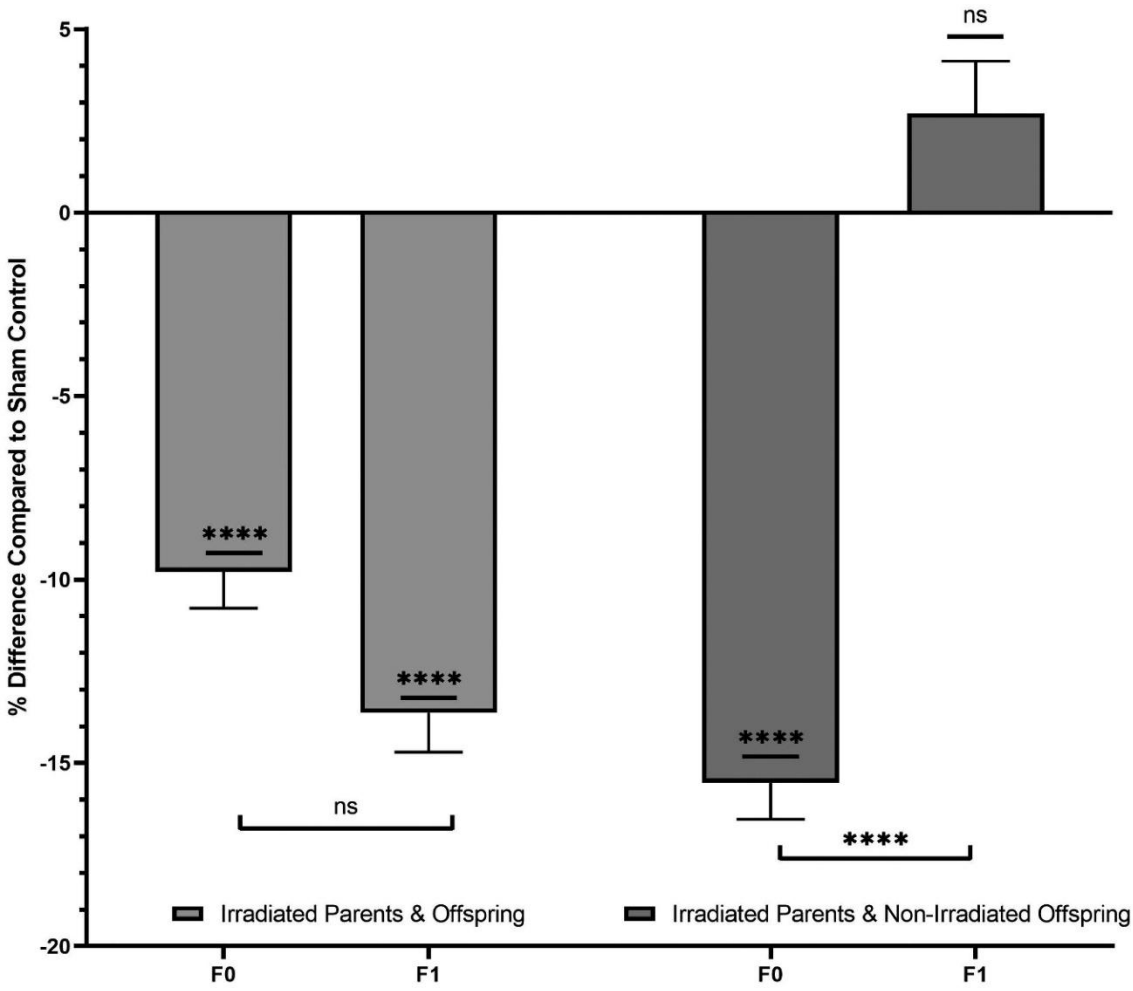

Figure 6.8.5. % Effects of early life ionizing radiation on average growth rate (mg/day) ± SEM of F0 and F1 female *Acheta domesticus* compared to Sham Controls. Percent difference in average growth rate of Irradiated Parents & Offspring (IPO) and Irradiated Parents & Non-Irradiated Offspring (IPNIO) compared to sham control. Sham crickets had no exposure to radiation but were exposed to same environmental stressors of being handled and traveling to the source. F0 and F1 Crickets in the IPO group were exposed to 13.92 Gy of ionizing radiation (dose rate = 0.58 Gy/min) at 14 d old. F0 crickets in IPNIO group were exposed to 13.92 Gy of radiation at 14 day old, F1 crickets were exposed to the same environmental stressors as the other groups but were not exposed to radiation. Radiation significantly decreased mean maturation size in crickets exposed to 13.92 Gy of radiation. F1 IPNIO crickets exhibits significant difference from the parents but no significant difference from the sham control. Dunnett's multiple comparison test indicating significant differences between IPO and IPNIO compared to sham, and significant differences within and between groups. *p < .05, **p < .01, ***p < .001

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