IONIZING RADIATION EFFECTS ON LIFE HISTORY AND IMMUNITY

IMPACTS OF IONIZING RADIATION ON LIFE HISTORY AND IMMUNITY IN THE CRICKET, *ACHETA DOMESTICUS* L.

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LAY ABSTRACT

High-dose ionizing radiation can have inhibitory effects on cellular systems while low doses can have lasting stimulatory effects. The latter phenomenon, known as hormesis, can act on growth, longevity, and immunity. We investigated the effects of early life gamma radiation exposure on life history traits and measures of innate immunity in the cricket (*Acheta domesticus*). We observed trade-offs between survival, growth, and immunity. We also tested late life radiation exposure to assess potential hormetic effects on innate immunity and redox status. Our results show immune stimulation with low doses but effects are complex and dependent on dose, type of immunity measured, and time of assessment.

ABSTRACT

Oxidative stress from high-dose ionizing radiation can contribute to accumulating cellular damage, affecting various fitness related traits. However, studies on low-dose ionizing radiation (LDIR) have shown hormetic effects on growth, longevity, and immunity. Comprehensive lifetime studies assessing LDIR effects and studies investigating LDIR immune stimulation in insects are limited. We utilized ^{137}Cs gamma radiation with a dose rate of 0.25 Gy/min. We examined the impacts of early-life exposure (doses: 0, 0.2, 0.5, 1, 2, 4, 7, and 10 Gy) on life history and immunity in *Acheta domesticus*. Moderate doses (above 4 Gy) increased mean longevity but decreased growth rate, adult body mass and innate immunity. We also performed a time course study in male *A. domesticus* to assess the acute effects of radiation (doses: 0, 0.2, 0.5, 0.75, 1, 5, and 15 Gy) on innate immunity and redox status. LDIR (below 1 Gy) generally achieved immune stimulation and improved the encapsulation response but effects were time dependent. Benefits could extend to improved immune responses and protection against infection. Our results provide evidence of immune stimulation with LDIR in insects but with potential trade-offs with life history traits when assessing early-life exposure. With increasing concern of radiation exposure in the environment, more comprehensive studies utilizing a multi-discipline approach will help to elucidate the full mechanism of hormesis.

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LIST OF ABBREVIATIONS

AMP – antimicrobial peptide

Gy - gray

LNT – linear-no-threshold

LDIR – low-dose ionizing radiation

ROS – reactive oxygen species

TPA – total phenoloxidase activity

DECLARATION OF ACADEMIC ACHIEVEMENT

All ideas expressed throughout the manuscript are those of **Jonathan Tran** unless otherwise referenced. The research direction was developed by **Jonathan Tran** with the guidance of **C. David Rollo**.

CHAPTER 1: Impacts of Early Life Ionizing Radiation on Life History Traits and Immunity in the Cricket (*Acheta domesticus*)

Animal care and data collection for the life history study were performed by **Jonathan Tran**, **Alex Shephard** and **Vadim Aksenov** under the supervision of **C. David Rollo**. Experimental design for life history study was created by **Jonathan Tran**, **Alex Shephard**, and **Vadim Aksenov**. Hemolymph collection for immune assays were collected by **Jonathan Tran**. Analysis of hemolymph for hemocyte concentration and total phenoloxidase activity were completed by **Jonathan Tran**. Data presentation and statistical testing were done by **Jonathan Tran** assisted by **Vadim Aksenov** and **C. David Rollo**.

CHAPTER 2: Low-Dose Ionizing Radiation Stimulates the Immune System of the Cricket (Acheta domesticus): Evidence for Hormesis

Animal care was performed by **Jonathan Tran**. Experimental approach was designed by **Jonathan Tran** under the supervision of **C. David Rollo**. Hemolymph collection was performed by **Jonathan Tran** with the assistance of **Zulal Ozkan**. All cellular and humoral immune assays were performed by **Jonathan Tran** assisted by

Matthew Costa-Parke, **Madeline Kubiseski**, **Mia Costa-Parke**, **Connor Nelson**, and **Joshua Silva**. Access to the Motic microscope, imaging software, and hemocytometer for the analysis of the encapsulation response and hemocyte concentration were provided by **Sinah Lee and Marvin Gunderman**. Collection and dilution of tissue samples for redox analysis were performed by **Jonathan Tran** and **Vadim Aksenov**. Hydrogen peroxide concentration and total antioxidant capacity were measured by **Jonathan Tran** and **Vadim Aksenov**. Data presentation and statistical analysis was finalized by **Jonathan Tran** and **Vadim Aksenov** with the assistance of **C. David Rollo**.

PREFACE

Dear Reader,

This manuscript is prepared in a "sandwich" format to facilitate the report on two distinct studies. Individual chapters are written in journal article style and each chapter is a stand-alone piece assuming no prior knowledge of other chapters. To simplify the reading process, all figures and references pertaining to a chapter are shown at the end of the chapter.

A main introductory section following the preface is provided detailing general background information for the significance of this work. Each chapter opens with its own respective introduction of background specific to the following results and discussion. A final conclusion is included at the end to summarize major findings, report significant implications of the study, and describe future research directions.

INTRODUCTION

This work examines the effects of ionizing radiation exposure on a wide range of fitness traits including physiological and molecular biomarkers of insect immunity. Determining dose response relationships of radiation remain an important focus to decipher between established hormetic and linear-no-threshold (LNT) models guiding the field.

Experimental findings in crickets (*A. domesticus*) are presented here serving as a model for studying the impacts of early- and late-life radiation exposure. Particular focus is involved in investigating potential hormetic effects of low-dose ionizing radiation (LDIR) on immunocompetence. Results presented throughout this work provide evidence and understanding of radiation hormesis, which has strong implications on environmental protection, health, and ecology.

This general introduction briefly outlines the current understanding of ionizing radiation effects in animals. Aspects pertaining to physiological trade-offs are considered. Finally, a brief description of our animal model and objectives are presented.

Radiation Effects in Animals

The health risks associated with high-dose ionizing radiation exposure are relatively well established (Deininger et al., 1998; De Santis et al., 2007). Ionizing radiation can generate reactive oxygen species (ROS) and although many free radicals are important signaling molecules (e.g. nitric oxide and hydrogen peroxide), increasing levels can contribute to accumulating damage to cellular organelles, DNA, lipids, and proteins.

This can lead to membrane destabilization and cell death (Leach et al., 2001; Elgazzar & Kazem, 2015). Available data suggests that radiation can induce cancer (Shah et al., 2014), cataracts (Ainsbury et al., 2009), cardiovascular disease (Sumner, 2007), and psychological alterations (Pastel, 2002). Rapidly dividing cells such as those lining the gastrointestinal tract, blood cells, and reproductive cells are generally most affected by ionizing radiation. Ultimately, damage to these areas would have severe consequences reflecting changes in digestion, immunity and reproduction, possibly causing long-term generational effects (Francois et al., 2013).

Although much work has been done on high-dose radiation exposure, our current understanding of low-dose radiation is largely incomplete. Research on low-dose ionizing radiation (LDIR) exposure has become increasingly important as a result of the growing nuclear energy industry and use of radiation in medicine (Tang & Loke, 2015). However, in the ecological literature, most work involving ionizing radiation has consisted of field studies on the molecular and morphological phenotypes of species from areas surrounding nuclear disaster sites such as Chernobyl in 1986 (Hinton et al., 2007; Moller & Mousseau, 2006) and Fukushima in 2011 (Beresford & Copplestone, 2011; Strand et al., 2014). These nuclear catastrophes resulted in enormous political, economic, social and psychological impacts deeply rooted in the fear of radiation, which was largely induced by the "Linear-No-Threshold" hypothesis.

Linear vs. Non-Linear Dose Response Models

Current recommendations for permissible radiation exposure in humans are based on the 'linear-no-threshold' (LNT) model (Vaiserman, 2010). Following health studies on the Japanese atomic bomb survivors that documented negative health effects associated with ionizing radiation exposure, the LNT model of radiation carcinogenesis became the dogma in radiation protection in 1958 (Brues, 1958). The LNT model extrapolates predictions of harm from high doses to predict risks at low-doses. Thus, with the LNT model, even a very low-dose of radiation could cause cancer or other pathological effects in the body (i.e. all doses are potentially harmful).

There is a growing body of experimental and epidemiological evidence that does not support the LNT model for estimating health risks at LDIR (Azzam et al., 1996; Boreham et al., 2006). Several studies have shown LDIR-induced hormesis, adaptive responses, and bystander effects (Tang & Loke, 2015). Radiation hormesis is characteristic of a biphasic J- or U-shaped dose response curve associated with LDIR stimulation. Low-dose stimulation often consists of 'beneficial' effects including increased longevity, enhanced growth, augmentation of immune responses, and improved cellular repair mechanisms (Wiegant et al., 2013). Another phenomenon known as the adaptive response is the concept where LDIR is used to stimulate cellular processes that result in enhanced resistance to a second later challenge thus improving health outcomes (Olivieri et al., 1984). Lastly, the bystander effect is the phenomenon in which unirradiated cells exhibit effects of radiation as a result of signals received from adjacent or proximally located irradiated cells (Mothersill & Seymour, 2001). Bystander effects

could be a potential mechanism of hormesis in biological systems. Studies on these low-dose effects are important to assess the legitimacy of the LNT model, which predicts harm at all doses (Vaiserman, 2010).

Potential Reasons for Fitness Trade-offs

From an ecological perspective, there have been relatively few dose-response studies investigating the impacts of ionizing radiation on broad life history traits (but see Han et al., 2014; Won & Lee et al., 2014). Early-life stress has been known to impact life history patterns, potentially linked to changes in resource allocation (Zera & Harshman, 2001). Assessment of integrated impacts on lifetime biomarkers is essential for determining the overall fitness of organisms. Thus, whether these biomarkers express linear or hometic dose responses to radiation demands careful investigation through detailed laboratory studies on model organisms.

Life history trade-offs under oxidative stress can emerge as changes in interconnected fitness traits (Monaghan et al., 2009). Oxidative stress results from the mismatch between the generation of ROS and the organism's capacity to mitigate their damaging effects. Managing oxidative stress is likely to be a major determinant of life histories as virtually all-cellular activities generate or are impacted by ROS (Monaghan et al., 2009). Hence, radiation impacts on various biomarkers must ultimately be gauged relative to the integrated phenotype, which can only be addressed through comprehensive life history studies.

Our Animal Model

Crickets (*Acheta domesticus*) possess relatively short lifespans and exhibit exceptional phenotypic plasticity in growth, longevity, morphology, immunity and behaviour in response to stressors (Lyn et al., 2011; 2012; Hans et al., 2015; da Silva et al., 2000a,b) Thus, *A. domesticus* is advantageous for life history studies to quantify impacts of radiation stress on life features, aging and immunity. There also exists a large literature studying the cricket immune system, providing many potential biomarkers to assess impacts of radiation stress (da Silva et al., 2000a,b; Pinera et al., 2013).

Goals of the Study

Our experimental protocols include assessment of dose response effects of irradiation on life history traits in addition to documenting impacts on cellular and humoral immunity. Assessment of cellular and molecular markers is of central importance to understanding the underlying mechanisms of hormesis. It is also crucial to take into account the temporal relationship and age of exposure when investigating potential hormetic effects. Throughout this work, we document stimulatory effects on immunity with LDIR in adult *A. domesticus*. However, these benefits are not necessarily cost-free. Rather, our results suggest potential trade-offs between growth, survival and immunity when assessing early life radiation exposure.

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CHAPTER 1

IMPACTS OF EARLY-LIFE IONIZING RADIATION ON LIFE HISTORY TRAITS AND IMMUNITY IN THE CRICKET (*ACHETA DOMESTICUS*)

1.1 INTRODUCTION

The phenomenon of hormesis is becoming more relevant with the increasing appreciation of stressors in the environment. Generally, hormesis can be defined as a dose-response relationship that is characterized by a reversal in response between low and high doses of a stressor (Kendig et al., 2010). Several studies investigating impacts of various stressors found stimulatory responses at low-dose that were deemed beneficial (Feinendegen, 2005). However, studies identifying beneficial influences of stress on fitness challenge our understanding of evolution because it would imply that life histories are generally suboptimal (Forbes, 2000).

Life history evolution is based on the idea that a phenotype consists of a series of traits connected to each other by constraining relationships called trade-offs, and it is the interaction among these traits that determine evolutionary fitness (Stearns, 1992). According to resource allocation theory, organisms have the flexibility to invest their resources in relation to different priorities (Williams, 1966; Stearns, 1992). For instance, life history theory predicts trade-offs between growth and self-maintenance (Constantini, 2014; Hall et al., 2010). Growth impinges on numerous possible mechanisms that target organismal growth and development. In contrast, self-maintenance would include a vast

array of processes including survival, immunity, and antioxidant defense (Hall et al., 2010). However, upon maturation in insects (i.e. adult stage), we would expect energy allocation strategies to shift from growth, towards reproduction and self-maintenance (Jager et al., 2013; Sousa et al., 2008).

Ionizing radiation has already been shown to increase life span in insects at low doses (Calabrese 2013), but at what cost? The stimulation of one or more traits would represent increased resource consumption, which must limit resource availability for another trait due to energetic constraints. Many studies have failed to assess early life dose-response effects of ionizing radiation on life history traits (but see Han et al., 2014; Won & Lee et al., 2014). For this reason, we have a limited understanding of the implications of hormesis on determining fitness outcomes. Therefore, comprehensive life history studies are required to explore a broad range of fitness-related traits and increase our understanding of hormetic effects.

The immune system is a key fitness factor and is extremely sensitive to oxidative stress (Cui et al., 2017). Exposure to low doses of ionizing radiation stimulate immune responses in mice (Marple & Collis, 2008; Nakatsukasa et al., 2010; Pollycove, 2007) and *Drosophila* (Seong et al., 2012). However, recent advances in ecological settings have concluded that the immune response is costly in terms of resources and is frequently traded-off against other life history traits/fitness elements (McClure et al., 2014; Schmid-Hempel, 2005; Sadd & Schmid-Hempel, 2009). Alternatively, a large literature has shown that mild stressors can simultaneously increase multiple fitness traits including longevity and reproduction (Parkhurst et al., 1981; Giesy et al., 2000; Calabrese and

Baldwin, 2003). Such discrepancies might be explained by the developmental conditions (i.e. stage of life cycle) under which stress is applied or duration of the exposure (i.e. acute vs. chronic).

There are several potential mechanisms driving fitness trade-offs (Constantini, 2014). Evolutionary ecologists and physiologists have generally relied on physiological mechanisms, such as energy production and expenditure, immune response or hormones to explain trade-offs related to stress. Given that organisms might be particularly sensitive to radiation exposure in early life, it has been suggested that oxidative stress might be a prime physiological mechanism mediating short- and long-term life history trade-offs. Fitness trade-offs would have implications on growth patterns, senescence, survival, and reproductive performance (Constantini, 2008; Dowling and Simmons, 2009).

In this study, we examined dose-response effects of early-life ionizing radiation exposure on a broad range of life history traits. We used the cricket (*Acheta domesticus*) model to study the relationship of early-life stress on global life history and immunity. Crickets have a relatively short lifespan and reach peak immune function shortly after maturation (Pinera et al., 2013), making immunity a good proxy of adult fitness. We can therefore assess the consequence of early life oxidative stress on late life parameters of immune function. We specifically measured survivorship, mean and maximal longevity, growth rate, maturation age and mass, and feeding rate as markers of life history. We also measured hemocyte concentration and total phenoloxidase activity (TPA) to establish dose-response relationships for innate immunity and assess potential fitness trade-offs.

1.2 METHODS

1.2.1 Animals

A total of 480 two-week-old crickets (*Acheta domesticus*) were collected from our genetically heterogeneous breeding colony and separated into eight experimental groups (n=60 per treatment group). They were irradiated at 0 (Sham), 0.2, 0.5, 1, 2, 4, 7, and 10 Gy (dose rate: 0.25 Gy/min) with ^{137}Cs gamma radiation (Taylor Radiobiology Source, McMaster University). The "sham" irradiated crickets represent the control treatment group. Crickets were housed in plastic containers (30x19x12cm) and maintained at 30±1°C, 12h light/12h dark photoperiod with egg carton shelters, water-soaked cellulose sponges and food *ad libitum*. All groups were raised on a diet based on guinea pig food (*Living World® Extrusion*) for the entire study (Lyn et al., 2012).

1.2.2 Life History Features

We measured standard life history features including mean and maximal longevity, growth rates, maturation age, and maturation mass. Groups were monitored daily for mortality to construct lifetime survivorship curves. Mean longevity was determined as the average age of mortality among all animals. Maximal longevity was determined as the average age of mortality for the remaining 20% of animals in the study. Juvenile crickets were periodically weighed from 2 weeks of age to just before maturation (~6 weeks of age). Maturation mass and age of each animal were also collected. The ratio of maturation mass to maturation age was used as a proxy for overall growth rate (see Lyn et al., 2012).

1.2.3 Food Consumption

Potential differences in juvenile food consumption could have critical impacts on developmental trajectories. Juvenile feeding rate was measured during 2-3 weeks of age (i.e. first week following radiation treatment) to assess potential acute effects of ionizing radiation on appetite. Food dishes were collected and dried in a 50°C oven prior to being weighed. Food consumption was reported as mg dry food/g cricket/day.

1.2.4 Innate Immunity Assessment

A subsample of 6 male crickets from each treatment group were separated from the life history study at maturity (~45 days of age). At 1-week post-maturation, crickets were anesthetized with CO_2. The technique for hemolymph collection was adapted from Pinera et al. (2013) and is described in more detail in Chapter 2. Briefly, 2 μl of hemolymph was removed from each cricket using a 27.5-gauge needle and diluted with 198 μl anticoagulant (98 mM NaOH, 146 mM NaCl, 16 mM EGTA, 10 mM citric acid, pH 6.5; see da Silva et al., 2000). Another 2 μl of hemolymph was removed with a sterile needle and diluted with 98 μl PBS (pH 7.4). All hemolymph samples were analyzed within 2 hours of collection.

A hemocytometer composed of nine (3x3) 1mm^2 grids was used to estimate the number of circulating hemocytes in the hemolymph. 10 μl of the hemolymph-anticoagulant mixture was loaded into the chamber of the hemocytometer. Using phase contrast microscopy (10x magnification), the total number of hemocytes were counted from five 1 mm^2 grids (four corners and center). The number of hemocytes per μl

hemolymph was estimated using the formula: cells/μl = (total number cells counted/5) x dilution factor (100) x volume of 10 μl.

Total phenoloxidase activity (TPA) was determined using a spectrophotometric assay modified from Pinera et al. (2013). 50 μl of each hemolymph-PBS sample was mixed with 70 μl of 1.3 mg/ml α-chymotrypsin from bovine pancreas (Sigma) in a 1.5 ml plastic cuvette and incubated at room temperature for 20 min. 600 μl of 0.15 M 3,4-Dihydroxy-L-phenylalanine (L-DOPA; Sigma) was added and the change in absorbance was measured at 490 nm with a spectrophotometer at 0, 8, 15, 23, 30, 45, and 60 min. The addition of L-DOPA represented time 0 min. TPA was reported as the slope of the absorbance from the linear phase of the reaction (8-30 min) multiplied by 10^3.

1.2.5 Statistical Analyses

The Kaplan-Meier (log-rank) test was used to compare survivorship among groups. Effects of radiation on all life history features were analyzed using a one-way ANOVA. Where significant differences were detected, data was analyzed with Tukey's HSD post-hoc test. Statistical significance was set at $p<0.05$. Analyses were performed on GraphPad Prism 6.0 software.

1.3 RESULTS

1.3.1 Life History

Crickets irradiated at doses above 2 Gy showed an alternate survivorship curve that exhibited improved mean survivorship compared to those irradiated at doses below 1

Gy but the log-rank test did not reveal significant differences in survival (Fig. 1.1). ANOVA detected significant differences in mean longevity ($F=4.248$, $df=7,472$, $p=0.0001$). Crickets irradiated at 4 and 7 Gy had improved mean longevity compared to controls ($p<0.05$) (Fig. 1.2). ANOVA revealed significant differences in maximal longevity ($F=4.976$, $df=7,32$, $p=0.0007$). Maximal longevity was enhanced in crickets irradiated at 0.2 and 7 Gy compared to controls ($p<0.03$) (Fig. 1.3).

Crickets were sexed at maturity so data for maturation age and mass are reported individually for males and females. The mean maturation age for females (Sham: 42 days) was generally earlier than males (Sham: 44 days) resolving an effect of sex (ANOVA: $p<0.0001$). ANOVA detected significant differences in maturation age in males ($F=27.23$, $df=7,192$, $p<0.0001$) and females ($F=39.29$, $df=7,179$, $p<0.0001$). However, maturation age for male and female crickets irradiated at 10 Gy was identical (~51 days). Time to maturation was increased at 7 and 10 Gy compared to controls for males ($p<0.003$) and females ($p<0.0002$) (Fig. 1.4).

Overall, females were larger in body mass compared to males (~424 mg vs. ~383 mg, respectively; $p<0.0001$). Significant differences in maturation mass was revealed by ANOVA in males ($F=24.45$, $df=7,192$, $p<0.0001$) and females ($F=25.54$, $df=7,197$, $p<0.0001$). 7 and 10 Gy exposures decreased adult body mass at maturation in males by 19% and 34%, respectively ($p<0.0001$) (Fig. 1.5a). Irradiation with 7 and 10 Gy also decreased female adult body mass by 17% and 37%, respectively ($p<0.0001$) (Fig. 1.5b). Significant differences in growth rate were detected in males (ANOVA: $F=44.14$, $df=7,185$, $p<0.0001$) and females (ANOVA: $F=42.86$, $df=7,179$, $p<0.0001$). Growth rates

were significantly decreased compared to sham in male crickets irradiated with 2 Gy ($p=0.04$), 4 Gy ($p=0.0073$), 7 Gy ($p<0.0001$) and 10 Gy ($p<0.0001$) (Fig. 1.6a). Growth rates were also slower in female crickets irradiated at 7 and 10 Gy compared to sham ($p<0.0001$) (Fig. 1.6b)

1.3.2 Feeding Rate

Feeding rates are important as they represent resource acquisition and reductions can induce dietary restriction impacts. Early life feeding rates were measured at 18, 19, and 22 days of age. Feeding rates were highly variable across development. ANOVA revealed significant differences in feeding rate ($F=70.88$, $df=7,24$, $p<0.0001$). Generally, food consumption was suppressed for crickets irradiated at 10 Gy at all time points ($p<0.0138$). At 18 days, food consumption was also decreased for crickets irradiated at 2, 4, and 7 Gy ($p<0.0016$) (Fig. 1.7).

1.3.2 Innate Immunity

Significant differences in hemocyte concentration between doses were detected (ANOVA: $F=2.768$, $df=7,40$, $p=0.0192$). Tukey's multiple comparisons found a significant decrease in hemocyte concentration with 10 Gy compared to SHAM ($p=0.017$) (Fig. 1.8). No other significant differences in hemocyte concentration were observed.

Significant differences in total phenoloxidase activity (TPA) were also detected with ANOVA ($F=4.291$, $df=7,40$, $p=0.0013$). Decreased TPA was observed at 7 and 10 Gy compared to SHAM ($p<0.02$) (Fig. 1.9).

1.4 DISCUSSION

Ionizing radiation is becoming increasingly relevant to global ecosystems requiring the investigation of radiation effects on organismal fitness. One method is by detecting impacts of radiation on broad life history traits. In this study, we investigated the effects of early life ionizing radiation stress on survival, growth and development, as well as measures of innate immunity in *A. domesticus*. Despite noticeable improvements in survival and mean longevity, development and growth rate was negatively impacted. Our results are consistent with the idea that animals shift their life histories in response to environmental stress (Tatar et al., 2003). However, we also observed immunological costs for treatments that improved survival. These observations could be explained by changes in resource allocation strategies between growth and self-maintenance during development (Stearns, 1992; Constantini, 2014; Hall et al., 2010).

There are two different phenomena that could potentially explain non-linear dose response effects. The first, as previously mentioned, is commonly associated with a biphasic response, with low-dose stimulation and high-dose inhibition, known as hormesis (Calabrese & Baldwin, 2002). Alternatively, a threshold model describes a case when there may be no noticeable effect until the level of stressor reaches a threshold value, above which, oxidative stress increases with dose (Constantini, 2014). Our data largely follows the threshold model as shown by the dose response effects on mean longevity (Fig. 1.2), maturation age (Fig. 1.4), maturation mass (Fig. 1.5), growth rate (Fig. 1.6), hemocyte concentration (Fig. 1.8), and TPA (Fig. 1.9).

It is often presumed that stimulatory effects are cost free (Rattan et al., 2009; Calabrese et al., 2012). As clearly shown from our data, early-life radiation stress inflicted fitness trade-offs. Improved mean longevity with 4 and 7 Gy (Fig. 1.2) resulted in deficits in growth rate (Fig. 1.6). We also observed longer maturation times (Fig. 1.4) and decreased adult body mass (Fig. 1.5) with 7 and 10 Gy. Overall, most inhibitory alterations of life history were observed with doses above 7 Gy, further suggesting a threshold response to radiation exposure.

All organisms must obey the conservation laws for mass and energy (Jager et al., 2013) and this should be taken into account when studying the effects of stressors on life history traits. The organism must invest energy and nutrients to sustain one aspect of its fitness, but resources are also required for other competing functions such as growth, self-maintenance or reproduction (Constantini, 2014). Given that resources are finite, trade-offs can arise between investment in lifespan versus the immune system, leading to complex relationships between life history decisions and levels of infection and parasitism (Sheldon & Verhulst, 1996; Norris & Evans, 2000; Schulenburd et al., 2009).

Trade-offs between longevity and immunity occur in the context of flight-or-flight stress (Adamo, 2010). We observed decreased hemocyte concentration (Fig. 1.8) and lower TPA (Fig 1.9) in crickets exposed to 7 and 10 Gy, which could be explained by a stress hormone response to oxidative stress. For example, stress hormone release (most notably octopamine in crickets) can have regulatory effects on immune surveillance and lipid transport, which both rely on apolipophorin III. However, its physiological function is specific to its protein conformation and thus apolipophorin III cannot perform both

functions simultaneously. Intense activity often leads to immunosuppression since apolipophorin III is co-opted into lipid transport and is thus, unavailable for immune surveillance (Adamo, 2008). Given that increased oxidative stress is linked to secretion of stress hormones such as adipokinetic hormone, radiation could likely induce a stress hormone response resulting in fitness trade-offs (Kodrik et al., 2015).

Fitness trade-offs induced by resource allocation could be explained by changes in molecular pathways (Barnes & Partridge, 2003). Although not directly measured, we would expect oxidative stress to activate stress resistance mechanisms (most notably forkhead transcription factors (FOXO)). FOXO are a class of highly conserved transcription factors that respond to diverse stressors including oxidative stress and energy deprivation (Brunet, 2004). For example, sestrins are conserved proteins that accumulate in cells exposed to stress leading to the potentiation of the cellular energy sensor, AMP-activated protein kinase (AMPK) along with FOXO activation (Greer et al., 2007; Lee et al., 2010).

These mechanisms play an important conserved role in stress resistance by upregulating a series of target genes including genes involved in removing ROS (i.e. the production of antioxidants) (Lee et al., 2010; Ogg et al.1997). FOXO and AMPK activation have an inhibitory effect on the target of rapamycin (TOR), which predominantly regulates growth processes including protein synthesis and cell growth (Martin et al., 2005), which could explain the deficits seen in growth rate (Fig. 1.6). Thus, through the modulation of AMPK and FOXO, and subsequent inhibition of TOR, we

would expect an inhibitory effect of ionizing radiation on growth during development as an indirect consequence of ROS production.

These molecular pathways could mediate trade-offs giving rise to the central concept in evolutionary ecology that all fitness-related traits cannot be simultaneously maximized because of constraints on trait expression and costs originating from life history strategies (Constantini, 2014). Thus, environmental conditions under which we investigate these trade-offs are very important to take into account because they can exacerbate or mitigate costs associated with certain strategies and shape ecological trade-offs (Jessup and Bohannan, 2008).

Radiation impacts on growth and survival could also be associated with changes in energy acquisition or assimilation efficiency (i.e. feeding rates). We reported decreased food consumption in groups exposed to higher doses of radiation (Fig. 1.7). Decreased uptake of energy would imply decreased growth rate and body size, and increased time to maturity, while the opposite would be true for increased energy uptake (Jager et al., 2013). In addition, digestion could be negatively affected as a result of digestive system damage or impacts on the gut microbiota, which would limit energy acquisition. Trade-offs with survival and longevity would be expected (i.e. increased survival probability with decreased growth) in a way that is similar to the caloric restriction paradigm (Bartke et al., 2013; Rollo, 2002). Therefore, we cannot rule out the potential role of radiation impacts on food consumption in shaping life history patterns. Future work should consider assessing radiation effects on digestive function and aspects related to the gut microbiome.

Epigenetics represents a new research frontier that could explain the life history effects of early-life radiation exposure. Ionizing radiation can promote long-lasting phenotypic effects through changes in gene expression as a result of altered DNA methylation or modifications in chromatic structure (Kim et al., 2013). In our study, it was remarkable that a single acute radiation dose during early-life affected adult phenotypes (i.e. adult body mass and immunity). This single stressful event during early- could have severe transgenerational impacts through epigenetics (Kim et al., 2013; Merrifield and Kovalchuk, 2013). For example, exposure to ionizing radiation can induce genome instability in germlines of exposed male mice, which was associated with genomic instability in the offspring (Luning et al., 1976). Therefore, future work on early-life radiation exposure should perform transgenerational studies to consider potential epigenetic effects.

Oxidative stress, the imbalance between the production of reactive oxygen species (ROS) and the level of antioxidant defences, could also explain the trade-offs observed between growth and self-maintenance (Hall et al., 2010). We did not measure redox status in crickets exposed to early life radiation stress but we could expect changes in oxidative stress given that ionizing radiation generates ROS (Ward, 1988). We could also predict organisms experiencing early life oxidative stress to upregulate antioxidant defenses in anticipation of future stressful environments (Constantini, 2014). This would be supported by studies showing adaptive responses reporting improved resistance to subsequent doses of radiation (Olivieri et al., 1984; Wolff, 1996). Future work

investigating developmental stress effects on fitness should also assess redox status to deduce potential oxidative mechanisms.

In summary, this work provides evidence that early life stress can have complex, long-lasting effects on life history and adult immunity. These results support the idea that fitness traits are regulated by developmental trade-offs and responses can either be stimulatory, sustained or inhibitory depending on the trait being assessed. While many studies suggest hormetic benefits to organismal fitness, we clearly show that they are not entirely cost-free. These outcomes likely depend on resource allocation strategies and regulation of molecular pathways. Future work investigating potential hormetic effects of ionizing radiation exposure should adopt a multidisciplinary integrated approach to assess the developmental outcomes of early life oxidative stress.

1.5 FIGURES AND TABLES

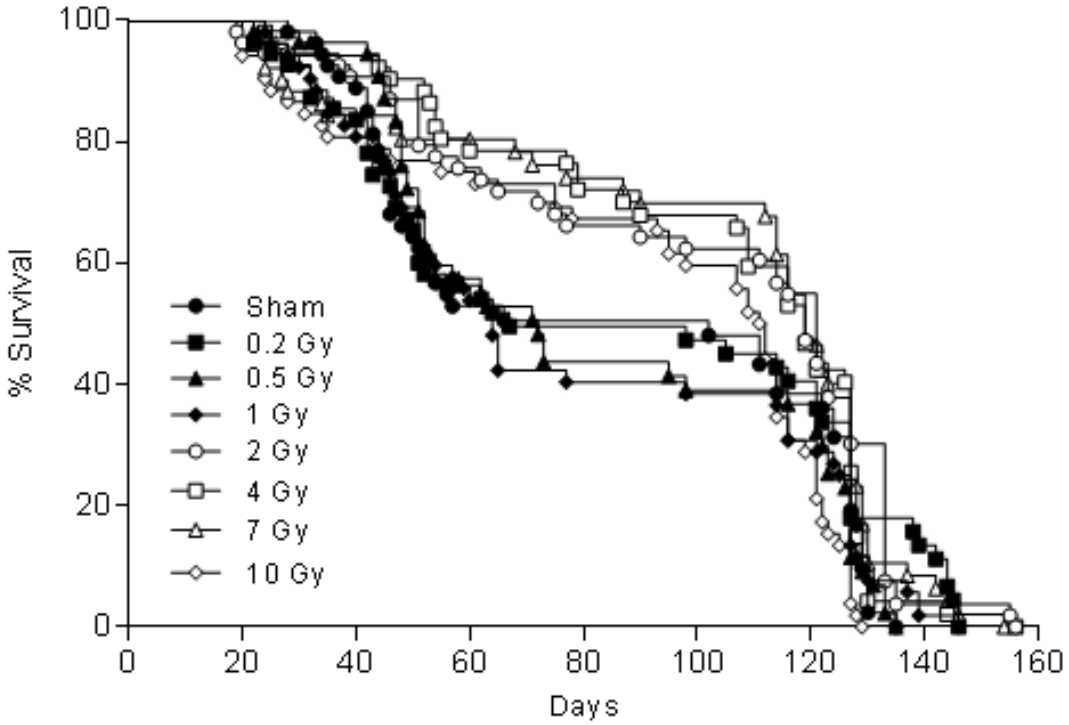

Figure 1.1. Survivorship curves for early life radiation exposure in *A. domesticus.* Groups of 60 crickets were irradiated at 14-days of age representing the start of the study and at doses of 0 (Sham), 0.2, 0.5, 1, 2, 4, 7, and 10 Gy.

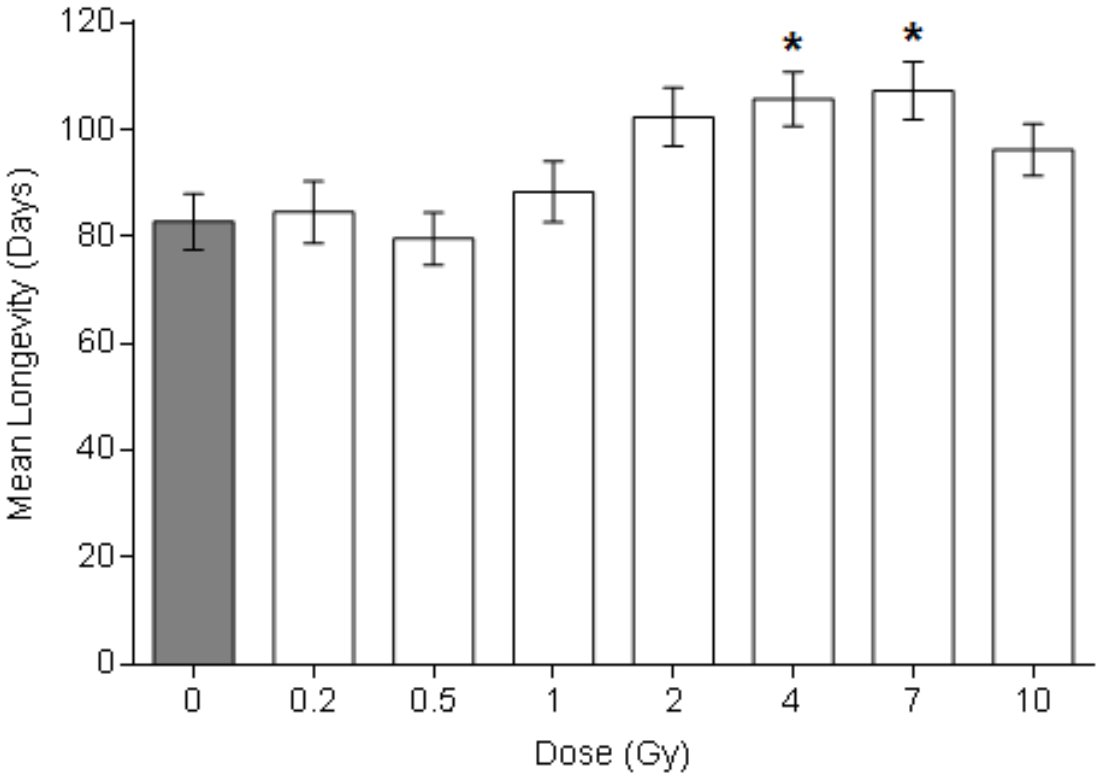

Figure 1.2. Dose response effects of gamma radiation exposure on mean longevity. *A. domesticus* were irradiated at 14-days of age at 0 (Sham), 0.2, 0.5, 1, 2, 4, 7, and 10 Gy. Mean longevity (mean ± SEM) represented the average age of mortality among all animals in the study. Tukey's HSD post-hoc test detected significant differences in mean longevity between 4 and 7 Gy compared to Sham ($p<0.05$).

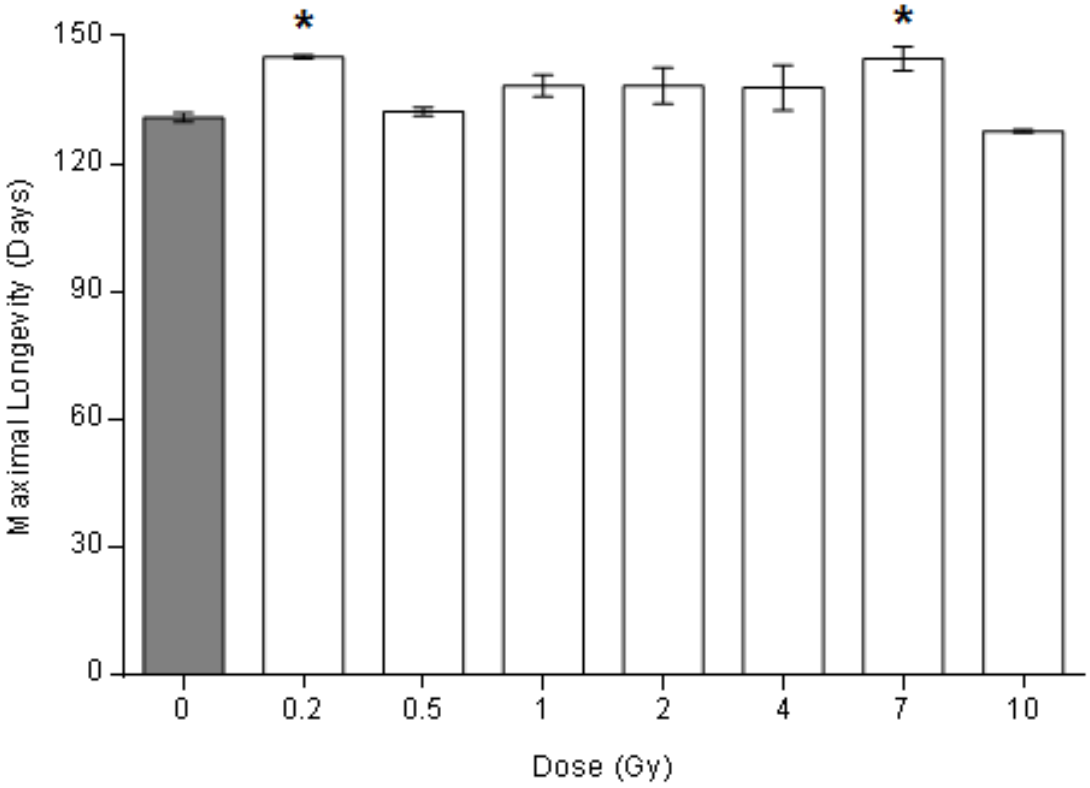

Figure 1.3. Dose response effects of gamma radiation exposure on maximal longevity. *A. domesticus* were irradiated at 14-days of age at 0 (Sham), 0.2, 0.5, 1, 2, 4, 7, and 10 Gy. Maximal longevity (mean ± SEM) was determined using the age of mortality of the last 20% of animals in the study. Tukey's HSD post-hoc test revealed a significant difference in maximal longevity between 0.2 Gy, 7 Gy and sham indicated by the asterisks ($p<0.03$).

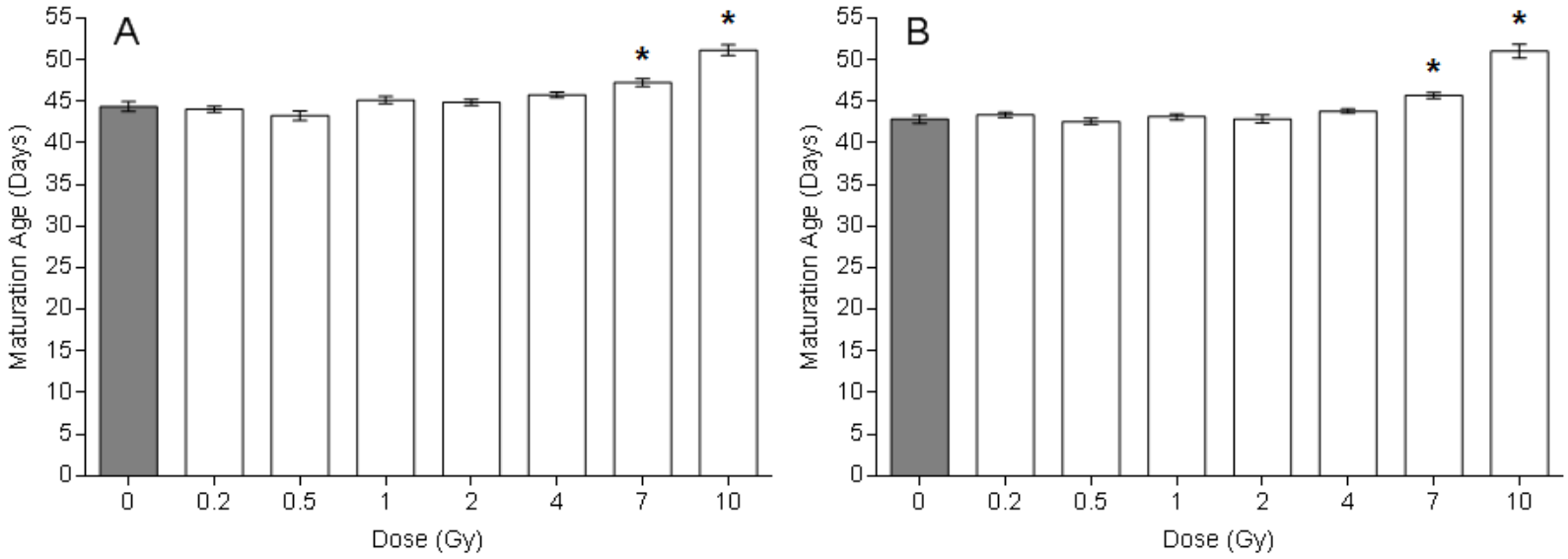

Figure 1.4. Sex-specific dose response effects of gamma radiation exposure on maturation age. *A. domesticus* were irradiated at 14-days of age at 0 (Sham), 0.2, 0.5, 1, 2, 4, 7, and 10 Gy. Male (A) and female (B) maturation ages (mean ± SEM) are reported. Tukey's HSD post-hoc test revealed significant differences in maturation age in males and females irradiated at 7 and 10 Gy compared to Sham ($p<0.003$).

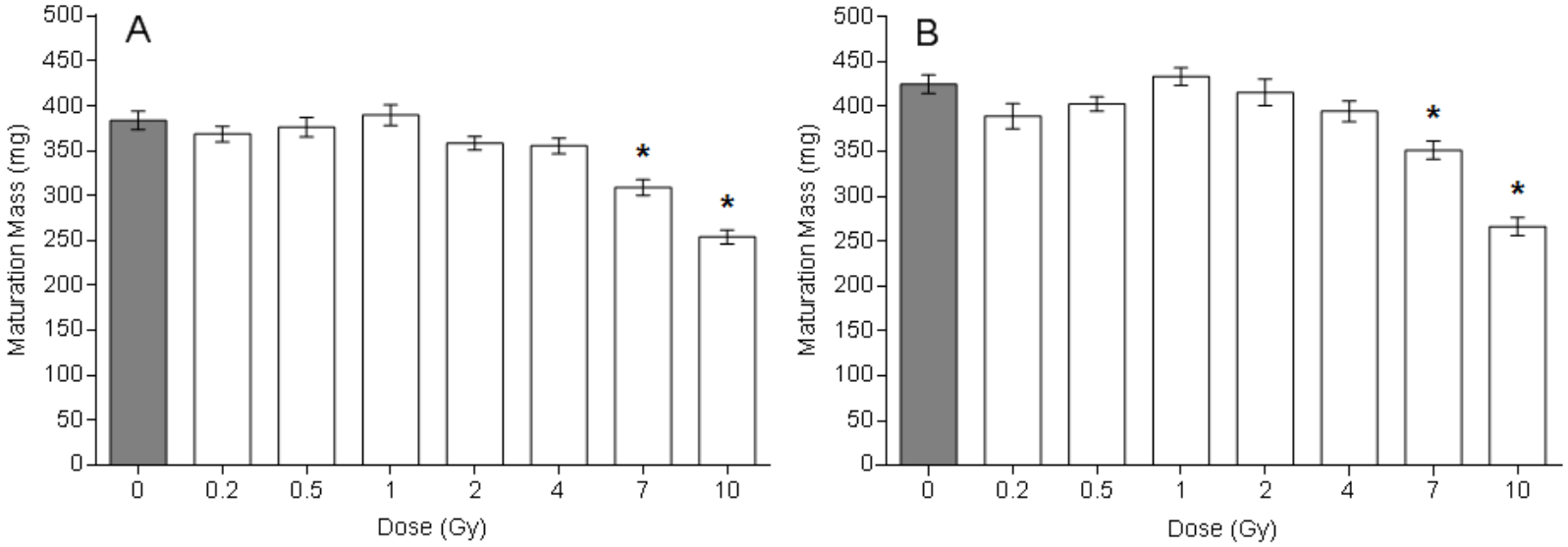

Figure 1.5. Sex-specific dose response effects of gamma radiation exposure on maturation mass. *A. domesticus* were irradiated at 14-days of age at 0 (Sham), 0.2, 0.5, 1, 2, 4, 7, and 10 Gy. Male (A) and female (B) mass at maturation (mean ± SEM) are shown. ANOVA revealed significant differences in maturation age in males and females irradiated at 7 and 10 Gy compared to Sham ($p<0.0001$).

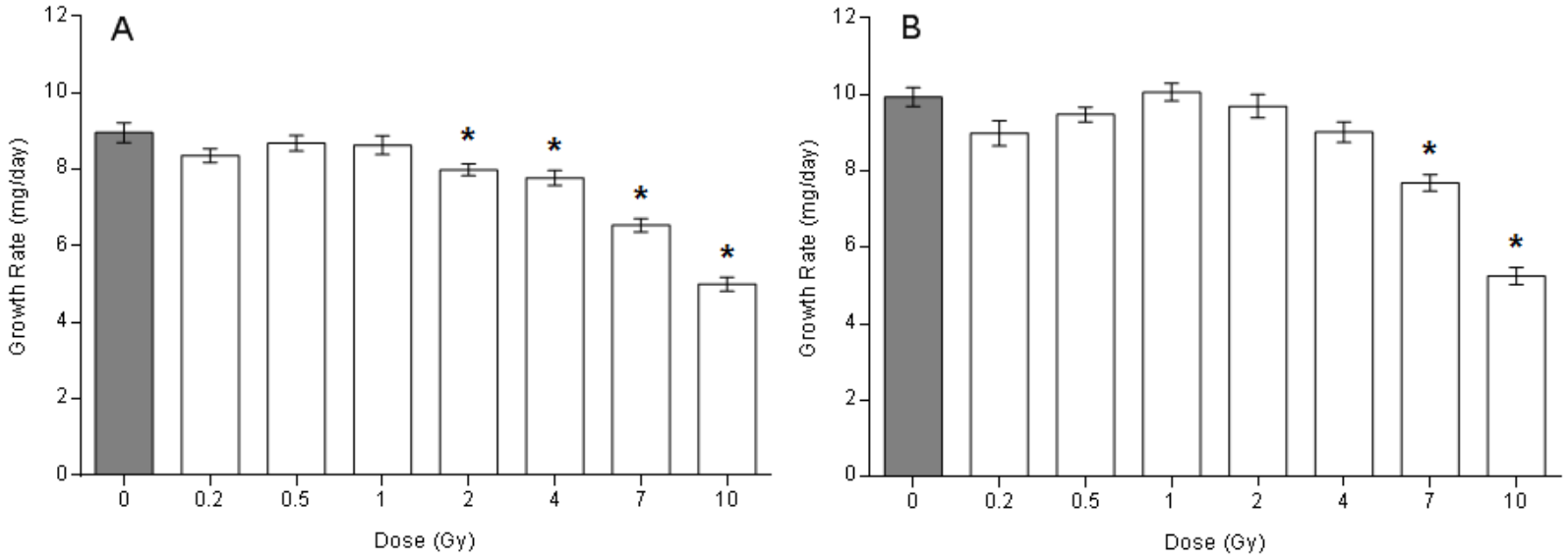

Figure 1.6. Sex-specific dose response effects of gamma radiation exposure on growth rate. *A. domesticus* were irradiated at 14-days of age at 0 (Sham), 0.2, 0.5, 1, 2, 4, 7, and 10 Gy. Male (A) and female (B) growth rates (mean ± SEM) are shown. Tukey's HSD post-hoc test revealed significant differences in maturation age in males irradiated at 2 Gy ($p=0.04$), 4 Gy ($p=0.0073$), 7 Gy ($p<0.0001$) and 10 Gy ($p<0.0001$) compared to sham. Significant declines were also observed in females at 7 and 10 Gy compared to sham ($p<0.0001$).

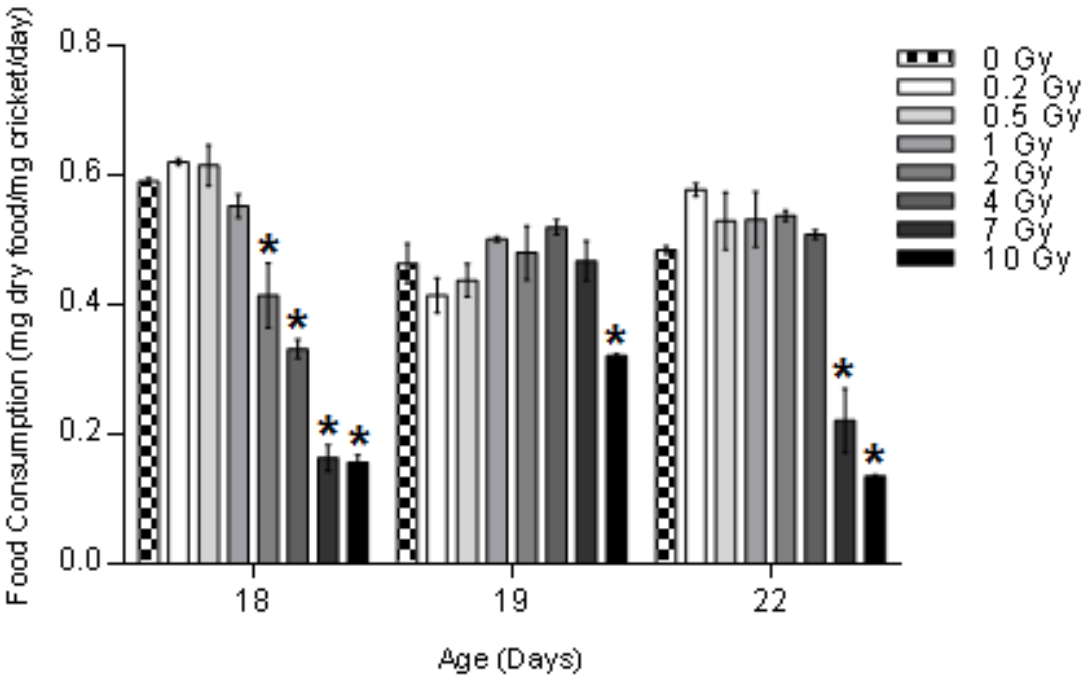

Figure 1.7. Dose response effects of gamma radiation exposure on juvenile feeding rates. Food consumption in *A. domesticus* were measured at 18, 19, and 22 days of age. Tukey's HSD post-hoc test detected significant differences in food consumption at 2, 4, and 7 Gy compared to sham at 18 days of age ($p<0.0016$). Significant differences in feeding rate were also revealed between 10 Gy and sham at each time point ($p<0.0138$).

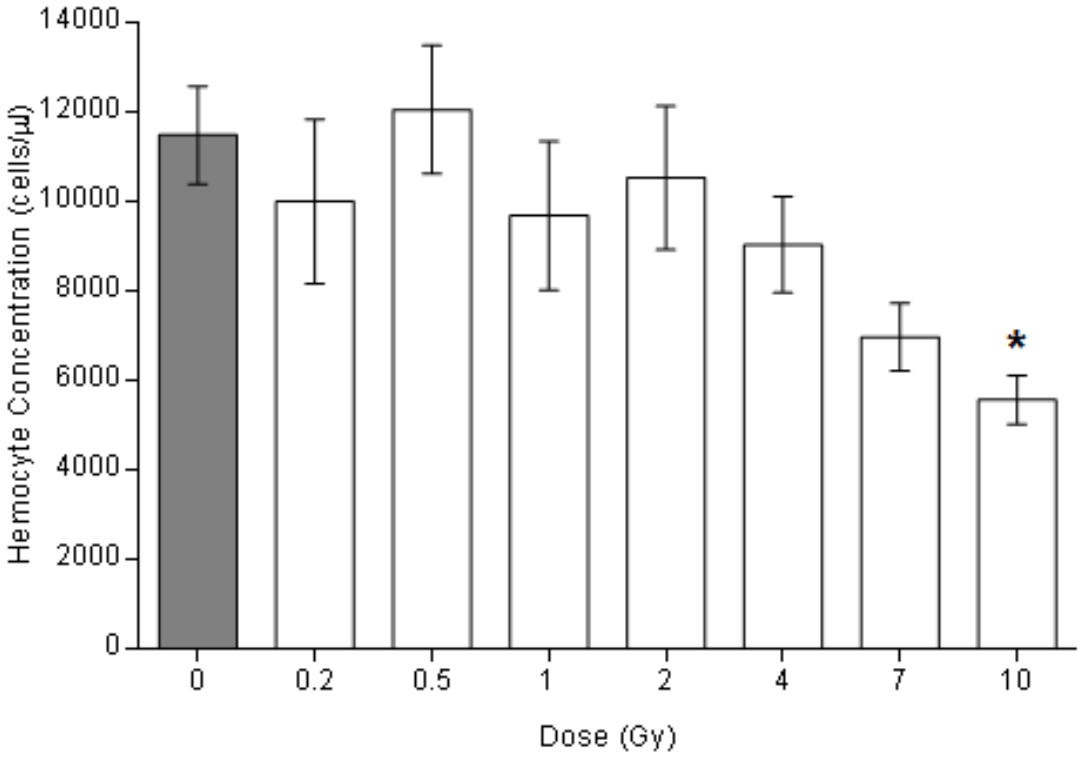

Figure 1.8. Dose response effects of gamma radiation exposure on hemocyte concentration in male crickets. Hemocyte counts were performed 1-week post-maturation in *A. domesticus* irradiated at 0 (Sham), 0.2, 0.5, 1, 2, 4, 7, and 10 Gy. N=6 crickets/treatment. Values are reported as means ± SEM. ANOVA detected a significant difference between 10 Gy and sham (p=0.017).

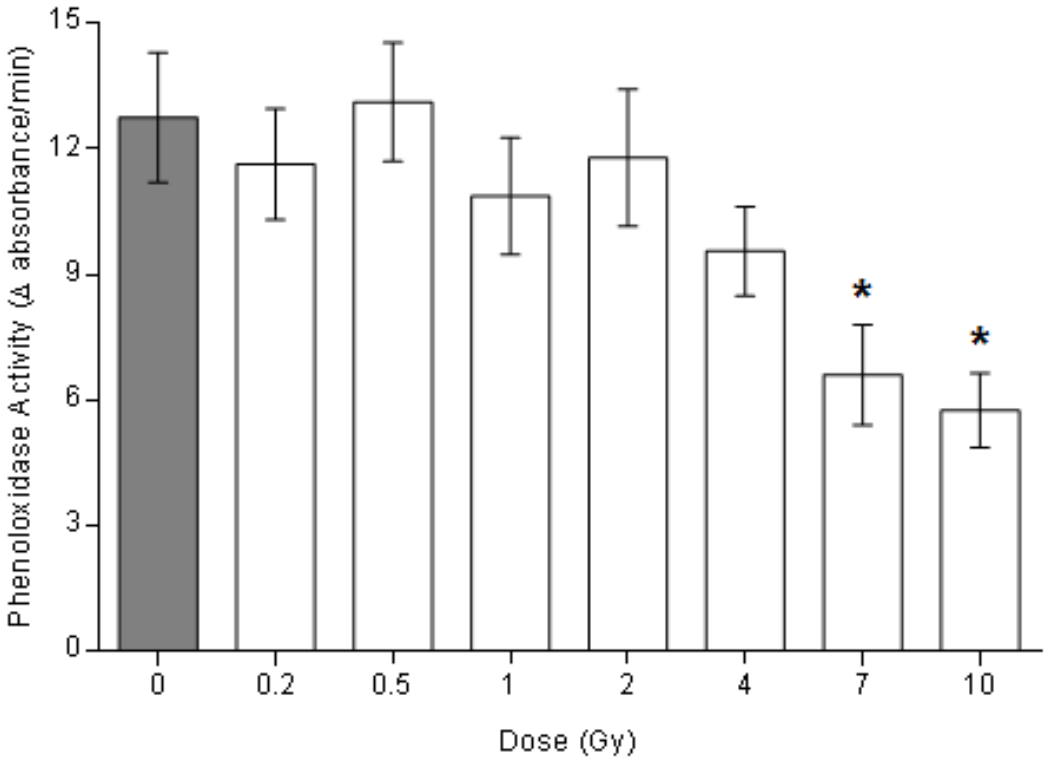

Figure 1.9. Dose response effects of gamma radiation exposure on total phenoloxidase activity in male crickets. Total phenoloxidase activity was assessed at 1-week post-maturation in *A. domesticus* irradiated at 0 (Sham), 0.2, 0.5, 1, 2, 4, 7, and 10 Gy. N=6 crickets/treatment. Values are reported as means ± SEM. ANOVA revealed significant differences in total phenoloxidase activity at 7 Gy and 10 Gy ($p<0.02$) compared to sham.

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CHAPTER 2

LOW-DOSE IONIZING RADIATION STIMULATES THE IMMUNE SYSTEM OF THE CRICKET (*ACHETA DOMESTICUS*): EVIDENCE FOR HORMESIS

2.1 INTRODUCTION

Animals encounter multiple stressors in their environment that can impact their fitness. Ionizing radiation can be detrimental via DNA damage, and generally elevated free radical stress (Ward, 1988). Current radiation regulations apply a linear-no threshold (LNT) dose-response curve that assumes that all doses are potentially damaging (Vaiserman, 2010). Exposure to high-dose radiation is certainly damaging (Elgazzar & Kazem, 2015; Shan et al., 2007) but low doses of ionizing radiation (LDIR) can stimulate biological systems to promote growth rate, slow aging or enhance immunity (Cui et al., 2017; Wiegant et al., 2013). This phenomenon of 'hormesis' may incur a fitness advantage to the organism depending on their environment and could therefore be considered adaptive, granting enhanced resistance to a second challenge (Olivieri et al., 1984).

Oxidative stress has emerged as a potential mediator of hormesis (Constantini, 2014). Ionizing radiation produces reactive oxygen species (ROS) and although many are important signaling molecules (e.g. nitric oxide and hydrogen peroxide), high levels can damage DNA, lipids and proteins (Leach et al., 2001; Elgazzar & Kazem, 2015). Oxidative stress emerges as an imbalance between pro- and anti-oxidant molecules

(Constantini, 2014; Finkel & Holbrook, 2000) so it is crucial that the antioxidant system protectagainst cellular damage.

The immune system is an important form of defense that is extremely sensitive to oxidative stress (Cui et al., 2017). The insect immune system relies on cellular and hormonal defense pathways that interact to mount effective defensive response. The cellular response largely consists of circulating hemocytes (i.e. the immune cells). Hemimetabolous insects like crickets are able to produce hemocytes throughout their lifespan (including the adult stage) via their lymph glands (Grigorian & Hartenstein, 2013) and thus are able to adjust hemocyte production in response to stress.

Hemocytes can be categorized by their morphology and physiological functions such as phagocytosis, encapsulation, and nodulation (Lavine and Strand, 2002). The encapsulation response requires the activity of the humoral defense pathway, involved with melanization. This process is largely accomplished by an enzyme known as phenoloxidase (Gonzalez-Santoyo & Cordoba-Anguilar, 2012). Another major component of humoral defense are antimicrobial peptides (AMPs) such as lysozyme, which break down bacterial cell walls. These AMPs are produced by the insect fat body and secreted into the hemolymph to combat infection (Pinera et al., 2013).

The impacts of stress (e.g. ionizing radiation) on the immune system are complex, multifactorial, and dependent on context, dose, time, and immune trait (Adamo, 2012; Cui et al., 2017). High-dose radiation generally supresses immunity, while low-dose radiation may enhance immunity and even improve health (Cui et al. 2017). LDIR triggers immune activity and antioxidative systems (Marples & Collis, 2008; Pollycove,

2007). LDIR is effective in treating some autoimmune-related diseases such as arthritis and encephalomyelitis by controlling overactive autoimmune reactions (Nakatsukasa et al., 2010; Tsukimoto et al., 2008). LDIR has also been shown to inhibit the development of infections (Nowoseilska et al., 2006; Seong et al., 2012).

Immune stimulation may derive trade-offs within the immune system, which could impact subsequent responses. For instance, increases in lysozyme concentration inhibits phenoloxidase activity under baseline conditions (Rao et al., 2010). Further, Bailey and Zuk (2008) reported an increase in phenoloxidase activity with a decrease in lysozyme activity in response to parasitoid infection in the field cricket (*Teleogryllus oceanicus*). However, no studies have assessed potential trade-offs within the innate immune system in response to LDIR (i.e. cellular vs. humoral or between components of the system). Moreover, few studies assess the temporal relationship of these stimulatory effects (i.e. when stimulation is observed following exposure and whether these physiological changes are sustained). Prior studies focused on early impacts of stressors on insect immunity (Seong et al., 2012) and rarely measure recovery potential (but see Charles & Killian, 2015).

Insects are an excellent model to study of innate immunity since key elements of the innate immune system are highly conserved (e.g. the Toll signaling pathway and immune deficiency pathway resemble the mammalian tumor necrosis factor pathway) (Hoffmann & Reichhart, 2002). Since different immune parameters may be differentially regulated and thus differentially impacted by ionizing radiation, it is important to measure multiple endpoints to properly assess an animal's immunocompetence (Adamo, 2004).

The dose-response relationship of immunological parameters following exposure to ionizing radiation can be affected by a number of factors. These include: the target cell under observation, dose range, spacing, and rate, as well as the temporal pattern of changes (Liu, 2003).

In this research, we examined the dose-response effects of late-life ionizing radiation exposure on several parameters of insect immunocompetence. Our results provide a comprehensive analysis of the impacts of radiation on immunity, a key fitness trait, crucial to survival. We investigated the impacts of LDIR in male crickets (*Acheta domesticus*) on hemocyte concentration and composition, total phenoloxidase activity, and lysozyme activity. These endpoints were assessed at various time points post-irradiation. We also performed an encapsulation assay as a functional test of the cricket's immune response. Dose-response effects on redox status (hydrogen peroxide concentration and total antioxidant capacity) were also measured.

2.2 METHODS

2.2.1 Animals

Two-week-old crickets (*Acheta domesticus*) were collected from our genetically heterogeneous breeding colony and separated into seven experimental groups. They were housed in plastic containers (30x19x12cm) and maintained at 30±1°C in a 12h light/12h dark photoperiod with egg carton shelters, water-soaked cellulose sponges and food *ad libitum*. All groups were raised on a guinea pig (*Living World® Extrusion*) control diet (Lyn et al., 2012). Male crickets were maintained until 1-week post-maturation and

groups were then irradiated with various doses (0, 0.2, 0.5, 0.75, 1, 5, and 15 Gy) at a dose rate of 0.25 Gy/min from a ^{137}Cs gamma source (Taylor Radiobiology Source, McMaster University). The 0 Gy or "sham" irradiated crickets provided controls. To avoid possible variation associated with mating we separated newly eclosed males daily. Animals were euthanized at 6h, 24h, 1-week or 2-weeks post-irradiation for immune assays.

2.2.2 Removal of Hemolymph for Immune Assays

This technique was adapted from Pinera et al. (2013). Crickets were weighed and anesthetized with CO_2. A 27.5-gauge needle sterilized with 80% ethanol was used to puncture the soft tissue between each cricket's pro- and mesothoracic leg, on the right side of the thorax. 2 μl of hemolymph was removed with a pipette and dispensed into a 1.5 ml eppendorf tube containing 198 μl anticoagulant (98 mM NaOH, 146 mM NaCl, 16 mM EGTA, 10 mM citric acid, pH 6.5; see da Silva et al. 2000). Another 2 μl was removed with the pipette at the same wound site and dispensed into a 1.5 ml eppendorf tube containing 98 μl PBS (pH 7.4). An additional 1 μl was removed and dispensed into a PCR tube containing 9 μl PBS (pH 7.4) and stored at -20°C until analysis of lysozyme activity was performed. Removal of hemolymph for the various immune assays took no longer than 2 min. Hemocyte counts, their classification, and total phenoloxidase activity (TPA) assays were performed within 1 hour of hemolymph collection. Lysozyme activity assays were performed within 2 months of hemolymph collection.

2.2.3 Tissue Sample Collection and Homogenization for Redox Assays

Following hemolymph removal, animals were sacrificed and various tissues were collected in cryogenic vials and immediately frozen in liquid nitrogen. We collected the head, legs, gut, and fat bodies (3 lobes) and all were stored at -80°C. Only fat bodies were used for the redox assays (see hydrogen peroxide and total antioxidant capacity). Fat bodies were homogenized in 500 μl cricket saline (100 mM NaCl, 8.6 mM KCl, 8.5 mM $MgCl_2 \cdot 6H_20$, 2 mM $CaCl_2 \cdot 2H_20$, 4 mM $NaHCO_3$, 4 mM $NaH_2PO_4 \cdot 1H_2O$, 25 mM MOPS, 24 mM glucose, 10 mM proline, pH 7.2; see Coast and Kay, 1994). Four 100 μl aliquots were stored at -80°C until used in redox assays.

2.2.4 Hemocyte Concentration and Classification

A hemocytometer composed of nine (3x3) $1mm^2$ grids was used to estimate the number of circulating hemocytes in the hemolymph. 10 μl of the hemolymph-anticoagulant mixture was loaded into the chamber of the hemocytometer. Using phase contrast microscopy (10x magnification), the total number of hemocytes were counted from five 1 mm^2 grids (four corners and center). The number of hemocytes per μl hemolymph was estimated using the formula: cells per μl = (total number cells counted/5) x dilution factor (100) x volume of 10 μl. We also classified hemocytes as plasmatocytes, granular cells, coagulocytes, or other based on the observed morphology. Classifications were performed with reference to previous reports (da Silva et al., 2000; Price and Ratcliffe, 1974).

2.2.5 Total Phenoloxidase Activity

TPA in a hemolymph sample was determined using a spectrophotometric assay (modified from Pinera et al. 2013). Each 100 μl hemolymph-PBS sample was vortexed and 50 μl was transferred into a 1.5 ml plastic cuvette. 70 μl of 1.3 mg/ml α-chymotrypsin from bovine pancreas (Sigma) made fresh in PBS was added, vortexed, and left to incubate at room temperature for 20 min. 600 μl of 0.15 M 3,4-Dihydroxy-L-phenylalanine (L-DOPA; Sigma) made fresh in PBS was added and the change in absorbance was recorded at 490 nm using a spectrophotometer at 0, 8, 15, 23, 30, 45, and 60 min following the addition of L-DOPA, with the 0 time point being immediately after addition. The reaction is light sensitive and was performed in a darkened room. TPA was reported as the slope of the absorbance from the linear phase of the reaction (8-30 min) multiplied by 10^3.

2.2.6 Lysozyme Activity

Lysozyme activity was measured using a turbidity assay (modified from Pinera et al. 2013). 10 μl of hemolymph-PBS mixture was loaded into a well of a 96-well microplate followed by 90 μl of *Micrococcus lysodeikticus* (0.5 μg/ml, Sigma). Absorbance at 450 nm was measured at 0, 5, 10, 15, 20, 25, and 30 min using a Spectramax Plus 384 plate reader with temperature at 30°C. Lysozyme activity was reported as the change in absorbance from the linear phase of the reaction (10-25 min) multiplied by -10^3. A greater decrease in absorbance indicated greater lytic activity.

2.2.7 Encapsulation Response

A 3-mm nylon thread (0.18 mm diameter) was knotted at one end, roughened with fine sandpaper to increase hemocyte affinity and soaked in 90% ethanol for sterilization, and dried. Crickets (1-week post-irradiation) were weighed and anesthetized with CO_2. A puncture was created with a sterile 30.5-gauge needed at the area between the second and third abdominal sternite on the left side of each cricket. The nylon thread was inserted anteriorly until the knot was touching the cricket's external cuticle. After 24h, the thread was dissected and placed in a PCR tube to air-dry overnight. Dried threads were kept in the freezer (-20°C) until ready to be photographed. Two faces of each thread were photographed at 10x with a Motic microscope. ImageJ software (Schneider et al., 2012) was used to analyze the level of encapsulation by taking the average gray value for a predetermined area overlaying each thread face. The box was placed 0.5 mm from the cut end of each thread to prevent inclusion of scar tissue. The same analysis was performed on an un-inserted control thread. The experimental value was then subtracted from the control threads. Darker values corresponded to smaller numerical values, therefore, larger differences between control and experimental threads indicated a greater level of encapsulation.

2.2.8 Redox Status

The hydrogen peroxide concentration was determined using a colorimetric assay kit (Sigma). Each 100 μl tissue homogenate was further diluted by 50x to ensure that readings were within the linear range of the standard curve. Each well contained the 25 μl

sample, red peroxidase substrate stock, horseradish peroxidase stock, and assay buffer as per protocol. Samples were incubated for 16 min (protected from light) at room temperature and absorbance was measured at 570 nm with a Spectramax Plus 384 plate reader. Hydrogen peroxide concentration was calculated from the H_2O_2 standard curve (Sigma).

Total antioxidant capacity was measured as the concentration of small molecule antioxidants using a colorimetric assay (Sigma). Tissue homogenates were further diluted by 60x to ensure that readings were within the linear range of the standard curve. Preliminary trials were run to ensure that readings were within the linear range of the standard curve. As per instructions, wells were filled with sample, water, and Cu^{2+} working solution. Samples were incubated for 45 min (protected from light) at room temperature. Absorbance was measured at 570 nm with a Spectramax Plus 384 plate reader. Small molecule antioxidant concentration was determined with a trolox standard curve.

2.2.9 Statistical Analyses.

Effects of radiation dose on all immune parameters (hemocyte concentration, phenoloxidase activity, lysozyme activity, and encapsulation ability) were analyzed using a one-way ANOVA. If significant differences were detected, a Tukey's HSD post-hoc test was used for multiple comparisons. A regression analysis was also performed to compare linear (first order polynomial) and parabolic (second order polynomial) fits to test between LNT and hormesis models. The linear fit represented the null hypothesis.

Where parabolic fits were better (indicated by smaller p-value), we tested whether the parabolic fit was significant to reject the linear fit. A correlation analysis was performed between total phenoloxidase activity and lysozyme activity. All data are presented as means ± standard error of the mean (SEM). Statistical significance was set at $p < 0.05$. Analyses were performed on Graphpad Prism 6.0 software.

2.3 RESULTS

2.3.1 Cellular Immune System

The concentration of hemocytes were highly variable across treatment groups. However, there was a significant difference in hemocyte concentration among radiation doses at 1-week post-irradiation (ANOVA: $F=8.225$, $df=6,99$, $p<0.0001$). Hemocyte concentration was increased at 0.5 Gy by ~80% compared to SHAM ($p=0.028$). There was also a ~58% decrease in hemocytes at 15 Gy compared to SHAM at 1-week post-irradiation ($p=0.001$). At 2-weeks post-irradiation, significant differences were also detected between radiation doses (ANOVA: $F=5.591$, $df=6,86$, $p<0.0001$). Hemocyte concentration was elevated for 0.5 and 0.75 Gy ($p<0.01$). No significant differences were detected at 6h or 24h post-irradiation (Fig. 2.1).

Hemocytes were visualized and classified into three morphologically distinct types: plasmatocytes, granular cells, and coagulocytes. A fourth small cell type was observed, which I classified as “unknown” (Table 2.1). Although total number of hemocytes differed between treatments, ANOVA did not detect any significant differences in the proportion of hemocyte types.

Regression analysis detected a preferred polynomial fit in hemocyte concentration at 24h (Table 2.3: $p=0.048$) and 1-week (Table 2.4 $p=0.089$) post-irradiation when assessing the low dose range. Analysis of the full dose range for hemocyte concentration appeared to exhibit a linear fit at all time points (Table 2.2, 2.3, 2.4, and 2.5).

2.3.2 Humoral Immune System

Significant differences in TPA were observed at 1-week post-irradiation (ANOVA: $F=5.183$, $df=6,101$, $p=0.0001$) and 2-weeks post-irradiation (ANOVA: $F=9.791$, $df=6,84$, $p<0.0001$). A trend for increased TPA at low doses (e.g. 0.5 Gy) at 24h was observed, however, differences fell short of significance ($p=0.0953$). Crickets irradiated at 15 Gy exhibited a decrease in TPA compared to SHAM at 1-week and 2-weeks post-irradiation ($p<0.0334$). A decline was also observed for 5 Gy at 2-weeks post-irradiation compared to control ($p<0.05$). TPA did not differ significantly at any dose below 5 Gy at 2-weeks post-irradiation (Fig. 2.2).

Significant differences in lysozyme activity were detected between radiation doses at 6h post-irradiation (ANOVA: $F=7.543$, $df=6,88$, $p<0.0001$) and 24h post-irradiation (ANOVA: $F=5.595$, $df=6,101$, $p<0.0001$). Lysozyme activity was significantly elevated at 0.75 Gy compared to SHAM at 6h and 24h post-irradiation ($p=0.0003$ and $p=0.0007$, respectively). There was a trend for lower lysozyme activity at higher doses (e.g. 5 and 15 Gy) when observed at 6h and 24h post-irradiation, however, no significant differences were found. No significant differences in lysozyme activity were observed at 1-week or 2-weeks post-irradiation (Fig. 2.3).

A significant moderate correlation between TPA and lysozyme activity was detected for 0.5 Gy at 1-week post-irradiation ($r=0.66$, $p=0.0054$; Fig. 2.4a). Correlations were not observed with any high doses (Fig. 2.4b).

TPA and lysozyme activity showed a significant linear fit at 1-week (Table 2.4: $p<0.043$) and 2-weeks (Table 2.5: $p<0.063$) post-irradiation when analyzing the full dose range. However, differences between linear or polynomial fits were not detected at any time point with the low dose range (Table 2.2, 2.3, 2.4, and 2.5).

2.3.3 Response to an Immune Challenge

Significant differences in encapsulation ability were observed between doses at 1-week post-irradiation (ANOVA: $F=4.984$, $df=6,76$, $p=0.0002$). There was an increased encapsulation response in crickets irradiated with 0.5 Gy ($p=0.0185$) and 0.75 Gy ($p=0.0224$) compared to SHAM. Exposure to 15 Gy decreased the encapsulation response by ~46% compared to controls but differences fell short of significance (Fig. 2.5). Preliminary data did not detect significant differences in the encapsulation response at 24h (data not shown).

Regression analysis of encapsulation showed a preference for a linear fit with the full dose range (Table 2.4: $p=0.070$). The low dose range did not detect a difference between linear or polynomial models (Table 2.4).

2.3.4 Redox Status

Effect of body mass on hydrogen peroxide or total antioxidant capacity was not detected (data not shown). Regression analysis showed a significant polynomial fit for hydrogen peroxide and total antioxidant capacity (Fig. 2.6c,f) at 2-weeks post-irradiation ($F=9.517$, $df=1,4$, $p=0.0368$ and $F=9.334$, $df=1,4$, $p=0.0378$, respectively). Differences between linear and polynomial fits were not resolved with 24h or 1-week time points with either hydrogen peroxide or total antioxidant capacity (Fig. 2.6a,b,d,e).

2.4 DISCUSSION

Results revealed apparent hormetic impacts of low-dose ionizing radiation (LDIR) on immunocompetence, particularly stimulating markers of cellular and humoral immune defense in crickets. Few studies have expressed clear evidence of LDIR activation in insects (but see Seong et al., 2012). We are the first to report stimulating effects of LDIR on innate immunity in crickets. Consistent with previous findings on radiation exposure in vertebrates (Cheda et al., 2004; Hashimoto et al., 1999; Yang et al., 2014), LDIR increased immune cell proliferation (Fig. 2.1) and function (e.g. encapsulation response: Fig. 2.4). Hemocyte concentration demonstrated a significant polynomial fit at low-dose (Table 2.3 and 2.4). LDIR exposure also increased aspects of the humoral system including lysozyme activity (Fig. 2.3). In contrast, high-dose ionizing radiation largely suppressed cellular (Fig. 2.1) and humoral (Fig. 2.2 and Fig. 2.3) components of the cricket immune system. Together, these results show that a single exposure to ionizing

radiation can have profound dose-dependent impacts on insect immunity, but more importantly, sustained stimulatory aspects of the immune system were detected.

Stressors may only show a characteristic hormetic curve at a certain time point after treatment. This time-dependent phenomenon for hormesis expression is known as 'overcompensation stimulation' (Calabrese, 2001). For example, Liu et al. (2000a) performed a time course study with several immune organs (e.g. thymus, spleen, lymph nodes) demonstrating a J-shaped dose response curve of apoptosis at 12h after whole-body irradiation in mice. Significant decreases in apoptosis rate at 0.05 and 0.075 Gy were detected (Liu et al., 2000a). However, they also revealed a brief increase in apoptosis rate 1h after irradiation at the same doses (Liu et al., 2000a). This could be explained by overcompensation stimulation whereby a certain magnitude of damage is required in order to stimulate a compensatory reaction (Calabrese, 2001). Alternatively, the immediate radiation stress could have simply upregulated apoptosis. Although we did not detect an initial decline in any immune parameters measured at LDIR, it is possible that our earliest time point of 6h was too late. Therefore, we cannot rule out the possibility of an earlier overcompensation stimulation mechanism.

Studies have also shown hormetic dose responses without the observation of an overcompensating response. For example, Liu et al. (2000b) showed a hormetic dose response curve of thymocyte cAMP/cGMP ratio after whole-body irradiation in mice. Time course studies showed no overcompensation even as early as 30 min after irradiation (Liu et al., 2000b). This could indicate that hormetic responses occur through direct stimulation (Calabrese, 2001). Our current results suggest a direct stimulatory

hormetic response with respect to immunity (e.g. hemocyte concentration: Fig. 2.1 and lysozyme activity: Fig. 2.3) since a disruption in homeostasis was not initially observed with LDIR. In this regard, one could expect direct stimulation to utilize fewer resources as compared to overcompensation stimulation since there is no obvious damage or disruption to the system. This could explain the lack of observed trade-offs within the components of the immune system (explained below). Therefore, it is imperative that time course studies be performed to fully understand the mechanisms of hormesis.

In contrast to the mammalian literature (Cheda et al., 2004; Hashimoto et al., 1999; Yang et al., 2014; Sonn et al., 2012), the impacts of LDIR on the insect immune system remain largely unexplored. Insect hemocytes are functionally similar to vertebrate macrophages critical for clearance of pathogens (Gordon et al., 2013; Gordon & Taylor, 2005). LDIR has been shown to promote macrophage differentiation in mice (Klug et al., 2013). We observed slight changes in hemocyte composition at 0.5 and 0.75 Gy potentially showing increased proportion of plasmatocytes but significance was not detected. More importantly, LDIR demonstrated a polynomial dose response as early as 24h (Table 2.3) and increased hemocyte proliferation until 2-weeks post-irradiation. Mechanistic studies in mammalian models have shown that direct effects of LDIR and indirect increases in ROS can lead to changes in the expression of surface molecules in T lymphocytes (e.g. CD2, CD28, TCR, CTLA-4). These changes in gene expression determine the outcome of the immune response (i.e. immunoenhancement or immunosuppression) (Liu, 2003). In insects, genes implicated in proliferation and differentiation of hemocytes include Janus kinase (JAK) - signal transducer and activator

transcription (STAT) signaling pathway (Dearolf, 1999; Myrick and Dearolf, 2000) and Toll receptors (Govind, 1999). In this regard, LDIR could alter protein/gene expression on hemocytes but additional studies in insects are required to fully resolve the mechanism.

A number of studies have characterized trade-offs within the insect immune system involving resource allocation and/or physiological constraints (Ardia et al., 2011; Bailey & Zuk, 2008; Rao et al., 2010; Siva-Jothy et al., 2005). Since the immune system is a unified defense system, utilizing one component of immunity may lead to changes in others. Many studies have found negative correlations between cellular immunity and antibacterial activity (Cotter et al., 2004; 2008) suggesting potential trade-offs between cellular and humoral components. In addition, exposure to a bacterial challenge frequently results in increased lysozyme but decreased phenoloxidase activities (da Silva et al., 2000; Moret & Schmid-Hempel, 2001; Rao et al., 2010). In contrast, other studies have found positive correlations between lysozyme and phenoloxidase activities (Adamo, 2004), as well as melanization, the process largely driven by phenoloxidase (Lambrechts et al., 2004).

Because of lags in activation, the potential links between lysozyme and phenoloxidase activity may differ depending on the type of stressor. Generally, lysozyme activity is increased by bacterial infection as a result of upregulation of gene expression (Gillespie et al., 1997; Zhang et al., 2009). Enhanced immune activity from LDIR in insects has been linked to increased expression of key proteins involved in the Toll signaling pathway (Seong et al., 2012). Although we did not measure gene expression,

our results are consistent with increased AMP production (lysozyme activity: Fig. 2.3) with LDIR at 6h and 24h post-irradiation. However, these changes were absent at 1-week and 2-week time points (Fig. 2.3). On the other hand, we observed no changes in TPA at any time point (although trends may suggest stimulation at lower doses). These results could be explained by constraints on the evolution of immune responses that prevent maximizing all aspects of immunity. Sorci and Faivre (2009) suggested that, to optimize immunity, selection may have evolved rapid activation and deactivation to minimize associated high costs. Therefore, regulation/coordination of these separate immune pathways is vital.

Interpretation of our humoral immune data could suggest a complex relationship between phenoloxidase and lysozyme activity given that we observed a moderate positive correlation at 1-week post-irradiation with 0.5 Gy (Fig. 2.4). Despite our measurements at several time points, we did not observe any declines in phenoloxidase activity associated with LDIR. This lack of impact could be explained by differences in the type of stress (i.e. radiation vs. immune challenge) and thus the mechanism of stimulation compared to previous works. In addition, whole-body irradiation affects all parts of an organism uniformly and avoids the issue of unintended localization of stimulation that likely occurs when testing a specific immune challenge (i.e. bacterial infection) (Koch & Hill, 2016). Therefore, LDIR could potentially upregulate several aspects of immunity without the need for decline in other components. Together, these works suggest that the immune response depends on the type of stressor.

Our data on encapsulation provided a functional test of immune responses to challenge. Given the importance of the encapsulation response in immune defense, this provides clear evidence of a fitness advantage from LDIR. Encapsulation refers to the binding of hemocytes to larger foreign invaders like parasitoids and nematodes to effect encapsulation and associated melanin deposition (Lavine & Strand, 2002). Improved encapsulation arguably represents increased defense against parasites. The two types of hemocytes most often involved in encapsulation are granular cells and plasmatocytes (Schmidt et al., 2001). The increase in total hemocyte concentration along with marginal increases in plasmatocytes at LDIR could explain the improved encapsulation ability. Although we did not observe a significant decline in encapsulation at 15 Gy (trending), it is likely that inhibition would occur at higher doses. For future studies, increasing the dose range would improve the chances of capturing a biphasic dose response curve.

Recovery from radiation damage is generally mediated by signaling pathways that upregulate antioxidants and DNA repair mechanisms (Spitz et al., 2004; Dauer et al., 2010). Radiation can also trigger the release of inflammatory cytokines linked to immune activation (Iyer & Lehnert, 2000; Kim et al., 2006; Jang et al., 2013). For example, increased expression of genes related to AMP production are correlated with increased resistance to oxidative stress in *Drosophila* (Zhao et al., 2011). Furthermore, a positive correlation was shown between immune cell activity and glutathione production (Kojima et al., 2004). Our results showed stimulation of lysozyme activity at 6h and 24h but total antioxidant capacity did not exhibit a hormetic dose response until 2-weeks post-irradiation (Fig. 2.6f). This suggests that although LDIR may rapidly stimulate immunity,

hormetic effects causing the upregulation of antioxidant production may take longer to develop. However, this effect could have long-term implications enhancing resistance to future oxidative challenge (Szumiel, 2012; Spitz et al., 2004).

Changes in hydrogen peroxide concentration and total antioxidant capacity do not necessarily reflect the potential for oxidative damage (i.e. lipid peroxidation, protein carbonylation). Since our only measurement of oxidative stress was hydrogen peroxide, we are unable to determine how other more damaging free radicals (e.g. superoxide) are affected. Although antioxidant capacity can predict protection from free radical damage (Riley, 1994), our analysis cannot differentiate between specific antioxidants (e.g. glutathione, superoxide dismutase, and catalase). Measuring one class of antioxdiants may give us a different picture from that obtained while measuring another (e.g. superoxide dismutase vs. glutathione). Paskova et al. (2008) showed that levels of two antioxidants, glutathione and glutathione peroxidase, may covary negatively after immune stimulation. Similar results emerged from other studies with catalase and superoxide dismutase (Koinarski et al., 2005; Georgieva et al., 2006). Furthermore, stronger hormetic effects might be seen with exposure to a subsequent dose of radiation. In light of this, future work should consider possible adaptive responses when investigating the effects of radiation on redox status.

In summary, a single exposure to ionizing radiation at doses below those causing immunosuppression was observed to augment and sustain various immune responses consistent with hormesis. Radiation hormesis is becoming more recognized, but clear evidence for hormesis in insects are limited. Results presented support the idea that

radiation stress has differential impacts on immunity depending on dose and time of assessment. This study provides evidence for the enhancement of innate immunity without any obvious assoiciated trade-offs. This study focused specifically on the responses of late-life acute ionizing irradiation on insect innate immunity. Although not measured, trade-offs with other aspects of fitness including longevity and reproduction are possible and should be assessed to fully understand global impacts and hormetic potential. Given the importance of understanding the impacts of radiation stress on physiological function, further studies of LDIR exposure in model systems will help elucidate the molecular mechanisms relevant to resistance, recovery, and hormesis.

2.5 FIGURES AND TABLES

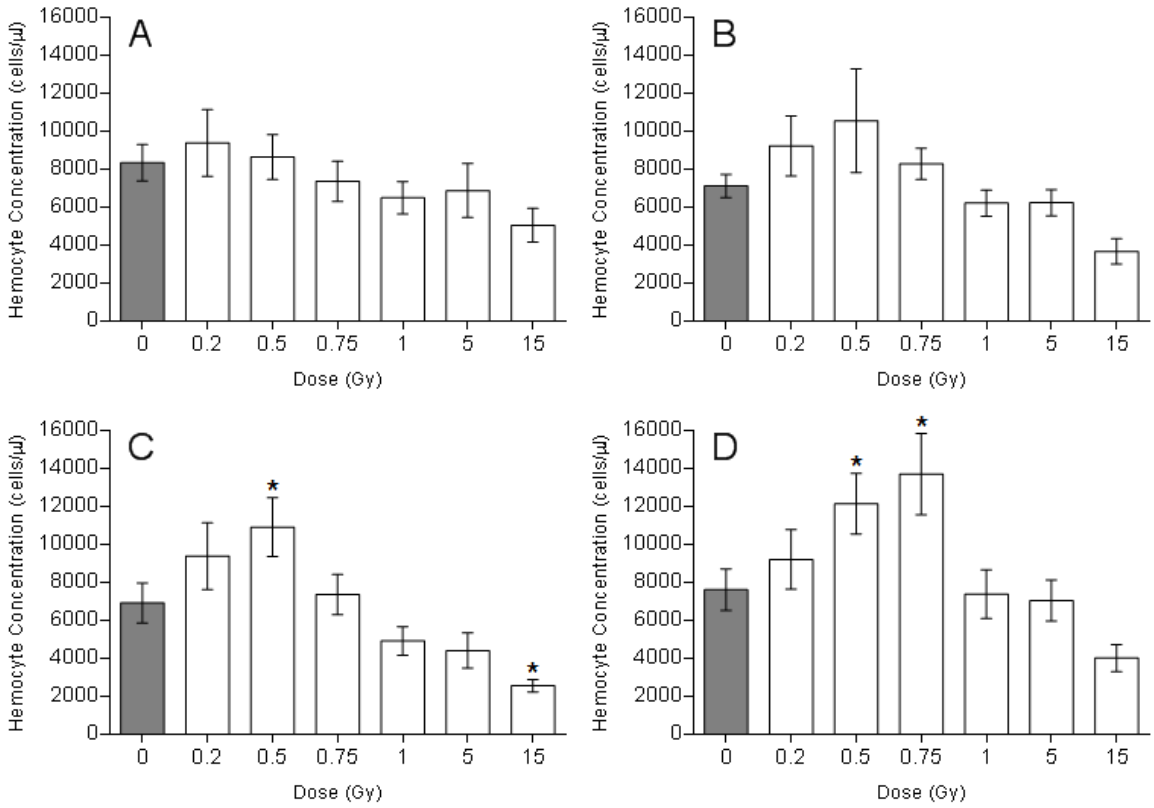

Figure 2.1. Dose response effects of ionizing radiation exposure on hemocyte concentration in male crickets (cells/µl hemolymph). Hemocyte counts were performed at 6h (A), 24h (B), 1-week (C), and 2-weeks (D) post-irradiation. Hemocyte concentration was elevated at 0.5 Gy ($p=0.028$) and depressed at 15 Gy at 1-week post-irradiation ($p=0.001$). Hemocte concentration was also elevated for 0.5 and 0.75 Gy at 2-weeks post-irradiation ($p<0.01$). N=15 crickets/treatment. Values are reported as means ± SEM. Asterisks indicate significant differences compared to 0 Gy treatment group (SHAM).

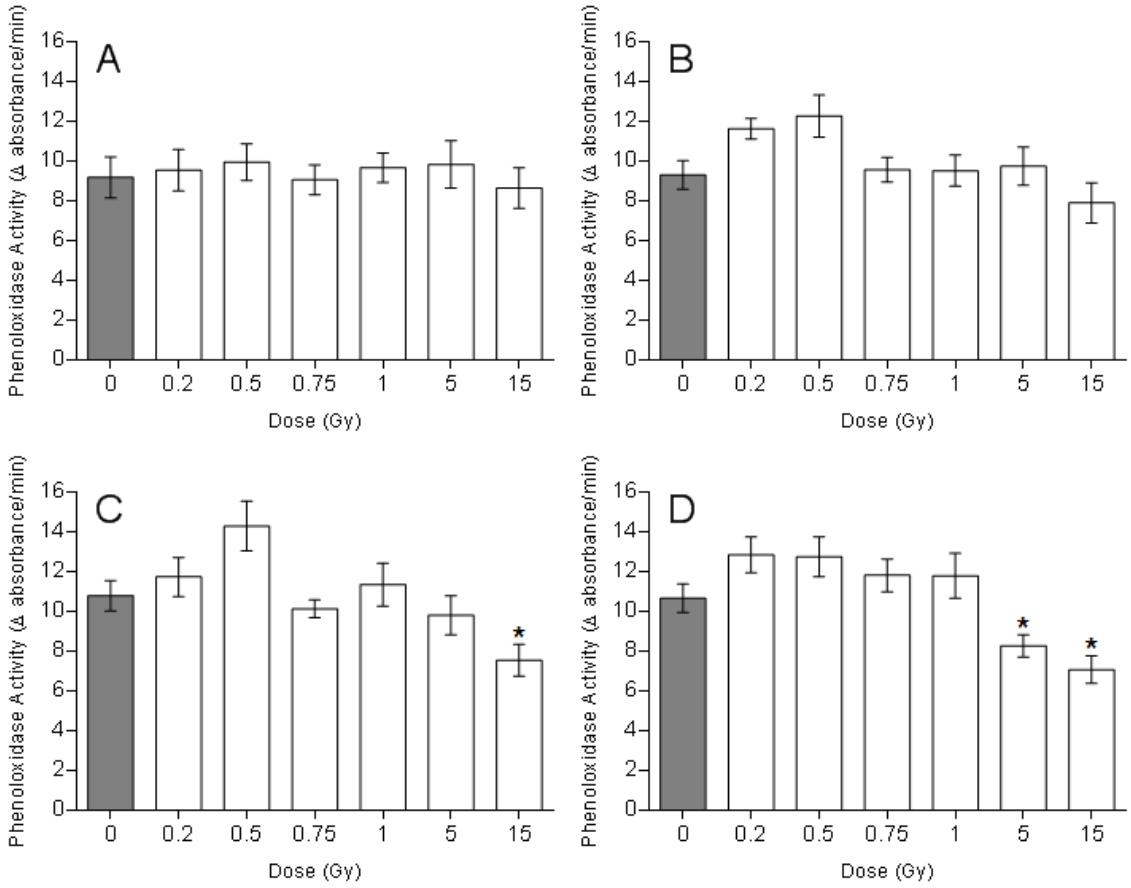

Figure 2.2. Dose response effects of ionizing radiation exposure on total phenoloxidase activity in male crickets (Δ absorbance/min x 10^3). Total phenoloxidase activity (TPA) was assessed at 6h (A), 24h (B), 1-week (C), and 2-weeks (D) post-irradiation. TPA was decreased at 15 Gy at 1-week and 2-weeks post-irradiation ($p<0.0334$). TPA was also depressed at 5 Gy at 2-weeks ($p<0.05$). N=15 crickets/treatment. Values are reported as means ± SEM. Asterisks indicate significant differences ($p<0.05$) compared to 0 Gy treatment group (SHAM).

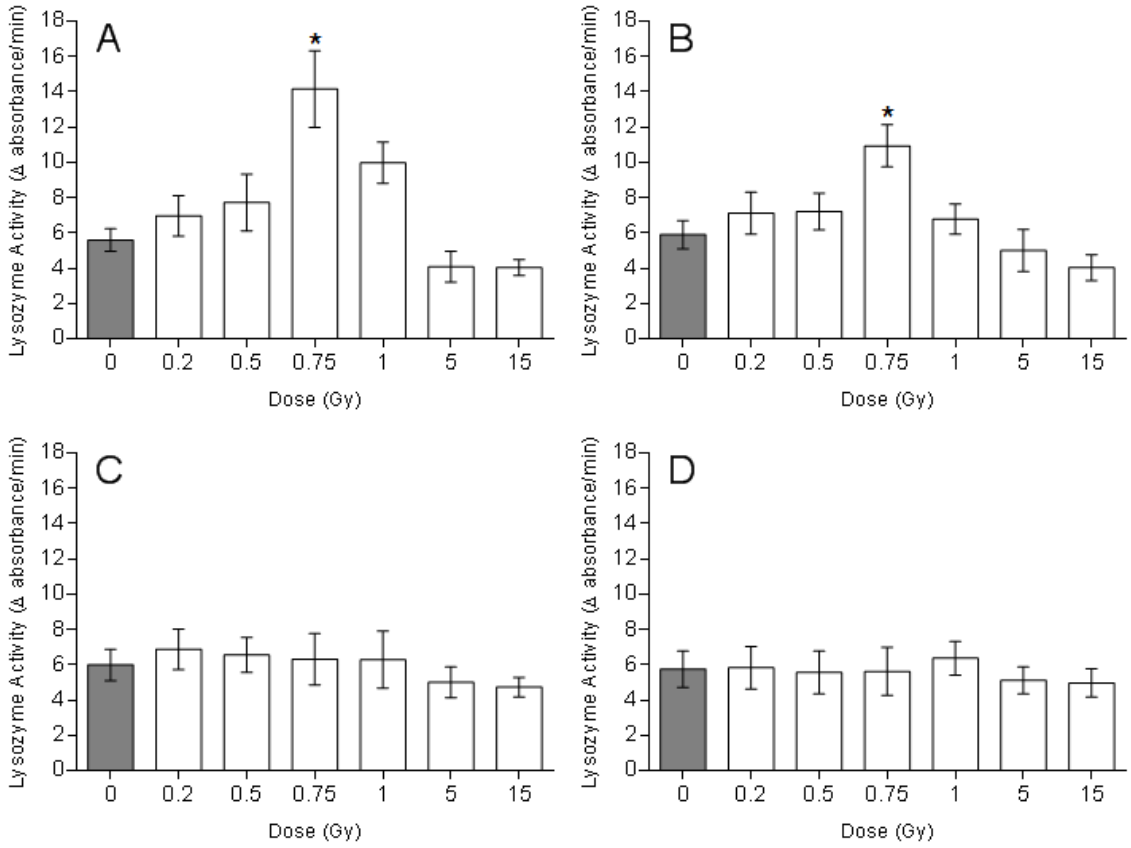

Figure 2.3. Dose response effects of ionizing radiation exposure on lysozyme activity in male crickets (Δ absorbance/min x -10^3). Lysozyme activity was measured at 6h (A), 24h (B), 1-week (C), and 2-weeks (D) post-irradiation. Lysozyme activity was elevated for 0.75 Gy at 6h ($p=0.0003$) and 24h ($p=0.0007$). N=15 crickets/treatment. Values are reported as means ± SEM. Asterisks indicate significant differences ($p<0.05$) compared to 0 Gy treatment group (SHAM).

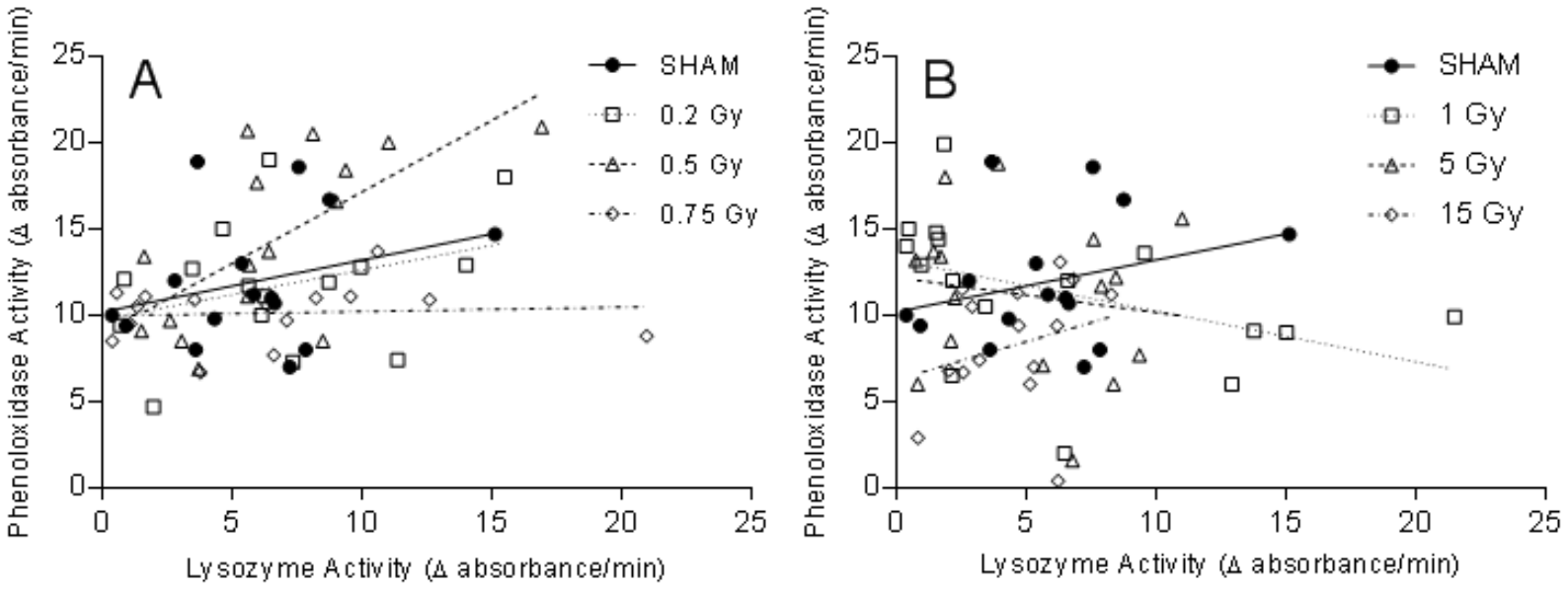

Figure 2.4. Relationship between total phenoloxidase activity (Δ absorbance/min x 10^3) and lysozyme activity (Δ absorbance/min x -10^3) in male crickets exposed to ionizing radiation. Low-dose (A; 0.2, 0.5, and 0.75 Gy) and high-dose (B; 1, 5, and 15 Gy) correlations were compared at 1-week post-irradiation. N=15 crickets/treatment. Regression analysis detected significance at 0.5 Gy (r=0.66, linear regression: p=0.0054, y=0.2855x + 9.771) and slopes significantly differed, p=0.0446.

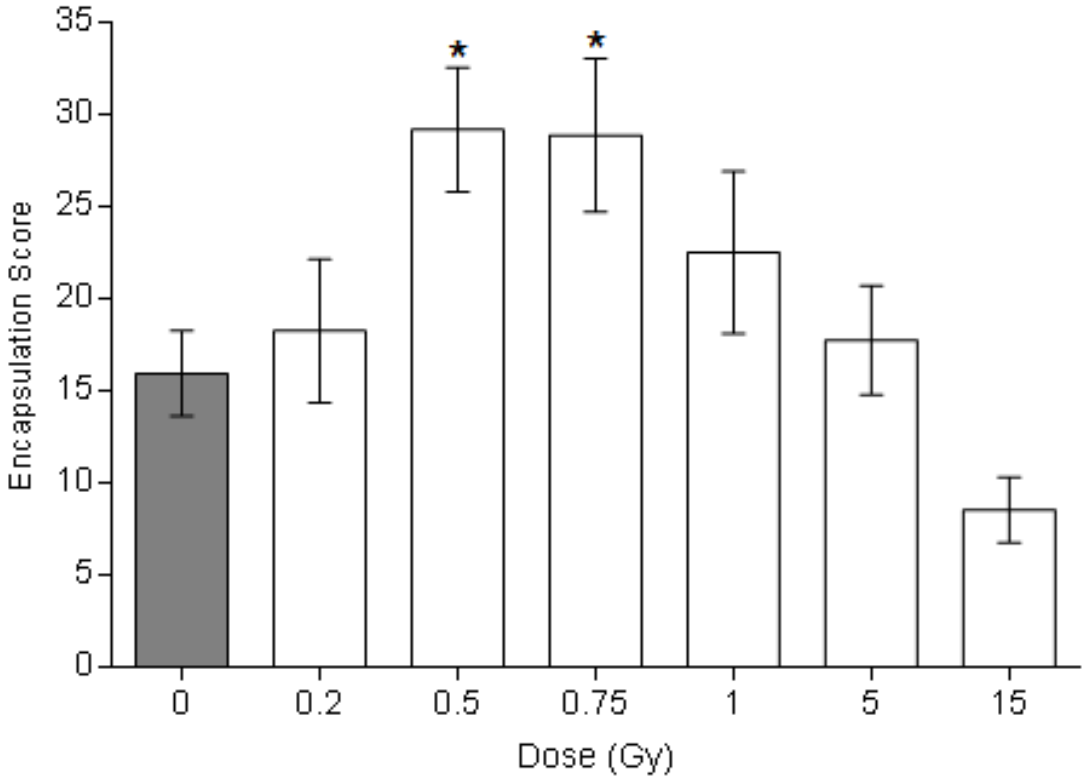

Figure 2.5. Dose response effects of ionizing radiation exposure on encapsulation ability in male crickets. Encapsulation ability was measured at 1-week post-irradiation. Encapsulation was elevated with 0.5 Gy ($p=0.0185$) and 0.75 Gy ($p=0.0224$). N=15 crickets/treatment. Values are reported as means ± SEM. Asterisks indicate significant differences ($p<0.05$) compared to 0 Gy treatment group (SHAM).

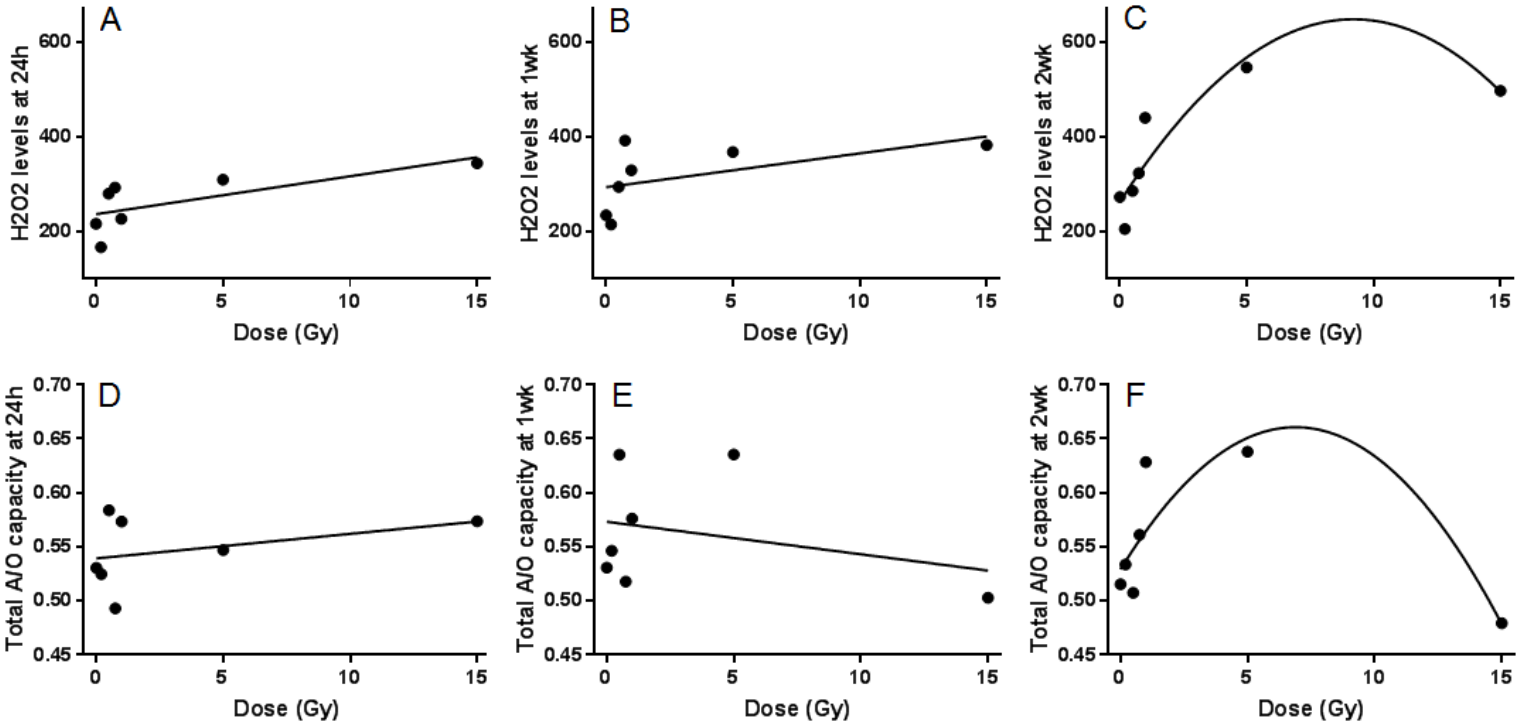

Figure 2.6. Relationship between dose of ionizing radiation and redox status. Hydrogen peroxide concentration was measured at 24h (A), 1-week (B), and 2-weeks (C) post-irradiation. Total antioxidant capacity was measured at 24h (D), 1-week (E), and 2-weeks (F) post-irradiation. Regression analyses obtained a significant parabolic fit for hydrogen peroxide concentration (r^2=0.84, polynomial regression: p=0.0368, $y=-4.5x^2 + 84x +26$) and total antioxidant capacity (r^2=73, polynomial regression: p=0.0378, $y =-0.00028x^2 + 0.038x + 0.53$) at 2-weeks post-irradiation. N=15 crickets/treatment.

Table 2.1. Percentage of hemocyte types in 1 μl hemolymph of *Acheta domesticus* across ionizing radiation dose. A fourth small cell type was observed and are classified as 'unknown'. ANOVA did not detect any significant differences in hemocyte composition. N=8/treatment group.

	% Total Hemocytes			
Dose (Gy)	**Plasmatocyte**	**Granular Cell**	**Coagulocyte**	**Unknown**
0 (SHAM)	58.7	28.0	10.9	2.4
0.2	59.9	28.6	8.1	3.4
0.5	60.0	29.7	8.3	2.0
0.75	60.9	28.3	8.2	2.6
1	60.6	28.2	8.5	2.7
5	58.2	30.2	8.8	2.8
15	57.9	30.5	9.5	2.1

Table 2.2. Linear and polynomial regressions for innate immunity (indicative of LNT versus hormetic models) at 6h post-irradiation. Data were analyzed with full dose range (0-15 Gy) and low dose range (0-1 Gy). Preferred model had the lowest significant p-value ($p<0.1$).

Trait	Dose Range	Equation	R^2	p-Value	Preferred Model
Hemocyte Concentraiton	Full	$y = -216x + 8146$	0.65	0.029	Linear
		$y = 15x^2 – 443x + 8329$	0.683	0.101	
	Low	$y = -2298x + 9173$	0.97	0.088	Linear
		$y = -4727x^2 – 2393x + 8626$	0.90	0.098	
Phenoloxidase Activity	Full	$y = -50x + 9574$	0.35	0.165	Neither
		$y = 14x^2 – 158x + 9406$	0.62	0.147	
	Low	$y = -194x + 9386$	0.05	0.729	Neither
		$y = -940x^2 – 1125x + 9277$	0.13	0.868	
Lysozyme Activity	Full	$y = -343x + 8601$	0.27	0.230	Neither
		$y = 34x^2 – 864x + 9020$	0.30	0.490	
	Low	$y = 6260x + 5811$	0.57	0.140	Neither
		$y = -6969x^2 – 13177x + 5004$	0.62	0.374	

Table 2.3. Linear and polynomial regressions for innate immunity (indicative of LNT versus hormetic models) at 24h post-irradiation. Data were analyzed with full dose range (0-15 Gy) and low dose range (0-1 Gy). Preferred model had the lowest significant p-value ($p<0.1$).

Trait	Dose Range	Equation	R^2	p-Value	Preferred Model
Hemocyte Concentraiton	Full	$y = -326x + 8377$	0.62	0.035	Linear
		$y = 16x^2 – 567x + 8571$	0.64	0.131	
	Low	$y = -2298x + 9173$	0.07	0.664	Polynomial
		$y = -4727x^2 – 2393x + 8626$	0.94	0.048	
Phenoloxidase Activity	Full	$y = -177x + 10555$	0.43	0.111	Neither
		$y = 0.740x^2 – 188x + 10564$	0.43	0.326	
	Low	$y = -721x + 10807$	0.04	0.734	Neither
		$y = -9191x^2 – 8401x + 9742$	0.60	0.409	
Lysozyme Activity	Full	$y = -251x + 7511$	0.39	0.133	Neither
		$y = 18x^2 – 524x + 7731$	0.41	0.346	
	Low	$y = 2166x + 6522$	0.20	0.447	Neither
		$y = -8643x^2 – 10744x + 5520$	0.46	0.540	

Table 2.4. Linear and polynomial regressions for innate immunity (indicative of LNT versus hormetic models) at 1-week post-irradiation. Data were analyzed with full dose range (0-15 Gy) and low dose range (0-1 Gy). Preferred model had the lowest significant p-value ($p<0.1$).

Trait	Dose Range	Equation	R^2	p-Value	Preferred Model
Hemocyte Concentraiton	Full	$y = -393x + 7902$	0.55	0.057	Linear
		$y = 47x^2 – 1101x + 8471$	0.63	0.139	
	Low	$y = -2421x + 9087$	0.18	0.478	Polynomial
		$y = -17388x^2 – 14838x + 7072$	0.91	0.089	
Phenoloxidase Activity	Full	$y = -288x + 11732$	0.59	0.043	Linear
		$y = 11x^2 – 455x + 11866$	0.60	0.160	
	Low	$y = -187x + 11750$	0.002	0.939	Neither
		$y = -7417x^2 – 7174x + 10890$	0.28	0.716	
Lysozyme Activity	Full	$y = -123x + 6357$	0.72	0.016	Linear
		$y = 17x^2 – 387x + 6569$	0.86	0.018	
	Low	$y = -34x + 6416$	0.002	0.947	Neither
		$y = -1839x^2 +1792x + 6203$	0.40	0.599	
Encapsulation Ability	Full	$y = -0.962x + 23$	0.51	0.070	Linear
		$y = -0.038x^2 – 0.393x + 22$	0.52	0.230	
	Low	$y = 9x + 18$	0.40	0.250	Neither
		$y = -36x^2 – 45x + 14$	0.90	0.133	

Table 2.5. Linear and polynomial regressions for innate immunity (indicative of LNT versus hormetic models) at 2-weeks post-irradiation. Data were analyzed with full dose range (0-15 Gy) and low dose range (0-1 Gy). Preferred model had the lowest significant p-value ($p<0.1$).

Trait	Dose Range	Equation	R^2	p-Value	Preferred Model
Hemocyte Concentraiton	Full	$y = -356x + 11888$	0.48	0.083	Linear
		$y = 40x^2 – 967x + 12380$	0.49	0.263	
	Low	$y = 406x + 11773$	0.07	0.664	Neither
		$y = -5854x^2 – 6217x + 11095$	0.78	0.219	
Phenoloxidase Activity	Full	$y = -216x + 8146$	0.75	0.011	Linear
		$y = 15x^2 – 443x + 8329$	0.86	0.021	
	Low	$y = -2298x + 9173$	0.03	0.767	Neither
		$y = -4727x^2 – 2393x + 8626$	0.60	0.405	
Lysozyme Activity	Full	$y = -61x + 5796$	0.53	0.063	Linear
		$y = 6x^2 – 166x + 5880$	0.60	0.158	
	Low	$y = 393x + 5629$	0.25	0.391	Neither
		$y = 2022x^2 – 1614x + 5864$	0.77	0.225	

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CONCLUSIONS

Important conclusions pertaining to the findings throughout this work are summarized at the end of each chapter. I presented and discussed comprehensive evidence on the impacts of ionizing radiation on life history and immunity.

Life history theory describes physiological trade-offs, which are usually thought of in terms of resource allocation. Although work on radiation hormesis have reported improved aspects of life history including longevity and growth, these 'benefits' would amount to no fitness advantage with a deficit in immune function. Prior to my work, LDIR has exhibited enhanced immune function but it is also important to assess its affects on other systems. Thus, the main objectives of my research were to investigate the impacts of gamma radiation from a multi-targeted approach and expanding on the effects of LDIR on immunity in an insect model.

Throughout this work, extensive immune tests were employed to investigate the impacts of ionizing radiation on cellular and humoral immunity in adult crickets, utilizing a range of molecular, and cellular biomarkers. Generally, LDIR stimulated immunity in adults suggesting potential hormetic effects but changes were dependent on when tests were measured post-irradiation. This only brings up the importance of performing time course studies as ionizing radiation can have complex effects depending on the target cell/system. In contrast, when assessing early life exposure, trade-offs between life history phenotypes and immunity are evident. It is remarkable that a single early-life stressful event had sustained late-life effects. Given a shift in resource allocation strategies post-maturation, trade-offs between immunity and reproduction could arise

resulting in transgenerational effects. Thus, future work should assess the effects of LDIR on both immunity and reproduction to determine potential epigenetic effects.

The digestive system and is a vital component of health and is closely linked to immunity. Future work should also consider radiation effects on the gut microbiome, which can have lasting effects on growth, development, longevity and immunity.

Collectively, these results suggest very complex, dose-dependent effects of ionizing radiation. These findings also address an important scientific question of relevance to the nuclear power industry: what are the effects of ionizing radiation at low doses? Current regulations assume a LNT model but we provided substantial evidence suggesting that biological endpoints do not necessarily exhibit a linear dose response relationship. This work will enhance our understanding of radiation effects in the environment, having implications for radiation protection policies and economics of nuclear power.