EFFECTS OF EARLY LIFE RADIATION EXPOSURE IN CRICKETS

EFFECTS OF EARLY-LIFE IONIZING RADIATION EXPOSURE ON THE LIFE-HISTORY OF THE CRICKET, *ACHETA DOMESTICUS*

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Abstract

Stressful experiences in early life can have profound and lasting impacts on phenotypic development. In anthropogenic environments, organisms are increasingly exposed to evolutionarily novel stressors that may play a major role in shaping the phenotypic variation upon which natural selection acts. For instance, ionizing radiation persists in areas affected by nuclear reactor accidents, nuclear weapons testing, and the nuclear power production process. This thesis explored the dose-response effects of early life ionizing radiation exposure on life-history traits in the cricket (*Acheta domesticus* L.). Specifically, this work had two goals: (1) to examine the effects of early life radiation stress on the potential tradeoff between growth and self-maintenance, and (2) to explore the dose-dependent effects of juvenile radiation exposure on adult trait expression (particularly fecundity, offspring investment, and offspring fitness).

It was found that exposure to moderate doses of radiation in early development resulted in a slower juvenile growth rate but increased survival in early adulthood, suggesting that self-maintenance was prioritized over growth. Despite the strong inhibitory effects of early life radiation on adult female body mass, age-specific fecundity was negatively impacted only at relatively high radiation doses. Crickets exposed to moderate radiation doses in early development laid larger eggs in adulthood relative to controls and these eggs had a greater hatching success, suggesting that radiation exposure had transgenerational effects on offspring performance. No noticeable effects of early life radiation exposure were detected on total and non-enzymatic antioxidant capacity or hydrogen peroxide levels in adult females. Together, this research indicates that a single, acute exposure to ionizing radiation in early life can affect phenotypic development in a complex, dose-dependent manner and that rather than being purely negative, phenotypic responses can be sustained or even enhanced.

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Thesis Organization and Format

This thesis is organized in a "sandwich" format, consisting of an introduction, three chapters, and a conclusion. Chapter 1 is a literature review of current concepts and knowledge in the field and provides a context for the empirical work performed in this thesis. Chapters 2 and 3 describe empirical work and are written as standalone manuscripts in preparation for future publication. The Conclusion provides a summary of the work performed in this thesis and describes directions for future research.

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List of Abbreviations

AKHs – Adipokinetic hormones

AMP – Adenosine monophosphate

AMPK – Adenosine monophosphate-activated protein kinase

ANCOVA – Analysis of covariance

ANOVA – Analysis of variance

ATP – Adenosine triphosphate

Eco-evo-devo – Ecological evolutionary developmental biology

FoxO – Forkhead box class O transcription factors

Gy - Gray

LNT – Linear-no-threshold model

NADPH - Nicotinamide adenine dinucleotide phosphate

PGC-1α - Peroxisome proliferator-activated receptor gamma coactivator 1-α

ROS – Reactive oxygen species

TOR – Target of rapamycin

Introduction

Development is not solely determined by genes, but can be influenced by environmental inputs, such as stress (West-Eberhard 2003; Sultan 2007). Broadly, developmental stress can be defined as any suboptimal condition experienced during pre- or postnatal development (e.g., exposure to extreme temperatures, food restriction, immune challenges, psychological stress, or exposure to anthropogenic factors) (Crino and Breuner 2015). Across animal taxa, developmental stress has been shown to influence life-history trajectories in complex and often predictable ways, and such effects can persist into adulthood and future generations (Burtron and Metcalfe 2014). However, the extent to which early life stress is detrimental to phenotypic development is poorly understood.

While many studies have associated early life stress with negative outcomes for physiological, morphological, and behavioral traits, a considerable body of evidence has shown that low to moderate levels of stress can sometimes elicit resilient or stimulatory phenotypic responses (reviewed in Costantini, Metcalfe & Monaghan 2010). This concept, known as hormesis (Southam and Ehrlich 1943), is well documented in the toxicological literature (reviewed in Mattson & Calabrese 2010), but we have a limited understanding of hormetic responses in a developmental and life-history context (Costantini, Metcalfe & Monaghan 2010).

Our understanding of the developmental and phenotypic effects of early life stress is particularly relevant to species living in anthropogenic environments, where pollutants can be a significant source of developmental stress. For instance, ionizing radiation is an anthropogenic pollutant affecting natural ecosystems. Concern over the ecological impacts of radiation has increased over the years following the devastating nuclear reactor disasters at Chernobyl in 1986

and Fukushima in 2011. To date, however, there are no comprehensive dose response studies of developmental radiation exposure across diverse life-history stages (e.g., growth, reproduction, maintenance, and longevity). Such research is crucial for understanding the fitness consequences of developmental radiation exposure as well as the life-history allocation strategies emerging under stress.

This work examines the effects of early life ionizing radiation exposure on developmental and life-history phenotypes in the cricket, *Acheta domesticus*. Specifically, this work addresses the following questions:

1. Does early life ionizing radiation hormesis reflect a tradeoff between growth and self-maintenance?
2. What are the dose-response effects of early life ionizing radiation exposure on adult life-history traits?

This thesis is organized in a 'sandwich' format and consists of three chapters. Chapter 1 is a literature review that provides context for the work performed by integrating various fields of relevant research. Chapters 2 and 3 are written as stand-alone manuscripts that address the research questions listed above. The Conclusion provides a summary and perspective on the work performed and suggests directions for future research.

The model system: *Acheta domesticus*

The work described in this thesis utilizes an insect model, the house cricket, *Acheta domesticus* (Orthoptera: Gryllidae). The house cricket is a hemimetabolous insect, meaning that there is no complete metamorphosis between juvenile and adult stages. After hatching, juveniles undergo consecutive moults until reaching sexual maturity. Adults can be distinguished from

juveniles by the presence of wings, and females can be identified by the presence of an elongated ovipositor (**Figure 1.1**). This species is believed to be native to the semi-arid regions of Southwestern Asia or Northern Africa, but due to its commercial use (e.g., as pet food or fishing bait), it has been distributed worldwide by humans (Ghouri 1961). In North America, wild populations exist throughout the Eastern United States and Southern California. However, most of what we know about the life-history and ecology of *A. domesticus* is from studying domesticated populations, and we know relatively little about its natural ecology, especially in geographical regions to which it has been introduced by humans (Marlene Zuk, personal communication).

Relative to mammals and birds, insects appear to be a class of animals for which there has been relatively little work examining the effects of early life stress on phenotypic development and life-histories. However, the cricket offers several advantages for conducting this type of work. For instance, *A. domesticus* has a relatively short lifespan (~120 d in the laboratory), with a juvenile period of approximately 6 weeks (Lyn *et al.* 2011), making it ideal for assessing early life interventions on adult phenotypes. Due to their small body size, crickets can be raised in large cohorts with sufficient sample sizes for dose-response studies of stress impacts. Adult females are ideal models for studies of reproductive investment, as their breeding strategy is relatively straightforward compared to many vertebrate models, which can be limited by complex breeding strategies (e.g., parental care or multiple breeding seasons) (Adamo 1999). After maturity, female crickets continuously develop eggs throughout adulthood and store them in their lateral oviducts (Woodring *et al.* 1979**)**. Once mated, females lay eggs daily in batches for several weeks. Importantly, crickets do not lay eggs unless mated, allowing for experimental

manipulation of reproductive timing. Crickets do not have parental care (Bate 1971), meaning that post-mating reproductive effort can be approximated by egg provisioning and output.

Insects are the most abundant and taxonomically diverse group in the animal kingdom. Given their crucial role in many ecosystem processes, it is important to understand the fitness impacts that environmental pollutants such as ionizing radiation might have on these organisms. Much of what we know about the effects of ionizing radiation on insects comes from pest control research, where high-dose gamma radiation is used to sterilize invasive insect species (i.e., the sterile insect technique) (reviewed by Hasan and Khan 1998).

In general, insects are a relatively radio-resistant group, due to their low levels of post-mitotic activity in adulthood (Newman 2009). However, insect radio-sensitivity tends to vary with life-history stage, and several trends have emerged regarding the effects of ionizing radiation exposure during development. First, larval stages are generally more sensitive to ionizing radiation than adults, but less so than eggs. Second, radiation sensitivity tends to vary with respect to larval stage, but larval radiation exposure is generally associated with prolonged development time and increased adult lifespan (Calabrese 2013). Third, mortality tends to be highest when insect larvae are irradiated prior to moulting. While this research provides a background for understanding how insects might be affected by ionizing radiation, relatively little work has been done to assess life-history effects in the low-dose range.

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Figure 1.1: Image of a female (left), male (centre) and ~28-day-old juvenile (right) *Acheta domesticus*.

Chapter 1

Ionizing radiation hormesis in development: an evolutionary and mechanistic perspective

Organisms are increasingly exposed to pollutants released from human activities. The degree to which anthropogenic pollutants are harmful to organisms is an important concern for biologists. For instance, ionizing radiation is a prevalent anthropogenic pollutant affecting both terrestrial and aquatic ecosystems. While life has evolved in the presence of low levels of ionizing radiation from sources such as cosmic rays and radionuclides in the Earth's crust (Schultz, Whicker & Klement 1974), background radiation levels in many geographical areas have risen significantly due to human activities. Common anthropogenic sources of radiation pollution include radioactive fallout from nuclear reactor disasters and nuclear testing for military purposes, contamination from agricultural processes and laboratory research, and radiation released from various stages of the nuclear power production process, including uranium mining (Little 2003; Tykva & Podracká 2005; Mothersill *et al.* 2013). Thus, the extent to which ionizing radiation is harmful to living organisms is an important issue relevant to environmental protection.

The degree to which radiation is detrimental to living systems is highly debated in the toxicological literature. Current guidelines for permissible radiation exposure are based on a linear-no-threshold model (LNT), which assumes that starting from zero radiation exposure, pathology increases linearly with radiation dose (Vaiserman 2010). However, the low-dose radiation risks predicted by the LNT are largely based on extrapolations of known harmful

effects at high doses rather than actual empirical evidence for low-dose harm (Calabrese 2009a). Thus, the reliability of the LNT for predicting low-dose radiation effects is highly debated among radiobiologists. In fact, a large body of empirical evidence suggests that radiobiological effects in the low-dose range often do not conform to the LNT and may be better described by an alternative model called hormesis (Calabrese *et al.* 2008; Vaiserman 2010).

The hormetic model describes a biphasic dose-response relationship characterized by reversal of the response between low and high doses of radiation exposure (typically, low-dose stimulation and high-dose inhibition). A great deal of evidence in favor of radiation hormesis suggests that the effects of low-dose radiation are often complex and may not be easily predicted by extrapolating from responses in the high-dose range. While radiation hormesis is supported by over 3000 studies (Luckey 2008**)**, most of the research on this topic has focused on human health effects (Liu 2003; Schöllnberger *et al.* 2004; Vaiserman 2010). In comparison, relatively little is known about the low-dose impacts of radiation on fitness related traits most relevant to organisms in natural ecosystems.

Early development is a time when organisms are particularly susceptible to stress, and this is also a period when hormetic responses are thought to be particularly robust (Costantini 2013, 2014; Monaghan & Haussmann 2015). For instance, developing organisms must generally trade off limited energetic resources among the competing life-history demands of growth and self-maintenance. Developmental exposures to stress may alter the balance of investment between growth strategy and self-maintenance, and this could have implications for later life-history stages (e.g., fecundity or longevity) (Costantini, Metcalfe & Monaghan 2010; Costantini 2014). Thus, early life could be a time where exposure to ionizing radiation could be particularly likely to affect organismal fitness by altering life-history trajectories.

At the proximate level, oxidative stress is thought to be a primary mechanism underlying developmental life-history tradeoffs (Dowling & Simmons 2009; Monaghan, Metcalfe & Torres 2009; Selman *et al.* 2012; Speakman & Garratt 2014). Oxidative stress is defined as the physiological imbalance between the production and removal of reactive oxygen species. While high levels of oxidative stress are damaging to living systems, low levels of reactive oxygen species have important cellular signaling properties and may serve as a mechanistic basis for hormetic responses. Given the direct linkage between ionizing radiation exposure and the production of reactive oxygen species (Koch & Hill 2017), oxidative mechanisms may be a primary mechanism underlying radiation hormesis.

This chapter considers ionizing radiation hormesis during development from an evolutionary and mechanistic perspective. Section 1 provides a review of phenotypic responses to early life stress and integrates hormesis into an evolutionary framework. Section 2 discusses the role of oxidative stress as a proximate mediator of developmental life-history tradeoffs and describes oxidative stress as a potential mechanism underlying radiation hormesis.

1.0. An evolutionary perspective on developmental stress and hormesis

1.1. Stress during early life

Development can be defined as the period that begins before conception and lasts until sexual maturation (Burton & Metcalfe 2014). It is now well established that development is not simply driven by genes, but is a result of the complex interaction between genetic background and environmental factors (Rollo 1994; Pigliucci 2001; West-Eberhard 2003; Sultan 2007). While genes set the potential range of phenotypes that can develop, environmental factors fine-tune gene expression to influence the resultant phenotype. The ability of a single genotype to

develop into different phenotypes in different environments is known as developmental plasticity (West-Eberhard 2003).

Environmental factors such as stress can influence the developing phenotype both directly and indirectly (Monaghan 2008; Monaghan & Haussmann 2015). Direct environmental factors affect the individual during post-natal development and occur from direct exposure to stressors (e.g., nutrient deprivation or toxins). Indirect environmental factors, in contrast, affect the developing individual through the parent, and usually occur through the maternal translation of stressful environmental stimuli into physiological signals (e.g., hormones, nutrient levels, antioxidants) that influence morphology, physiology, and behavior of the developing offspring (Sheriff & Love 2013).

The developmental impacts of early life stress are extremely complex and do not appear to conform to any particular type of evolved strategy (Monaghan 2008; Costantini 2014). For instance, susceptibility to the stressor may depend on the stage of development at which the stress is experienced or may vary with respect to the life-history strategy of the organism. Such impacts can be reversible or they can persist throughout the organism's lifetime (Monaghan & Haussmann 2015) and even into subsequent generations (Burton & Metcalfe 2014). In many cases, however, it is unclear whether phenotypic responses to developmental stress are pathological (i.e., beyond the coping ability of the organism) or reflect phenotypic adjustments to the stressor that could potentially have adaptive value (Monaghan & Haussmann 2015).

For instance, impaired growth during foetal development in mammals is well known to coincide with adverse health conditions later in life (e.g., heart disease, diabetes and hypertension) (Barker *et al.* 1993). These conditions occur due to maternal stress responses (e.g., overexposure to glucocorticoids) that are triggered by exposure to environmental stressors (e.g.,

food deprivation) (Benediktsson *et al.* 1993). While such effects could be considered pathological, they have also been hypothesized to reflect maternal adaptations that "program" the stress response of the developing offspring to better cope with adverse conditions in later life (Seckl 1998). Thus, in many cases, the developmental impacts of early life stress can be context-dependent and the adaptive value of such responses is often unclear.

1.2. Is developmental stress always harmful?

The extent to which early life stress is detrimental to phenotypic development is a matter of debate (Monaghan 2008; Costantini 2013; Drummond & Ancona 2015; Monaghan & Haussmann 2015). The predominant view is that exposure to stress in early life induces phenotypic deficits, and there is extensive evidence of such effects on behavioral, reproductive, and longevity related traits in birds and mammals (Monaghan 2008), including humans (Lummaa & Clutton-Brock 2002). However, the degree to which developmental stress is harmful could depend on the magnitude of the stressor, as some work has reported resilient or even stimulatory responses to moderate levels of early life stress where detrimental effects were expected (Crino *et al.* 2014; Drummond & Ancona 2015; Philips, Kobiela & Snell-Rood 2017). Such responses are consistent with a phenomenon known as hormesis (Southam & Ehrlich 1943) (Calabrese & Baldwin 2002), where low levels of stressors or toxins can have stimulatory or beneficial effects even if high levels are damaging.

Hormetic responses are well characterized in the toxicological and medical literatures (reviewed in Mattson & Calabrese 2010), but their significance in an ecological and evolutionary context is less appreciated, especially when examining the effects of early life stress on trait performance. Therefore, empirical studies that acknowledge the possibility that early life stress

can be inhibitory but perhaps also beneficial for some aspects of phenotypic development are needed to broaden our understanding of phenotypic evolution as well as to help build a framework for understanding phenotypic responses to unpredictable environments.

1.3. How can hormesis be interpreted from an evolutionary perspective?

The concept of hormesis has gained a lot of attention in the fields of toxicology and medicine largely due to evidence that low-dose stress exposures may be able to improve health or extend lives (Calabrese 2009b; Mattson & Calabrese 2010). Indeed, hormetic responses are widespread and have been noted to occur in response to over 1000 types of low-dose stressors or non-essential substances across unicellular, plant, and animal taxa. However, most work on hormesis has focused on human health (Vaiserman 2010), where the primary interest lies in the protection of individuals. An ecological approach, in contrast, focuses on the protection of populations of organisms, and this requires hormesis to be studied within the framework of evolutionary fitness (Jager, Barsi & Ducrot 2013).

The branch of evolutionary biology that aims to explain how organismal phenotypes contribute to evolutionary fitness is called life-history theory (Stearns 2000). As fitness is not a singular concept but consists of multiple interacting components, an organism's life-history strategy is defined by the age-or stage-specific patterns of major fitness related traits occurring over the lifespan (e.g., size at birth, age and size at maturity, fecundity, and longevity). Classic life-history theory recognized that since organisms cannot simultaneously maximize all aspects of their fitness (e.g., produce unlimited offspring or grow indefinitely large), the evolution of organismal life-history strategies must be subject to constraints (Stearns 1992; Roff 1992**)**. These constraints arise from the fact that organismal energy resources are limited and must therefore be

traded off among competing life-history traits (e.g., growth vs. self-maintenance). Therefore, life-histories are assumed to have been shaped by natural selection to achieve a balance of resource allocation among competing life-history traits in such a way that optimizes fitness (Zuk & Stoehr 2002). Thus, understanding the way in which early life stress impacts organismal fitness should come from studying its effects on life-history allocation strategies.

1.3.1. Hormesis and fitness

From a life-history perspective, hormesis raises an interesting problem. The idea that low levels of stress can have stimulatory effects on some aspects of organismal performance might suggest that hormesis can improve organismal fitness (Forbes 2000; Jager *et al.* 2013). However, this would imply that fitness is generally suboptimal, which is at odds with the assumption that life-histories are in evolutionary equilibrium. This is the basis for Forbes (2000) argument that although hormetic stimulation of life-history traits can occur, this should not be expected to translate into an overall fitness increase, as such stimulation should be counterbalanced by reductions in other fitness components.

The idea that hormetic responses result in fitness-related tradeoffs has been supported by a handful of studies (Maynard Smith 1958; Krebs & Loeschcke 1994; Sørensen *et al.* 2007; Mcclure *et al.* 2014), although some work has reported that hormetic stimulation of life-history traits can occur without apparent tradeoffs (Cutler 2009; Le Bourg & Rattan 2014). It is important to note, however, that it may not always be possible to identify tradeoffs associated with hormetic responses, since costs may arise in traits, life-history stages, or under specific environmental conditions that are not examined by the experimenter (Jager *et al.* 2013). This illustrates the importance of monitoring multiple traits and time points when investigating the life-history consequences of hormesis.

Other work has suggested that costs of hormetic responses can be context-specific. Support for this comes from a study in zebra finches showing that individuals exposed to mild heat stress in early life were better able to endure exposure to more severe heat stress later in life compared to birds that did not receive the early life priming (Costantini, Monaghan & Metcalfe 2012). However, birds receiving the early life mild stress but not the adult stress treatment displayed lower survival than birds that received both treatments (Costantini, Monaghan & Metcalfe 2014). This study provides support for the idea that hormetic responses could be associated with phenotypic adjustments that prime the organism to withstand stressful conditions at a later period, but in the absence of environmental stress, such adjustments could be costly. This is consistent with the concept of the plasticity-history limit (Auld, Agrawal & Relyea 2010), whereby phenotypic changes induced by environmental conditions in early ontogeny are irreversible and therefore constrain the potential for plasticity in adulthood. This limit is thought to be a primary constraint on the evolution of phenotypic plasticity, and in a similar manner, may also constrain the evolution of hormetic responses (Costantini *et al.* 2012).

The idea that hormetic responses might be associated with physiological tradeoffs or that they might impose fitness costs under certain environmental contexts exposes an important point about hormesis as a general phenomenon: hormetic responses need not be beneficial (Costantini *et al.* 2010; Costantini 2014). Given that hormetic responses are often observed in important fitness- or health-related traits (e.g., growth, DNA repair, or longevity), this important point is often overlooked. Additionally, many descriptions of hormesis often associate the phenomenon with the term "adaptive" (Mattson & Calabrese 2010). However, "adaptiveness" in an evolutionary sense refers specifically to a trait that has a positive effect on organismal fitness in a given environmental context (Williams 1966; Sober & Wilson 2011). Thus, a stimulatory

hormetic effect must only be considered adaptive under environmental contexts in which the effect provides the organism with a fitness benefit.

1.3.2. Evolution of hormetic mechanisms.

The idea that hormetic responses may be costly raises another important evolutionary question about hormesis: why did it evolve? This question has been addressed by several authors. Calabrese and Baldwin, for instance, wrote "the hormetic phenomenon response is a common, evolutionarily-based strategy to carefully regulate resource allocation in a definable range within the context of the re-establishment and maintenance of homeostasis (Calabrese and Baldwin 2002, p. 333)." Parsons (2001) addressed this issue by arguing that "[hormesis] derives from metabolic reserves that are maintained as an adaptation to environmental stresses through evolutionary time (Parsons 2001, p. 459)". While these are interesting hypothesis, they are limited by one crucial factor: a lack of mechanistic clarity.

In fact, as pointed out by Thayer *et al.* (2005), many evolutionary explanations for hormesis have been solely based on empirical evidence of the hormetic dose-response relationship across taxa (i.e., the hormetic performance of traits themselves), with little concern for the mechanistic basis of these phenomena. However, understanding the mechanistic details underlying a biological trait is crucial for understanding its evolutionary significance. Just as an engineer might try to determine the purpose of a newly discovered piece of machinery by studying its component parts and figuring out how they operate together, biologists are faced with a similar task when trying to determine the evolutionary origins of a trait (Shephard *et al.* 2016). Thus, investigations attempting to determine why hormetic responses might have evolved must do so by studying the underlying mechanisms of the response in sufficient detail to understand how they might contribute to a particular functional role.

An additional point of confusion surrounding evolutionary explanations for hormesis is whether hormetic responses can be explained by a single underlying mechanism or whether multiple types of mechanisms can give rise to hormetic responses. This highlights the importance of distinguishing between two key concepts: hormesis as a dose-response phenomenon and hormetic mechanisms. The hormetic dose-response phenomenon is only a description of the biphasic dose-response curve (i.e., low-dose stimulation and high-dose inhibition) and is therefore independent of any trait or underlying mechanism. Hormetic mechanisms, as described above, must be responsible for producing a trait's hormetic response and are physiological in nature. Given that hormetic responses have been observed for a wide variety of traits (e.g., growth, reproduction, longevity responses) in highly divergent taxa and in response to hundreds of different types of stressors, hormesis should not necessarily be expected operate through a single evolved biological mechanism.

2.0. Radiation hormesis and life-history traits: a potential mechanistic basis

2.1. Oxidative stress as a potential mechanism underlying radiation hormesis

Given the distinction between hormetic dose-response relationships and their underlying mechanisms, we can now consider possible mechanisms through which ionizing radiation might induce hormetic effects on life-history traits. One likely mechanism that could underlie hormetic responses to radiation is oxidative stress. Oxidative stress is a key adaptive challenge for aerobic organisms, as they are constantly threatened by the potentially damaging effects of reactive oxygen species (ROS) (Finkel & Holbrook 2000). ROS are endogenously produced in response to metabolic challenges (e.g., temperature stress, exercise, or activation of the acute stress response), and if not controlled they can cause damage to biomolecules including DNA, lipids,

and proteins (Dotan, Lichtenberg & Pinchuk 2004). To avoid this, organisms have evolved a complex antioxidant defense system consisting of both enzymatic and dietary antioxidants. While any imbalance between ROS production and their removal by antioxidants might be expected to be damaging, low levels of ROS are known to act as important signaling molecules that regulate biological functions such as antioxidant production, repair mechanisms, and growth processes (D'Autréaux & Toledano 2007; Ristow & Zarse 2010; Ristow 2014). In this regard, low levels of ROS can have hormetic properties.

Here, I will introduce the known relationship between ionizing radiation and oxidative stress production, and then I will review some evidence in support of the hypothesized role of oxidative stress as a proximate mediator of life-history tradeoffs. Finally, I will describe some oxidative stress mechanisms that might be expected to underlie hormetic effects of low-dose radiation on life-history traits.

2.1.1. Ionizing radiation and oxidative stress

Historically, radiation biology has been dominated by the belief that the primary biological target of ionizing radiation is DNA (Sax, 1938; Elkind and Whitmore 1967). However, this view is rapidly changing, largely due to the more recent appreciation of the importance of biological effects of radiation that do not involve direct DNA damage (Mothersill & Seymour 2012). Oxidative stress may be a primary mechanism through which radiation elicits such effects. The relationship between ionizing radiation and ROS production is direct and occurs through the ionization of water, which makes up most of the molecules within animal cells (Riley 1994). Ionized water rapidly reacts with other water molecules to form ROS such as the superoxide radical, hydroxyl radicals, and hydrogen peroxide. The relationship between ionizing radiation and ROS production broadens our understanding of radiobiology from a

simplified DNA damage model to a paradigm involving physiological and regulatory systems responsive to oxidative stress (Rollo 2006**)**. Recent ecological evidence supporting a link between radiation and oxidative stress comes from a meta-analysis showing that both oxidative damage and antioxidant responses were significantly associated with chronic low-dose radiation exposure in animals from areas containing radioactive fallout from nuclear accidents (Einor *et al.* 2016).

Additionally, some evidence suggests that antioxidant systems in bird species from the Chernobyl region show signs of long-term adaptation to chronic low-dose radiation exposure (Galván *et al.* 2014). Thus, it seems possible that oxidative stress mechanisms could be associated with many of the physiological, morphological and life-history changes observed in animals from these areas (for review, see Møller and Mousseau 2006).

2.1.2. Oxidative stress during development

As described in section 1.3, classic life-history research aimed to explain variation in life-history strategies by understanding the nature of tradeoffs between life-history traits (e.g., reproduction and longevity). Although classic theory recognized that the currency being traded off among competing traits must have some sort of physiological basis (Stearns 1992), there was little consideration as to what these mechanisms could be. However, over the past couple of decades, it has become increasingly recognized that identifying the proximate mechanisms underlying life-history tradeoffs is crucial to understanding the diversity of life-history strategies that have evolved (Zera & Harshman 2001; Flatt & Heyland 2011).

Over the past decade, oxidative stress has emerged as a candidate physiological mechanism underlying life-history tradeoffs (Dowling & Simmons 2009; Monaghan *et al.* 2009; Costantini 2014). The basis for this hypothesis is that oxidative stress generated as a byproduct

metabolic processes (e.g., growth and reproduction) should have negative consequences for other fitness components (e.g., survival) and may therefore serve as a mechanistic basis for many of the tradeoffs studied by classic life-history theory (e.g., the tradeoff between reproduction and longevity). Although complex antioxidant systems have evolved to prevent oxidative damage, antioxidant production itself is costly and must be balanced against investment in other traits. Therefore, the fitness consequences of oxidative stress critically depend on the extent to which cumulative oxidative damage ultimately constrains the organism's lifetime reproductive success (i.e., by negatively affecting fitness components such as growth strategy, reproductive output, or longevity) (Monaghan *et al.* 2009).

Several lines of evidence have implicated a role for oxidative stress as a key mediator of life-history tradeoffs during development. For instance, ROS damage has been recognized as a cost of rapid or compensatory growth, suggesting that it could play a role in mediating the tradeoff between growth and self-maintenance (Costantini 2014). In zebra finches, for example, compensatory growth following a period of reduced growth rate was associated with greater oxidative damage (Alonso-Alvarez *et al.* 2007), and antioxidant supplementation allowed birds to achieve a higher growth rate without suffering greater oxidative damage (Hall *et al.* 2010). Additionally, mice with overexpressed growth hormone grew twice as fast but had a reduced lifespan, possibly due to their elevated levels of oxidative damage that had accumulated during growth (Rollo *et al.* 1996). Indeed, cross-species comparative studies in reptiles (Bronikowski 2008), birds (Galvan *et al.* 2012), and mammals (Brunet-Rossinni 2004) have demonstrated that increased resistance to oxidative stress tends to be associated with life-history strategies characterized by slow growth and extended longevity.

Evidence from across animal taxa also suggests that development could be a time of particularly high susceptibility to oxidative stress. This is supported by demographic studies of bird (Alonso-Alvarez *et al.* 2006) and mammal (Isaksson *et al.* 2011) populations showing that total antioxidant capacity tends to be lowest in early and late life age groups, but relatively higher in early adulthood. In reptiles, enzymatic antioxidant levels have been shown to increase from the hatchling to juvenile stage, but stabilize by early adulthood (Hermes-Lima *et al.* 2012). Ontogenetic changes in enzymatic antioxidant activity have also been noted in amphibians (Rizzo *et al.* 2007), fish and crustaceans (Fontagné *et al.* 2008). While these studies demonstrate that development appears to be a time of lower antioxidant activity across species, the degree to which this translates into increased susceptibility to oxidative stress is unclear.

Oxidative stress during development has also been shown to influence adult life-history traits, although most of this research has been conducted in birds. For instance, pre-fledgling oxidative damage levels in the European shag (*Phalacrocorax aristotelis*) were positively associated with reduced fecundity in adulthood (Noguera, Kim & Velando 2012). In another study, Alonso-Alvarez *et al.* (2006) demonstrated that zebra finches raised in crowded broods during early life delayed their age at first reproduction, and this was associated with increased longevity and reduced fecundity. Importantly, survival was associated with increased resistance to oxidative stress. Together, these results suggest that brood size in early life can influence life-history strategies in adulthood partly through mechanisms involving oxidative stress physiology.

2.1.3. Mitochondrial hormesis as a potential mechanism underlying hormetic effects of radiation on life-history traits

Multiple stressors are known to exert their hormetic effects via mechanisms involving ROS production in the mitochondria (Schulz *et al.* 2007; Ristow & Zarse 2010; Ristow 2014).

Low levels of ROS produced by the mitochondria serve as important signaling molecules that activate organismal stress responses. This phenomenon is known as mitochondrial hormesis (hereafter, mitohormesis). Given the direct linkage between ionizing radiation and oxidative stress, it seems likely that radiation hormesis could also operate through this type of mechanism. In this section, some common mitohormetic mechanisms are discussed, followed by an explanation of their potential relevance to radiation hormesis of life-history traits.

One of the most well described mitohormetic mechanisms is the AMPK-FOXO pathway, which is a highly conserved mediator of life extension responses to mild stressors in animals (Greer *et al.* 2007). This pathway is activated by adenosine monophosphate-activated protein kinase (AMPK), which is a central regulator of energy metabolism that is highly sensitive to changes in cellular energy levels. Under stressful conditions, such as exercise or caloric restriction, when cellular energy levels decline (i.e., the AMP:ATP ratio is high), AMPK inhibits energy consuming processes by deactivating various cell signaling pathways involved in growth. One of the key growth pathways inhibited by AMPK is the target of rapamycin pathway (TOR), which regulates protein synthesis (Wullschleger, Loewith & Hall 2006). When these growth pathways are downregulated, AMPK acts to restore cellular energy balance by activating pathways involved in generalized stress-resistance.

One of the primary ways in which this occurs is through the AMPK-mediated production of NAD+ metabolites (Cantó *et al.* 2009). These metabolites are intimately involved in redox processes and lead to the production ROS signaling molecules in the mitochondria. ROS signals are necessary for the activation of factors that initiate the transcription of stress response genes. One of the primary families of transcription factors activated through ROS signaling mechanisms is the forkhead transcription factors (FOXO) (Brunet 2004). FOXO upregulates numerous stress

responses, including the production of antioxidant enzymes, detoxification enzymes, heat shock proteins, and activation of the mitochondrial unfolded protein response. These responses enhance organismal stress resistance and therefore contribute to increased health and longevity.

Another important mitohormetic pathway mediated by ROS signaling involves the activation of mitochondrial biogenesis. This process also occurs in response to energetically stressful situations and functions to homeostatically restore ATP by increasing cellular mitochondria levels (Cantó & Auwerx 2009). Similar to FOXO activation, mitochondrial biogenesis begins when AMPK is activated in response to energy deficiencies. AMPK stimulates ROS production in the mitochondria, and ROS signaling activates a protein called peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1alpha), which is considered the master coordinator of molecular biogenesis (Fernandez-Marcos & Auwerx 2011). PGC-1alpha activates a family of transcription factors called the nuclear respiratory factors (NRF), which initiate mitochondrial biogenesis.

The direct involvement of ionizing radiation in ROS production suggests that ROS-mediated mitohormertic pathways may provide a mechanistic basis for radiation-induced hormesis of life-history traits. Indeed, some of the most commonly reported hormetic effects of ionizing radiation include increased longevity (Mitchel, Jackson & Carlisle 2004; Cameron 2005) and increased resistance to subsequent stressors of greater severity (Boreham & Mitchel 1991; Azzam *et al.* 1996). Both responses are consistent with FOXO-mediated stress resistance, suggesting involvement of a mitohormetic mechanism. This possibility should be explored by future research.

Other evidence has suggested a link between ionizing radiation and mitochondrial biogenesis. For instance, in human lung carcinoma cells, ionizing radiation exposure was shown

to increase mitochondrial ROS production, respiration, membrane potential, and ATP production (Yamamori *et al.* 2012). These changes were associated with increased cellular mitochondrial mass and DNA levels, suggesting that radiation-induced upregulation of ATP resulted from mitochondrial biogenesis. While a direct link between mitochondrial biogenesis and hormesis on life-history traits has not been reported, this could be a potential mechanism explaining why life-history tradeoffs sometimes do not occur when expected.

For instance, several studies that have attempted to demonstrate that oxidative damage is a cost of reproductive investment (and therefore might mediate the tradeoff between reproduction and longevity) have counterintuitively shown that breeding animals have lower oxidative damage levels compared with non-breeding animals (reviewed in, Metcalfe & Monaghan 2013; Speakman and Garratt 2014). These results have lead several authors to argue that assuming reproductive effort is stressful, breeding could mitigate oxidative stress by activating mitohormetic mechanisms (e.g., by repair increasing repair or energetic capacity through mitochondrial biogenesis) (Zhang and Hood 2016; Alonso-Alvarez *et al.* 2017). While this hypothesis remains untested, it does suggest that in some cases mild stress might be able to prevent expected life-history tradeoffs by increasing respiratory capacity.

3.0. Conclusion

Ionizing radiation pollution from human activities is a significant concern for species living in natural environments, and it is becoming increasingly important to understand the fitness effects of radiation exposure. This introductory literature review has integrated ionizing radiation hormesis into an evolutionary and developmental framework. Emphasis was placed on early life exposures to low levels of stress and the potentially important role that hormesis can

play in facilitating life-history plasticity. I also discussed the importance of understanding hormetic responses from not only the organismal level, but also the underlying mechanisms giving rise to these effects. As the field of radiation ecology continues to develop, future work should explore the mechanisms giving rise to the complex dose-dependent effects radiation might have on fitness-related traits. Given the intimate relationship between ionizing radiation and oxidative stress, coupled with the strong theoretical and empirical evidence for oxidative stress as a proximate mediator of life-history strategies, oxidative mechanisms provide a promising avenue for exploring the mechanistic bases of the developmental and life-history effects of ionizing radiation in animals.

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Chapter 2

Does hormesis associated with juvenile ionizing radiation reflect a tradeoff between growth and self-maintenance?

Abstract

Exposure to low levels of ionizing radiation are well known to have stimulatory effects on organismal fitness components such as growth, repair, and stress-resistance. This concept, known as hormesis, is well described in the toxicology literature, but the evolutionary relevance of such responses has received relatively little attention. In particular, it is often unclear whether hormetic responses of fitness-related traits are cost-free or result in tradeoffs with other fitness components. During development, organisms generally face a tradeoff between investment in growth processes and self-maintenance (i.e., defense, repair, and survival). This study aimed to test whether early life ionizing radiation hormesis reflects a tradeoff between these two fitness components. Using the short-lived cricket (*Acheta domesticus*) as a model system, we show that exposure to moderate doses of ionizing radiation in early juvenile development resulted in higher mean survivorship but a slower growth rate relative to controls. This indicates that early life radiation hormesis inflicted a cost, and this may reflect a shift in life-history strategy in which self-maintenance is prioritized over growth.

Introduction

For species in anthropogenic environments, pollutants released from human activities can be a significant source of stress and may have drastic fitness impacts. Ionizing radiation is a predominant anthropogenic pollutant affecting natural ecosystems. Ionizing radiation exists in the form of high energy rays (e.g., α-, ß-, or γ-rays) generated through the decay of radioactive nuclides (Sobolev *et al.* 1994), which can be released into the environment through human activities such as nuclear weapons testing, nuclear reactor accidents, and various stages of the nuclear power production process (e.g., uranium mining) (Tripathi *et al.* 2012; Mothersill *et al.* 2013).

The primary biological effect of ionizing radiation is the production of reactive oxygen species (ROS). This occurs when ionizing rays eject outer orbital electrons from water molecules within cells, leading to the propagation of highly reactive compounds such as hydrogen peroxide, hydroxyl radicals, and superoxide anions (Lane 2002) (Riley 1994; Koch & Hill 2017). If not controlled by exogenous protection mechanisms (e.g. enzymatic or dietary antioxidants), ROS can result in oxidative damage to biomolecules such as proteins, DNA, and lipids, and this can have detrimental effects on cellular and organ function (Dotan, Lichtenberg & Pinchuk 2004). Given the potential dangers of ionizing radiation pollution in natural ecosystems, there is a significant need to understand its fitness impacts.

The negative fitness consequences of high-dose ionizing radiation exposure are unambiguous and occur at all levels of biological organization, from molecules to life-history traits. Common effects of radiation damage include DNA strand breakage, chromosome aberrations, protein damage, and early death (Sudprasert *et al.* 2006; Won & Lee 2014). However, relatively low doses of ionizing radiation can stimulate what are known as stimulatory

or hormetic responses (Luckey 2008; Vaiserman 2010), suggesting that in some cases, the fitness impacts of low-dose radiation exposure may be positive.

In animals, hormetic effects of ionizing radiation often include stimulation of traits related to self-maintenance processes, such as enhanced DNA repair (Boreham & Mitchel 1991), increased longevity (Cameron 2005; Calabrese 2013), and activation of physiological systems that prime the organism to better withstand subsequent exposure to more severe stressors (Costantini 2013). Ionizing radiation has also been shown to have hormetic effects on growth-related traits in both invertebrates and vertebrates. For instance, in populations of *Daphnia*, continuous low-dose gamma radiation exposure was shown to increase the intrinsic rate of population growth, and this was associated with higher individual growth rates (Marshall 1962).

Radiation-induced stimulation of juvenile growth rate has also been reported in the blue crab (Engel 1967) and southern toad (Stark *et al.* 2015). The mechanisms responsible for radiation-induced growth hormesis are not known, but it has been suggested that this could reflect a compensatory response analogous to "catch-up" growth commonly observed in animals following periods of food restriction (Johnston, Bower & Macqueen 2011; Lee, Monaghan & Metcalfe 2012a).

From a life-history perspective, the seemingly positive fitness impacts of hormesis on traits such as growth and self-maintenance raise an interesting theoretical problem. Our understanding of life-history evolution is based on the principle that there are tradeoffs among fitness-related traits such as growth, maintenance, and reproduction (Stearns 1992; Roff 1992). Thus, any increased investment in one life-history trait should result in reduced investment in another trait, and this explains why organisms cannot simultaneously maximize all aspects of their fitness (e.g., grow indefinitely large or produce unlimited offspring) (Zuk & Stoehr 2002).

The idea that there are tradeoffs among competing life-history traits would therefore suggest that the hormetic stimulation of one fitness component should be expected to result in a tradeoff with another. While this has been suggested theoretically (Forbes 2000; Costantini, Metcalfe & Monaghan 2010; Jager, Barsi & Ducrot 2013), there have been relatively few experimental tests of whether hormesis is associated with life-history tradeoffs (but see McClure *et al.* 2014; Saul *et al.* 2013).

Early juvenile development is thought to be a time when hormetic responses are most robust (Yahav & McMurtry 2001; Burger *et al.* 2007; Costantini 2013). For instance, developing organisms must generally face a tradeoff in resource allocation between growth and self-maintenance, and it has been suggested that the hormetic stimulation of one of these traits could reflect one side of this tradeoff (Costantini 2014). Indeed, experimental manipulations of growth trajectories in animals have revealed maintenance costs of increased growth rates, supporting a general tradeoff between growth and self-maintenance. For instance, compensatory growth following periods of experimentally induced growth restriction have been shown to result in such costs as reduced longevity (Lee *et al.* 2012a), reproductive performance (Lee, Monaghan & Metcalfe 2012b), starvation resistance (Gotthard 2008), and locomotor ability (Lee, Monaghan & Metcalfe 2010), as well as increased oxidative stress (De Block & Stoks 2008).

Additionally, exposure to mild heat stress in early juvenile development conferred enhanced resistance to severe heat stress later in life (a hormetic response) (Costantini, Monaghan & Metcalfe 2012). However, if the subsequent heat shock was not experienced, animals receiving the initial mild heat stress showed poorer survival than untreated controls (Costantini, Monaghan & Metcalfe 2014). This suggests that in some cases, hormetic responses that involve increased investment in self-maintenance processes may have a positive effect on

fitness only if the stress is subsequently encountered, but may carry costs that arise under environmental mismatch.

In this study, we aimed to broaden our understanding of the fitness consequences of hormetic responses by testing the hypothesis that a hormetic response to ionizing radiation exposure during the early stages of organismal development reflects a tradeoff between growth and self-maintenance. To test this, we made use of a model with a relatively short juvenile development time, the house cricket (*Acheta domesticus*). We employed a dose-response approach, where populations of juvenile crickets were exposed to single dose of ionizing radiation in early juvenile development (14 d of age), and each population was monitored over the lifetime for juvenile growth rate and survivorship.

Methods

Breeding Colony

All experimental *Acheta domesticus* originated from a genetically heterogeneous breeding colony housed in an acrylic terrarium (93 x 64.2 x 46.6 cm) maintained at 30± 1°C with a 12 h light/12 h dark photoperiod. The colony was enclosed with 1.5-cm-thick Durofoam® to provide insulation. To prevent escape and provide ventilation, the top of the terrarium was covered with 1 mm^2 plastic mesh. Chicken feed (Quick Feeds©) and dechlorinated water (provided in soaked cellulose sponges) were provided *ad libitum*, and habitat features consisted of egg carton shelters and moist paper towels. Oviposition medium consisted of Pro-mix® soil provided in shallow plastic containers.

Experimental groups

At 14 days of age, juvenile *Acheta domesticus* were isolated from the breeding colony and were separated into 7 experimental groups. Each group was exposed to one of the following doses of ionizing radiation: 0.5, 1, 2, 4, 7, 10, and 0 Gray (Gy) (n = 180 per group), or 7 Gy (n = 90). Irradiation took place at the Taylor Radiobiology Source at McMaster University (dose rate = 0.25 Gy/min). Immediately following irradiation, crickets were housed in plastic containers (30x19x12cm; n = 45 per container) and maintained at 30˚C on a 12h light/12h dark photoperiod with egg carton shelters, water-soaked cellulose sponges, and *ad libitum* access to a diet of ground guinea pig food (*Little Friends*^{TM}: 16.5% crude protein, 4% crude fat and 19% crude fiber) mixed with deionized water (3g of food per 8 mL of water).

Mass-specific feeding rate

Differences in growth rates among radiation treatment groups could partly reflect variability in mass-specific food consumption. Food consumption was recorded throughout the juvenile period. For each radiation treatment group, feeding rate of the whole population was calculated 2-3 times per week over the juvenile period. Mass of crickets in each population was recorded before and after each feeding period. Repeated measurements of mass-specific food consumption over the entire juvenile period in each radiation treatment group were calculated and reported as: dry food mass consumed (mg)/cricket mass (mg) /day.

Juvenile growth rates and survivorship

Measurements of juvenile growth were recorded by weighing crickets four times following irradiation (24, 28, 32, and 36 days of age) in groups of 6 (crickets were assigned to groups randomly). At the mature molt (recognized by the full development of wings and sexual organs), juvenile development time (days) and maturation mass (mg) were recorded (see

supplementary **Figures S2.1 – S2.6** for dose-response relationships for mass at maturity and juvenile development time). Juvenile growth rate (calculated as the quotient of maturation mass and maturation age) provided an estimate of mass gain (mg/day) across juvenile development. Mature males and females were housed in separate containers to prevent mating. Mortality was recorded daily throughout the duration of the experiment.

Statistics

For growth rate and mass-specific feeding rate, all results are presented as mean +/- standard error. Prior to analyses, all data were checked for normality using the D'Agostino-Pearson omnibus normality test. All data were analyzed using one-way ANOVA followed by a Tukey's HSD post-hoc test. Criterion for statistical significance was $p < 0.05$. For survival analyses, the Gehan-Breslow-Wilcoxon test was applied to test for differences in survivorship between the 0 Gy treatment and each of the radiation dose treatment groups. Maximal longevity was analyzed using ANOVA on the average age of the last 6 remaining crickets (10% of the initial population) in each radiation treatment group. All statistical analyses were conducted using Prism Graph Pad.

Results

Juvenile growth

Juvenile growth rates were calculated for males and females separately, as well as the sexes combined. For males and females combined (**Figure 2.1**), growth rates did not change significantly throughout the 0 – 1 Gy dose range. However, in the 2, 4, 7, and 10 Gy treatment groups, growth rates were approximately 10% ($p = 0.01$), 11% ($p = 0.0024$), 23% ($p < 0.0001$), and 45% ($p < 0.0001$) slower than controls respectively. For females (**Figure 2.2**), growth rate

was unaffected throughout the 0 – 4 Gy dose range. However, females in the 7 and 10 Gy treatments grew significantly slower than 0 Gy females by 33% ($p < 0.0001$) and 48% ($p < 0.0001$) respectively. Male growth rates did not differ in the 0 – 2 Gy dose rage (**Figure 2.3**), but were significantly lower at the 4, 7, and 10 Gy doses by 13% ($p = 0.033$), 27% ($p < 0.0001$), and 43% ($p < 0.0001$) respectively.

Survival and Longevity

Relative to the 0 Gy treatment group, the Gehan-Breslow-Wilcoxon test established that mean survivorship was significantly higher in the 4 ($p = 0.033$) and 7 Gy ($p = 0.049$) treatment groups. Survivorship in the 2 Gy treatment was also higher than the 0 Gy group but the difference was not significant ($p = 0.077$). In each of these three comparisons, the largest separation in survival appeared to occur within the 50 – 125d period (**Figure 2.4b**). ANOVA followed by a post-hoc Tukey's HSD test on the average age of the last six surviving individuals from each radiation treatment group revealed that the 7 Gy group had a ~10% greater maximal longevity than the 0 Gy group, but this difference was marginally significant ($p = 0.067$).

Mass specific feeding rate

No differences in mass specific feeding rate were detected among any of the radiation treatment groups (**Figure 2.5**).

Discussion

The idea that low levels of stressors or toxins can have positive impacts on performance through hormesis has long fascinated biologists (Southam and Ehrlich 1943; Calabrese and Baldwin 2002; Costantini 2014), but its relevance to evolutionary fitness has been a point of controversy (Forbes 2000; Thayer *et al.* 2005; Jager *et al.* 2013; McClure *et al.* 2014; Le Bourg

and Rattan 2014). For instance, life-history theory is established on the assumption that due to internal resource limitations, any increase in one fitness-related trait must exact a cost that is "paid for" in terms of reduced investment in other traits (Stearns 1992). This would imply that hormetic trait responses to low-dose stressors might not reflect an overall increase in fitness *per se*, but rather a reallocation of limited energetic resources in which certain fitness components are prioritized over others.

In this study, we tested the hypothesis that hormetic responses to ionizing radiation exposure in early juvenile development reflect a tradeoff between growth and self-maintenance. Using *A. domesticus* as a model system, we found that crickets exposed acutely to ionizing radiation in early development in the dose range of 2 – 7 Gy had a significantly higher mean survivorship relative to non-irradiated controls (**Figure 2.4b**). However, this hormetic effect on survivorship was associated with a slower juvenile growth rate (**Figure 2.1**). Consistent with a general tradeoff between growth and self-maintenance, our results suggest that crickets responded to early life radiation stress by prioritizing investment in survivorship but at a cost to growth rate. Here, we discuss the possible ultimate and proximate explanations for these results and describe directions for future research.

Our results are consistent with previous work showing that ionizing radiation exposure during early juvenile development can have hormetic effects on self-maintenance related life-history traits. For instance, early life radiation exposure has been shown to extend lifespan in several insect taxa, including Diptera (Dauer 1965; Willard 1965), Lepidoptera (White and Hutt 1970), and Orthoptera (Menhinic & Crossley 1968). This is consistent with our finding that crickets subjected to the 7 Gy radiation dose in early life had a ~10% higher maximal longevity than controls (**Figure 2.4c**). Previous work in insects has also shown that increased lifespan

following juvenile radiation stress is associated with prolonged larval development, consistent with the idea that hormetic effects on self-maintenance processes could occur as a consequence of reduced growth rates (for review, see Calabrese 2013). Given that juveniles tend to be more susceptible than adults to ionizing radiation across insect taxa (Hasan and Khan 1998), investment in self-maintenance at the expense of growth could be a general strategy employed by insects to respond to developmental radiation stress.

Previous work has also reported increased longevity in insects exposed to moderate doses of ionizing radiation in adulthood (Calabrese 2013). In *A. domesticus* particularly, moderate levels of radiation exposure in adulthood were shown to increase longevity in females but not males (Hunter and Krithayakiern 1971). Interestingly, life extension in females was associated with reduced fecundity, suggesting that this response could reflect an increase in energy available for self-maintenance processes resulting from reproductive impairment. In our results, the hormetic increase in mean survivorship observed in crickets experiencing early life radiation was not sex-specific. This raises the possibility that the nature and strength of hormetic responses may differ with respect to the stage of the life-history at which the stressor is received.

Why should individuals respond to early life stress by upregulating self-maintenance processes if this can be costly to other fitness components such as growth? One possible explanation for why a strategy such as this should evolve comes from the environmental matching hypothesis (Love & Williams 2008; Chaby 2016) (also referred to as conditioning hormesis, *sensu* Calabrese and Baldwin 2002). This hypothesis proposes that stressful experiences in early development should induce phenotypic changes that prime the organism to better endure stressful environments later in life. The main constraint on the evolution of such a

response is that it should be costly under environmental mismatch (e.g., when the adult environment is not stressful).

This likely explains why this phenotypic response requires a priming process to occur (Costantini *et al.* 2010; Costantini 2013). Support for this hypothesis comes from previous research demonstrating that organisms experiencing mild stress in early life are more resistant to multiple types of stressors later in life (Le Bourg & Minois 1997; Le Bourg 2005). Although we did not test the environmental matching hypothesis directly, the increased survival observed in the groups experiencing moderate early life radiation doses suggests that these individuals were likely more stress-resistant. Future work could address this environmental matching hypothesis directly by testing whether crickets experiencing the early life radiation stress do better after receiving a subsequent exposure relative to untreated controls.

We did not investigate the proximate mechanisms responsible for the life-history effects of developmental radiation exposure observed in our study. However, given the direct relationship between ionizing radiation and ROS production, it seems likely that these effects could be partly attributed to mechanisms involving oxidative stress physiology. In recent decades, oxidative stress has emerged as a primary physiological constraint mediating life-history tradeoffs, including the tradeoff between growth and self-maintenance (Monaghan, Metcalfe & Torres 2009; Metcalfe & Alonso-Alvarez 2010; Costantini 2014). Early development is a period when oxidative stress levels are high, since ROS are generated as a byproduct of elevated metabolic activities required for growth (Alonso-Alvarez *et al.* 2007; De Block & Stoks 2008; Hall *et al.* 2010).

To protect tissues from oxidative damage, organisms must invest in self-maintenance processes, such as antioxidant production. Thus, it has been suggested that increased growth rate

may be constrained by the need to invest in antioxidant protection or other repair mechanisms to maintain somatic function (Metcalfe & Alonso-Alvarez 2010; Costantini 2014). It might therefore be expected that exposure to oxidative challenges during development may disrupt growth trajectories by shifting allocation away from growth and towards antioxidant production. The need to prioritize self-maintenance over growth in this way could potentially explain the tradeoff between survivorship and growth rate we observed in treatment groups exposed to moderate levels of early life radiation.

Early life radiation exposure may also mediate a tradeoff between growth and self-maintenance by activating highly conserved cellular signaling pathways responsive to oxidative stress. Across animal taxa, diverse types of stressors (including caloric restriction and ROS) converge on a group of transcription factors known as the Forkhead box class O transcription factors (FoxO) (Eijkelenboom. & Burgering 2013). In its inactive form, FoxO is present in the cytosol, but under conditions of oxidative stress, FoxO is translocated to the nucleus of the cell to initiate transcription of diverse stress response genes, including genes coding for antioxidant enzymes Brunet 2004).

In insects, translocation is thought to be initiated by adipokinetic hormones (AKH), which are a primary class of stress response hormones released from the brain in response to ROS stress (Krishnan & Kodrík 2011; Kodrík *et al.* 2015). The activated form of FoxO is involved in the upregulation of a cellular energy sensor called AMP-activated protein kinase (AMPK) (Hay 2011). Activated AMPK inhibits the target of rapamycin (TOR), which primarily regulates growth processes, such as cellular proliferation and protein synethesis. The FoxO stress response pathway indirectly inhibits growth pathways through its modulation of AMPK.

At the organismal level, FoxO activation regulates responses to diverse stressors and promotes longevity (Eijkelenboom. & Burgering 2013; Kodrík *et al.* 2015). Therefore, it can be speculated that the stimulatory effects of early life ionizing radiation exposure on survival and longevity phenotypes in our study might be explained by the activation of FoxO pathways. Additionally, the reduction in growth phenotypes may be attributed to the antagonistic effects of FoxO activation on TOR growth pathways. Future research should explore how these highly conserved cellular signaling pathways might contribute to life-history tradeoffs at the organismal level.

Despite previous research demonstrating hormetic effects of low-dose radiation exposure on juvenile growth in animals (Marshall 1962; Engel 1967; Stark *et al.* 2015), we found no evidence for growth hormesis in our study. Radiation-induced growth hormesis might be expected to occur through several possible mechanisms. For instance, it has been previously suggested that growth rate hormesis might reflect a compensatory response following a period of reduced growth (Johnston *et al.* 2011), such as what has been observed in fast-refeeding studies (Mangel & Munch 2005; Inness & Metcalfe 2008).

In our experiments, if initial radiation stress required growth to be temporarily reduced to prioritize investment in antioxidant or repair mechanisms, a compensatory response might be expected to help "catch up" for lost growth during this period. If growth rate were to be measured during this time, then the compensatory responses would appear hormetic. Our experiment did not allow us to monitor individual growth rates over the course of the juvenile period, so we were unable to directly test whether individuals in any of the radiation treatments exhibited a compensatory growth response. However, when comparing average masses for groups of individuals from each treatment group at several time points over the juvenile period,

there was no evidence that any of the radiation treatment groups were larger than controls at any point during development (**Figure S2.7**). Additionally, average mass-specific feeding rates were consistent throughout all radiation dose treatment groups, arguing against the possibility that any of the treatment groups were compensating for reduced growth by eating more than their body mass (**Figure 2.5**).

Another possible mechanism through which radiation-induced growth hormesis may occur is through the induction of intracellular ROS signaling pathways that promote cellular proliferation and differentiation (reviewed in Sauer, Wartenberg & Hescheler 2001). In animal cells, ROS signaling molecules are produced by the mitochondrial respiratory chain or through enzymatic processes. One of the most important intracellular generators of ROS is membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Abid *et al.* 2000; Chen *et al.* 2008).

In response to extracellular growth-promoting factors, NADPH generates a transient increase in intracellular ROS. These ROS signals are thought to engage various intracellular receptors, protein kinases, protein phosphatases and transcription factors involved in growth and differentiation (Sauer *et al.* 2001). Importantly, low-dose ionizing radiation has been shown to upregulate growth and differentiation processes in a variety of cell lines by activating various ROS-mediated second messenger pathways (Makinodan and James 1990; Liang *et al.* 2011) , suggesting that even exogenous sources of ROS production may be able to amplify growth processes. It is unclear, however, whether this ROS-mediated hormetic effect at the cellular level can translate into whole-organism growth hormesis.

The fact that ROS can activate cellular growth and proliferation pathways but are also known to engage stress-resistance pathways that inhibit growth (e.g., FoxO) creates somewhat of

a paradox. One likely possibility is that the response activated depends on the magnitude of ROS present (i.e., high levels of potentially damaging ROS activate cellular stress-resistance pathways at the expense of growth, while relatively low levels may amplify intracellular signaling pathways that promote growth). Our findings that crickets exposed to early life radiation tended to have greater survivorship but a slower juvenile growth rate are consistent with the hypothesis that radiation-induced ROS may have activated stress-resistance pathways while downregulating growth. While this pattern was observed in the moderate-to-high range of radiation doses tested in our study (2 – 7 Gy), it was not observed in the lower dose range (0.5 – 1 Gy) (**Figure 2.1**). However, even in the low dose range, we did not observe an increase in growth-related traits, which could have been expected under the hypothesis that relatively low levels of ROS stimulate growth pathways (Sauer *et al.* 2001).

One possible explanation for this is that any increase in growth following low-dose radiation exposure was transient and therefore might have been missed by our experimental protocol where the first measure of juvenile growth was taken at 24 days of age (10d after the crickets were irradiated). Another possibility is that even the low doses tested in our study were too high to stimulate a growth response. In other invertebrate species, radiation-induced growth hormesis occurred in response to radiation dose exposures in the milliGray range (Marshall 1962; Engel 1967).

Mode of radiation exposure might be another factor that could influence the likelihood that growth hormesis will occur. For instance, in our experiments, all radiation doses were delivered acutely, rather than chronically. It seems possible that chronic, low dose exposures delivered at a lower dose rate may be more likely to have a hormetic effect on growth, as this might be expected to provide cells with a constant supply of low-dose ROS to stimulate growth

pathways over a longer time period. Comparable doses delivered at a higher dose rate may overwhelm the system and activate stress-resistance pathways. Indeed, chronic low-dose exposures would be more consistent with the types of radiation doses experienced by animals living in radio-contaminated areas, such as Chernobyl (Galván *et al.* 2014).

The lack of observed hormetic effect on growth in our study could also be due to other genetic or environmental factors specific to laboratory conditions that could influence growth rate. For example, the animals used in our study were always provided with *ad libitum* access to high quality pet food. It is unlikely that this species would have access to these conditions in its natural environment. In the absence of resource limitations, it is possible that crickets could attain a growth rate close to their evolved limit such that a hormetic increase in growth rate following radiation exposure may not be possible. This possibility could be tested by repeating this experiment under conditions where crickets were provided with restricted access to food.

While food availability might be expected to influence growth rates, another possibility is that laboratory environments could select for growth rate strategies where organisms perform close to their evolved maximal rates. For instance, it is often seen that wild organisms do not grow at the maximal rate possible, even when provided with *ad libitum* access to resources (Gotthard 2008; Dmitriew 2011; Lee, Monaghan, & Metcalfe 2012), suggesting that there are extra costs to growing fast that could constrain the evolution of growth strategies. However, in organisms bred for many generations under stress-free laboratory conditions (e.g., unlimited food, optimal temperatures, no predators), one might expect selection to favor a growth strategy in which organisms grow at a rate closer to their evolved maxima, since there are perhaps fewer costs to doing so. Given their short generation times, insects are well known to undergo rapid selection under laboratory conditions to the extent that they can become significantly different

from their wildtype counterparts at the genetic level. This is particularly problematic in populations of invasive insect species bred in isolation for use in the sterile insect technique, where sterilized laboratory insects are genetically isolated to the degree that they will no longer mate with their wildtype counterparts (Matos *et al.* 2000; Mudavanhu, Addison & Conlong 2017). Given that the crickets used in these experiments were taken from a long-term laboratory breeding colony, we cannot rule out the possibility that selection for exaggerated traits such as increased growth rate may have occurred, which could limit the available scope for hormetic growth responses. An interesting test of this hypothesis could involve repeating this experiment by comparing individuals from our laboratory colony against individuals from a field population of *A. domesticus* to see whether the populations differ in the expression of hormetic responses.

In summary, this study provides evidence that early life radiation hormesis results in a tradeoff between growth and self-maintenance. Specifically, crickets exposed to moderate levels of ionizing radiation in early juvenile development (2 – 7 Gy dose range) displayed increased survivorship, but had a lower rate of juvenile growth, suggesting that these individuals adopted a life-history strategy where maintenance was prioritized over growth. This work provides empirical support for the suggestion that hormetic effects on life-history traits result from tradeoffs that may reflect organismal modifications in resource allocation strategies for coping with stress (Forbes 2000; Costantini *et al.* 2010; Jager *et al.* 2013; Mcclure *et al.* 2014).

Future work should consider the mechanistic changes that may be responsible for such tradeoffs, as well as the fitness consequences of these responses under variable environments. For instance, it is not known whether hormetic responses that favor stress-resistance can be costly under environmental mismatch (e.g., when the environment is not stressful). Importantly, this work suggests that ecological risk models must take into consideration the possibility of

tradeoffs when evaluating the fitness consequences of low-dose stress exposures in natural ecosystems.

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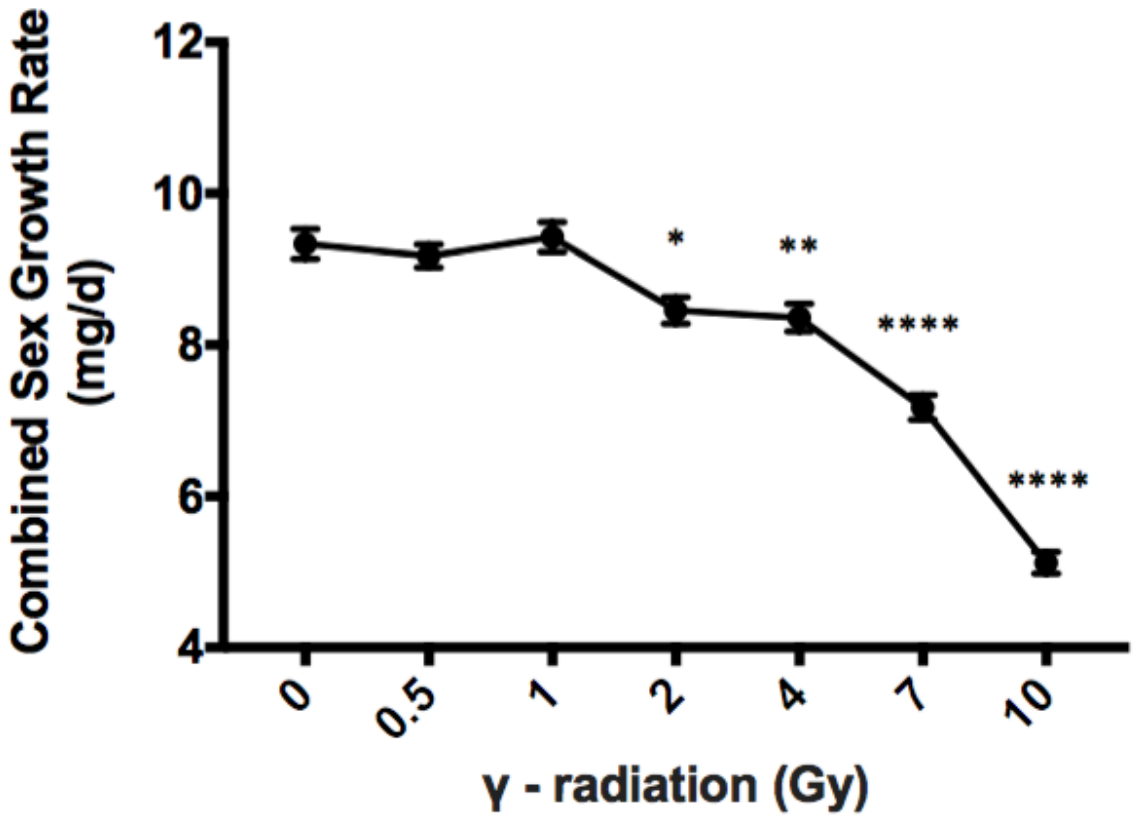

Figure 2.1: Dose-response effects of early life gamma-radiation exposure on combined male and female cricket juvenile growth rates. Growth rates were estimated by dividing mass at maturity (mg) by total development (d) for each individual. All crickets were irradiated at 14 d of age at the following radiation doses: 0 (n = 42), 0.5 (n = 50), 1 (n = 47), 2 (n = 51), 4 (n = 53), 7 (n = 51), and 10 Gray (Gy) (n = 48) (dose rate = 0.25 Gy/min). Values are reported as means ± SEM (* $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$). All significant differences reported are in comparison to the 0 Gy treatment group as revealed by Tukey's HSD test.

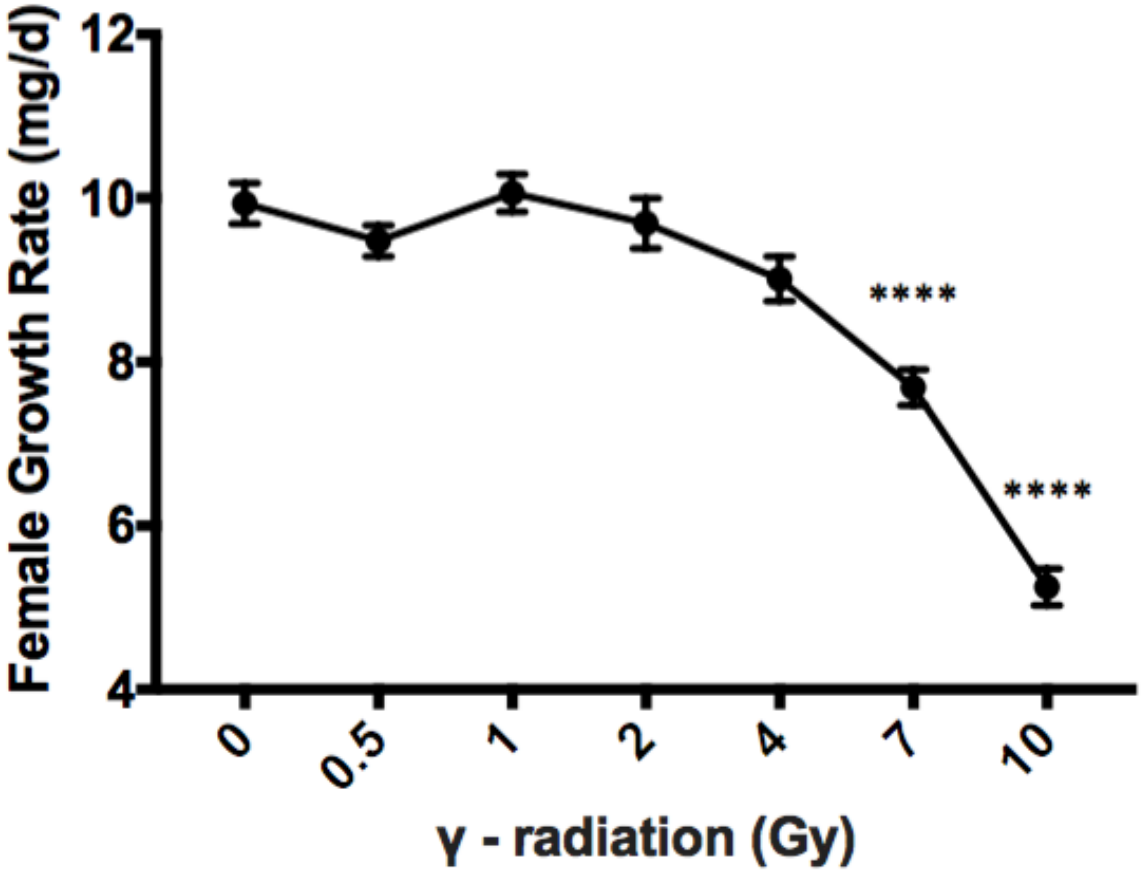

Figure 2.2: Dose-response effects of early life gamma-radiation exposure on female cricket juvenile growth rates. Growth rates were estimated by dividing mass at maturity (mg) by total development (d) for each individual. All crickets were irradiated at 14 d of age at the following radiation doses: 0 (n = 22), 0.5 (n = 31), 1 (n = 26), 2 (n = 14), 4 (n = 25), 7 (n = 28), and 10 Gray (Gy) (n = 23) (dose rate = 0.25 Gy/min). Values are reported as means ± SEM (**** $p < 0.0001$). All significant differences reported are in comparison to the 0 Gy treatment group as revealed by Tukey's HSD test.

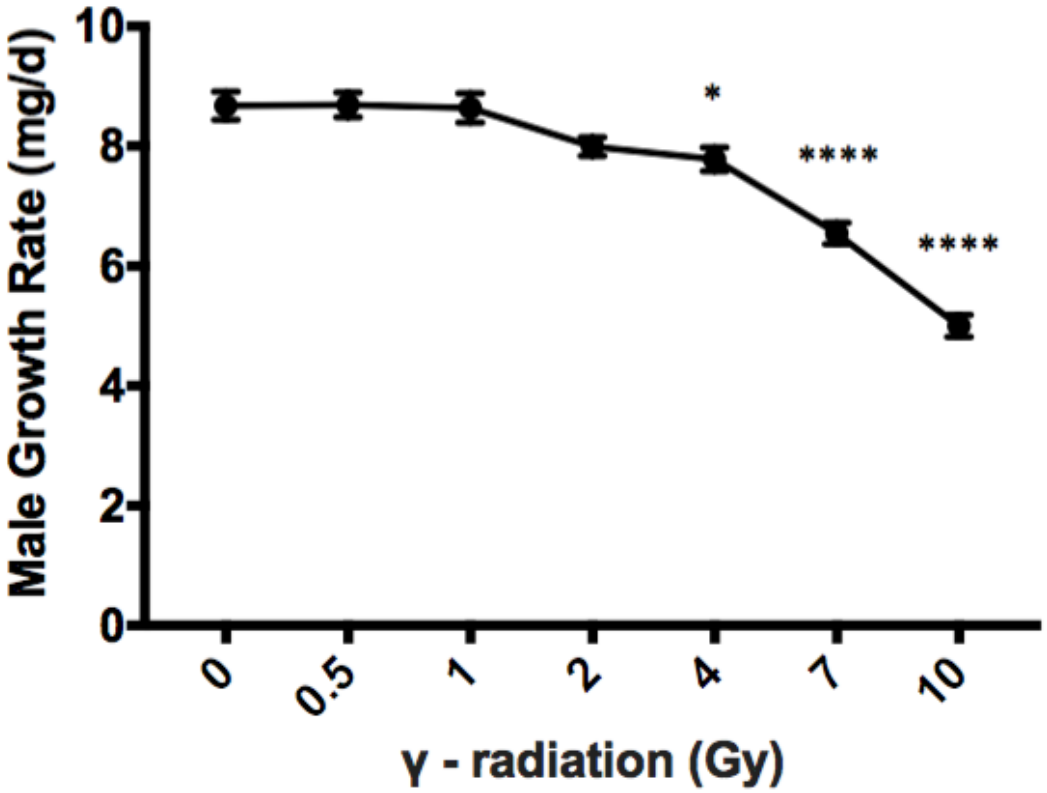

Figure 2.3: Dose-response effects of early life gamma-radiation exposure on male cricket juvenile growth rates. Growth rates were estimated by dividing mass at maturity (mg) by total development (d) for each individual. All crickets were irradiated at 14 d of age at the following radiation doses: 0 (n = 20), 0.5 (n = 19), 1 (n = 21), 2 (n = 37), 4 (n = 28), 7 (n = 23), and 10 Gray (Gy) (n = 25) (dose rate = 0.25 Gy/min). Values are reported as means ± SEM (* $p < 0.05$, **** $p < 0.0001$). All significant differences reported are in comparison to the 0 Gy treatment group as revealed by Tukey's HSD test.

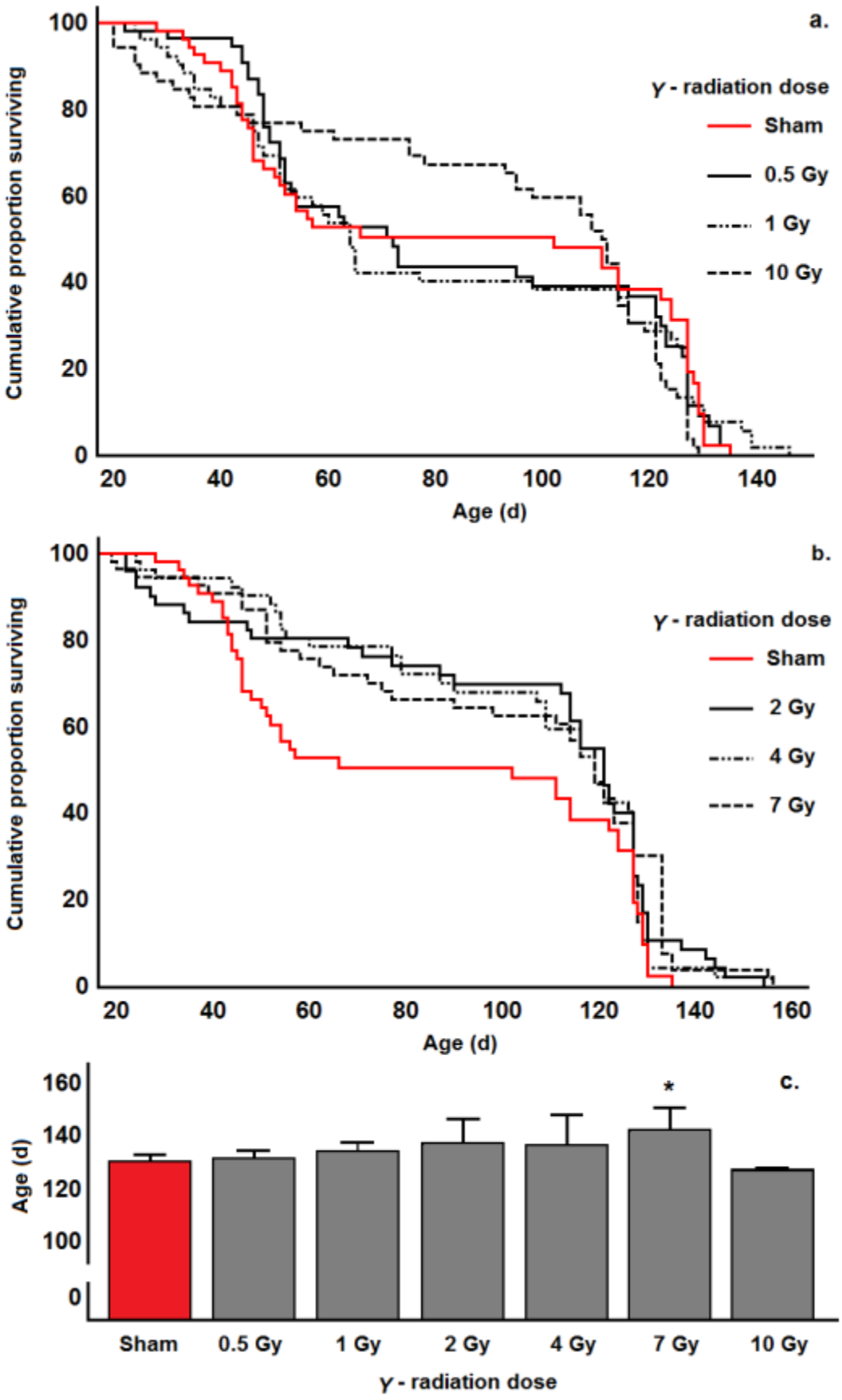
a.
Cumulative proportion surviving
100
80
60
40
20
0
20
40
60
80
100
120
140
Age (d)
γ - radiation dose
Sham
0.5 Gy
1 Gy
10 Gy
b.
Cumulative proportion surviving
100
80
60
40
20
0
20
40
60
80
100
120
140
160
Age (d)
γ - radiation dose
Sham
2 Gy
4 Gy
7 Gy
c.
Age (d)
160
140
120
100
0
*
Sham
0.5 Gy
1 Gy
2 Gy
4 Gy
7 Gy
10 Gy
γ - radiation dose

Figure 2.4: Kaplan-Meier survival curves for populations of 60 crickets subjected to a single dose of ionizing radiation at 14d of age. All radiation was delivered at a dose-rate of 0.25 Gray (Gy)/min. The Gehan-Breslow-Wilcoxon test revealed significant differences in mean survivorship between the 0 and 4 Gy treatments ($p = 0.033$), 0 and 7 Gy treatments ($p = 0.049$) and a marginally significant difference between the 0 and 2 Gy treatments ($p = 0.077$) (b). No significant differences in mean survivorship were found between the 0, 0.5, 1, and 10 Gy treatment groups (a). ANOVA with a post-hoc Tukey's HSD test on the last 10% surviving individuals in each population revealed a marginally significant increase in maximal longevity in the 7 Gy treatment relative to 0 Gy ($p = 0.067$) (c).

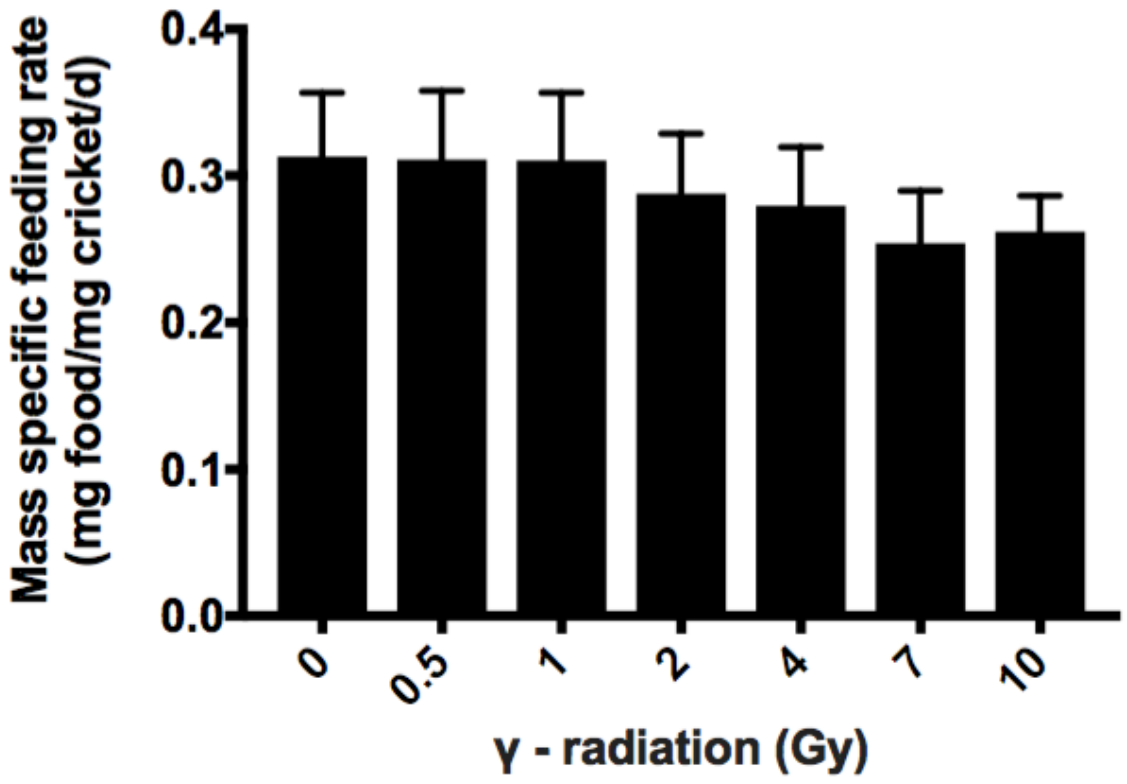

Figure 2.5: Dose-response effects of early life gamma-radiation exposure on average mass-specific feeding rates of juvenile crickets (mg dry food consumed/ cricket weigh/day). Repeated measurements for mass-specific feeding rates were taken for each radiation dose treatment group 2-3 times per week over the duration of the juvenile period. All crickets were irradiated at 14 d of age at the following radiation doses: 0 (n = 11), 0.5 (n = 11), 1 (n = 11), 2 (n = 11), 4 (n = 11), 7 (n = 11), and 10 Gray (Gy) (n = 11) (dose rate = 0.25 Gy/min). Values are reported as means ± SEM. Significant differences were tested in comparison to the 0 Gy treatment group with Tukey's HSD test.

Chapter 3

Early life ionizing radiation exposure has mixed effects on adult life-history traits

Abstract

Stressful experiences in early life can have lasting impacts on phenotypic development that can affect adult trait expression. However, the degree to which early life stress is detrimental to the adult phenotype is a matter of debate. In modern environments, ionizing radiation is a predominant source of pollution that may be a significant source of developmental stress. The goal of this research was to assess the dose-response impacts of early juvenile ionizing radiation exposure on adult life-history traits in the house cricket, *Acheta domesticus*. Dose-response effects of early life radiation exposure were assessed on measures of fecundity, adult oxidative stress physiology, offspring investment, and offspring fitness. Early life radiation had strong inhibitory effects on adult female body mass, but age-specific fecundity was negatively impacted only at relatively high radiation doses. Crickets exposed to relatively moderate radiation doses in early life laid larger eggs than controls and these eggs had greater hatching success, suggesting that radiation exposure can have transgenerational effects on offspring performance. No noticeable effects of early life radiation were detected on total and non-enzymatic antioxidant capacity or hydrogen peroxide levels in adult females. Together, these results demonstrate that a single exposure to ionizing radiation in early life can have diverse, dose-dependent impacts on trait development and that rather than being purely detrimental, responses can be sustained or enhanced.

Introduction

Stressful experiences early in life can have profound impacts on phenotypic development, and these effects can persist into adulthood (Monaghan 2008; Costantini 2013, 2014; Monaghan & Haussmann 2015). However, the extent to which early life stress is detrimental to adult trait performance is far from clear. For instance, early life stress has often been associated with negative impacts on adult life-history stages, and there is extensive evidence of such effects on physiological, morphological, reproductive, and behavioral traits across animal taxa (Lummaa & Clutton-Brock 2002; Monaghan 2008). However, a significant body of work has reported sustained or stimulatory effects of developmental stress on adult phenotypes where negative effects were expected (Costantini 2014; Crino & Breuner 2015; Monaghan & Haussmann 2015).

Such responses are consistent with a phenomenon known as hormesis (Southam and Ehrlich 1943), where low levels of stressors or toxins can have stimulatory or beneficial biological effects even if high levels are damaging. Hormetic dose-response relationships are well studied in the toxicological and medical literatures (reviewed in Mattson and Calabrese 2010), but the potentially important role they play in a life-history context is just beginning to be realized (Costantini, Metcalfe & Monaghan 2010; Costantini 2014). Thus, empirical studies are needed that acknowledge the possibility that the complex phenotypic outcomes of early life stress might crucially depend on the severity of the stressor.

Oxidative stress is emerging as a primary physiological mechanism linking early life stressful experiences to changes in the adult phenotype, including hormetic responses (Costantini 2014). All aerobic organisms are constantly threatened by the potentially toxic effects of oxidative stress through exposure to reactive oxygen species (ROS) (Finkel & Holbrook 2000). ROS are generated endogenously in response to metabolic challenges (e.g., temperature stress,

exercise, pollutants, or activation of the acute stress response), and if not controlled, they can cause oxidative damage to lipids, proteins, and DNA (Dotan, Lichtenberg & Pinchuk 2004).

To prevent this, organisms have evolved a complex antioxidant defense system consisting of both enzymatic and non-enzymatic antioxidants. In this regard, oxidative stress is generally defined as the physiological imbalance between the production of ROS and their removal by antioxidants. Early life is a time when organisms might be particularly sensitive to oxidative challenges (Costantini 2013), given that rapid growth is associated with high ROS production (Rollo 1996; Alonso-Alvarez *et al.* 2007) and that enzymatic antioxidant systems tend to be underdeveloped in early life stages across animal taxa (Isaksson *et al.* 2011; Hermes-Lima *et al.* 2012).

Indeed, oxidative challenges during early life have been shown to have lasting negative effects on fecundity and longevity (Alonso-Alvarez *et al.* 2006; Noguera, Kim & Velando 2012), suggesting that developmental oxidative stress can be an important life-history constraint (Metcalfe & Alonso-Alvarez 2010). However, other research has shown that exposure to mild stress in early life can lead to increased resistance to oxidative stress in adulthood, suggesting that oxidative mechanisms may also play a crucial role in facilitating hormetic responses (Costantini, Monaghan & Metcalfe 2012). Indeed, low levels of ROS are involved in important regulatory functions, such as the upregulation of antioxidant production, repair processes, and mitochondrial biogenesis (D'Autréaux & Toledano 2007; Ristow 2014), all of which could serve as important mechanisms underlying hormetic responses to oxidative challenges during development (Zhang & Hood 2016).

Our understanding of the life-history effects of oxidative challenges in early development has broad implications for species living near anthropogenic environments, where pollutants can

be a significant source of oxidative stress, even at low levels (Amado *et al.* 2006; Sureda *et al.* 2006; Yousef *et al.* 2017). For instance, radionuclide pollution is a significant environmental concern, as it is known to persist in low levels in geographical regions affected by the nuclear power production process (e.g., uranium mining or fallout from nuclear power plants) and nuclear reactor accidents.

Radionuclides are high-energy atoms that generate ionizing radiation in the form of α-, ß-, or γ-rays through the radioactive decay process (Sobolev *et al.* 1994; Won & Lee 2014). At the cellular level, radiation can induce damage directly through the breakage of chemical bonds in biomolecules (e.g., DNA, lipids, or proteins), but more commonly through the production of ROS via the ionization of water, which is the most abundant molecule in animal cells and is therefore the most likely cellular target of radiation (Riley 1994; Lane 2002; Koch and Hill 2017). Thus, ionizing radiation exposure during development is most likely to exert its phenotypic effects through the alteration of oxidative stress mechanisms.

In an ecological context, most work involving ionizing radiation has consisted of field studies of plants and animals from the radio-contaminated areas surrounding the nuclear disaster sites at Chernobyl (Kovalchuk *et al.* 2003; Møller & Mousseau 2006) and Fukushima (Beresford & Copplestone 2011; Mousseau & Møller 2014; Strand *et al.* 2014). This research has provided evidence that chronic low-dose radiation levels in these regions have affected organisms at the genetic, morphological, and demographic levels. It has also provided some support for the involvement of oxidative stress mechanisms underlying responses to radiation in wild animals (Einor *et al.* 2016), including evidence for the potential adaptation of antioxidant mechanisms to chronic radiation environments (Galván *et al.* 2014). However, there have been relatively few laboratory dose-response studies investigating the effects of ionizing radiation on

life-history traits (but see Hiyama *et al.* 2012; Mothersill *et al.* 2013), especially during the early stages of development when organisms might be most sensitive to oxidative stress (Costantini 2013). This has limited our ability to understand the potentially important role that hormesis might have in determining fitness outcomes of radiation exposure. Thus, detailed laboratory investigations in model organisms are needed to complement the field research and broaden our understanding of the fitness impacts of radiation, particularly in the low dose range.

In this research, we examined the dose-response effects of early life ionizing radiation exposure on a broad range of adult life-history and oxidative stress phenotypes. Our results provide a comprehensive analysis of how early life stress can affect the development of a broad suite of traits in a complex, dose-dependent manner. Given the challenges associated with establishing dose-response relationships for multiple trait responses, we made use of an insect model with a relatively short and straightforward life cycle, the house cricket (*Acheta domesticus*). This species has a juvenile period of about 6 weeks, making it suitable for assessing effects of early life stress on adult phenotypes. Due to their small body size, crickets can be raised in large cohorts to obtain sufficient sample sizes for dose-response studies (Lyn *et al.* 2012; Hans *et al.* 2015). We specifically established dose-response relationships for life-history traits relevant to fecundity (adult body size and age-specific reproductive output), offspring investment (egg size) and offspring fitness (hatching success and starvation resistance). Dose-response effects of total antioxidant capacity, non-enzymatic antioxidant capacity, and hydrogen peroxide levels were also assessed in adult females.

Methods

Experimental groups

We isolated 14-day-old juvenile *Acheta domesticus* from a genetically heterogeneous breeding colony as described previously (see Methods of Chapter 2 and Lyn *et al.* 2011) and separated them into seven experimental groups. Each group was subjected to one of the following acute doses of ionizing radiation: 0.5, 1, 2, 4, 5.5, 0 Gray (Gy) (n = 180 per group), or 7 Gy (n = 90). All crickets were irradiated at the Taylor Radiobiology Source at McMaster University (dose rate = 0.25 Gy/min). Following irradiation, crickets were housed in plastic containers (30x19x12cm; n = 45 per container) and maintained at 30˚C on a 12h light/12h dark photoperiod with egg carton shelters, water-soaked cellulose sponges, and *ad libitum* access to a diet of ground guinea pig food (*Little Friends*^{TM}: 16.5% crude protein, 4% crude fat and 19% crude fiber) mixed with deionized water (3g of food per 8 mL of water).

Mating

On post-maturation day 13, we individually mated 12 to 20 females from each experimental radiation group for one night with an age-matched, non-irradiated male. Females that failed to mate (as indicated by failure to oviposit throughout the course of the experiment) were excluded from our analyses of reproductive performance.

Measures of fecundity

Prior to mating, we estimated lifetime fecundity of females to approximate of lifetime fecundity. Mass measurements were taken at this time rather than on the day of maturity as females accumulate significant post-maturation mass through egg development and lipid storage (Hans *et al.* 2015). Adult body mass is a strong predictor of lifetime fecundity in Orthoptera (Whitman 2008).

Following mating, we recorded the oviposition rate (calculated as eggs laid over a 4-day period) of each female for two separate periods over the course of the study: an early oviposition period (post-maturation day 14 to 18) and a late oviposition period (post-maturation day 20 to 24). Oviposition substrate (moist medical gauze in a petri dish) was replaced daily and eggs remained incubated at 30˚C until later use. Food and water were provided *ad libitum* through the oviposition periods. Lifetime fecundity was estimated by measuring the mass of females prior to mating.

Measures of offspring investment and offspring fitness

We measured one proxy for offspring investment (egg size) and two proxies for offspring fitness (hatching success and hatchling starvation resistance) for the offspring of females from each radiation treatment group. Egg size (calculated as egg length x width) was measured under a (microscope name) for 5 to 11 randomly selected eggs (average = 6.7) laid by each female during the early oviposition period. All egg size measurements were taken within one day from the time eggs were laid. For hatching success, we incubated 20 eggs per female in moist medical gauze at 30˚C and measured percentage of eggs hatched. Starvation resistance was measured for 3 hatchlings from each female by monitoring the number of days hatchlings could survive without food (a proxy for initial energy reserves).

Measures of oxidative status

We performed measurements of oxidative status in heads removed form adult female crickets from each radiation treatment group. Measurements of oxidative status included hydrogen peroxide concentration (a measurement of ROS levels), total antioxidant capacity, and non-enzymatic antioxidant capacity. At post maturation day 14, adult female crickets were anesthetized with carbon dioxide and decapitated. Heads were homogenized in 500µL and

homogenates were aliquoted into separate 100µL vials and stored at -80˚C. Prior to analyses, homogenates were brought to 0˚C on wet ice. For antioxidant assays, homogenates were diluted by 50x in double distilled water. Total antioxidant capacity and non-enzymatic antioxidant capacity were measured using the Total Antioxidant Capacity Assay Kit® (Sigma-Aldrich®, St. Louis, MO, USA, catalog #MAK187). For the hydrogen peroxide assay, homogenates were diluted by 50x in assay buffer prior to analysis. Hydrogen peroxide concentration was measured using the Fluorimetric Hydrogen Peroxide Assay Kit® (Sigma-Aldrich®, St. Louis, MO, USA, catalog #MAK165). For all bioassays, measurements were corrected for body mass.

Statistics

Radiation dose-response relationships were established for each trait measured. As a preliminary test for hormesis, we utilized a general linear models approach. Here, regression analyses were performed where the dose variable was expressed as either a monotonic function with a linear term or a biphasic function with both a linear and quadratic term (**Table 3.1**). A hormetic response was determined only if the quadratic function explained more of the variance in the model than the linear function and if the fit was significant ($p < 0.05$).

For some traits (e.g., average egg size), neither a linear nor quadratic fit could adequately describe the dose-response model. Therefore, as a secondary test, all data were analyzed using a one-way ANOVA followed by a Tukey's HSD post-hoc test (**Fig. 1 – 4**). Differences between means were considered significant at $p < 0.05$. Prior to analyses, normality was confirmed using the D'Agostino-Pearson omnibus normality test. All statistical tests were performed using Prism 7.

Results

Fecundity

Fecundity was assessed by measuring adult female body mass and oviposition rate over two 4-day periods. There was a strong negative linear effect of early life radiation exposure on adult female body mass (**Table 3.1**). ANOVA revealed that relative to the 0 Gy treatment, female body mass was significantly lower at doses of 1 (~9%, $p = 0.033$), 2 (~12%, $p < 0.0001$), 4 (~15%, $p < 0.0001$), 5.5 (~25%, $p < 0.0001$), and 7 Gy (~32%, $p < 0.0001$) (**Figure 3.1**). Adult body mass did not significantly differ from 0 Gy in the 0.5 Gy treatment. Early adulthood oviposition rate (measured 14 – 18 d post maturation) remained relatively stable within the 0 – 2 Gy range, but was significantly lower than the 0 Gy group at 4 (~40%, $p = 0.002$), 5.5 (~72%, $p < 0.0001$), and 7 Gy (90%, $p < 0.0001$) (**Figure 3.2**). Late adulthood oviposition rate (measured 20 – 24 d post maturation) appeared to decrease in dose-dependent manner, but only became significantly lower than 0 Gy in the 4 (~38%, $p = 0.027$), 5.5 (73%, $p < 0.0001$), and 7 Gy (~94%, $p < 0.0001$) treatments (**Figure 3.3**). Fecundity was almost completely arrested at 7 Gy (**Figure 3.2, 3.3**). Both early and late adulthood oviposition rate were significantly described by a linear function (**Table 3.1**).

Offspring investment and offspring fitness

As an indicator of offspring investment, we measured size of eggs laid by females subjected to developmental ionizing radiation. Effect of dose on egg size was not significantly described by either a linear or quadratic function (**Table 3.1**). However, ANOVA revealed that females irradiated at the 1 and 2 Gy doses laid eggs that were ~11% ($p < 0.0001$) and ~9% ($p = 0.0016$) larger than those laid by females in the 0 Gy treatment (**Figure 3.4**). No significant effects of radiation on egg size were observed in the 0.5 Gy treatment or 4 – 5.5 Gy dose range.

As indicators of fitness for the offspring from irradiated females, we measured hatching success and hatchling starvation resistance. Hatching success was significantly described by a quadratic function (**Table 3.1**), with peak hatching success occurring in the 2 Gy treatment (**Figure 3.5**). ANOVA revealed that starvation resistance was not affected throughout the 0 – 2 Gy dose range, but was significantly lower than controls in the 4 (~19%, $p = 0.012$) and 5.5 Gy (~23%, $p = 0.012$) dose groups (**Figure 3.6**). Starvation resistance was significantly described by a linear function (**Table 3.1**).

Oxidative status

After using body mass as a covariate, Tukey's HSD test revealed no significant differences between the 0 Gy radiation treatment group and any of the other treatment groups in terms of hydrogen peroxide concentration (**Figure 3.7**), total antioxidant capacity (**Figure 3.8**), or non-enzymatic antioxidant capacity (**Figure 3.9**).

Discussion

Ionizing radiation pollution is a significant threat to species in natural ecosystems, and because of this, radiation research is transforming from a field that predominantly focuses on human health to one that must acknowledge the fitness effects of radiation in ecosystems. Over the past several decades, there has been a multitude of field research examining populations of organisms in radio-contaminated areas (Møller & Mousseau 2006; Buesseler, Aoyama & Fukasawa 2011; Strand *et al.* 2014). However, it is becoming increasingly acknowledged that detailed laboratory studies of the fitness impacts of ionizing radiation are required to help reduce uncertainties related to radiation effects in the field (Bréchignac *et al.* 2016).

Our results provide a comprehensive overview in a single model organism of the fitness impacts of radiation exposure in early development, a time when organisms are particularly susceptible to stress. Specifically, we investigated the effects of early life ionizing radiation exposure on measures of fecundity, offspring investment, and oxidative stress phenotypes in adult female *A. domsesticus*. Despite noticeably negative effects of developmental radiation exposure on adult body mass throughout the dose range tested (**Figure 3.1**), fecundity (measured as oviposition rate) was negatively impacted by only relatively high radiation doses (**Figure 3.2, 3.3**). Additionally, crickets exposed to moderate levels of developmental radiation laid larger eggs than controls (**Figure 3.4**). No effects of developmental radiation exposure were observed on adult total antioxidant capacity, non-enzymatic antioxidant capacity, or hydrogen peroxide levels (**Figure 3.7 – 3.9**). Together, these results show that stress in early development can affect the adult phenotype in a complex, dose-dependent manner, and that rather than being purely detrimental, developmental stress can have sustained or positive effects on phenotypic development. In the following sections, we provide potential explanations for our data and suggest directions for future work.

Effects on adult body mass

In Orthoptera, female adult body mass is a strong predictor of lifetime fecundity (Whitman 2008). In these experiments, we found that adult body mass was the most sensitive life-history trait to early life radiation exposure, as size was significantly inhibited by doses as low as 1 Gy (**Figure 3.1**). This is consistent with previous research in insects showing that developmental ionizing radiation exposure has strong inhibitory effects on growth rates and adult body size (see Hasan and Khan 1998). For instance, high doses of ionizing radiation are well known to inhibit cellular growth, particularly in rapidly growing tissues, by inducing apoptosis

or by delaying cell division by inhibiting various checkpoints throughout the cell cycle (reviewed in Maity *et al.* 1994; Iliakis *et al.* 2003). Another possible way by which radiation could reduce growth is through its inhibitory effects on feeding (Watters and MacQueen 1967). Reduced feeding can result from radiation-induced behavioral changes (e.g., lethargy) (York *et al.* 2012) or decreases in metabolic demand. Relatively low doses of ionizing radiation have also been shown to inhibit food processing in insects by inducing direct damage to midgut cells (Hasan and Khan 1998).

The negative effect of developmental radiation exposure on adult body size may also be explained by developmental growth deficits mediated by highly conserved cellular signaling pathways responsive to oxidative stress. In insects, increased oxidative stress is closely linked to the secretion of adipokinetic hormones (AKH) from neuroendocrine centers in the brain, and these hormones play a key role in coordinating anti-ROS responses (Krishnan & Kodrík 2011; Kodrík *et al.* 2015). This includes the upregulation of antioxidant enzymes, such as catalase, superoxide dismutase, and glutathione (Zou *et al.* 2011; Večeřa *et al.* 2012). Under conditions of oxidative stress, AKH is thought to initiate these stress responses by triggering the activation of the Forkhead box class O transcription factors (FoxO) (Bednářová, Kodrík & Krishnan 2015). FoxO are a highly conserved family of transcription factors that that regulate cellular responses to diverse types of stressors, including energy deprivation and oxidative stress (Brunet 2004). When active, FoxO is translocated to the nucleus where it is involved in the transcription of numerous stress response genes, including antioxidants. The activated FoxO is also crucially involved in the upregulation of a key cellular energy sensor called AMP-activated protein kinase (AMPK) (Greer *et al.* 2007). When AMPK is activated, it has an inhibitory effect on the target of rapamycin (TOR), a predominant cellular pathway controlling growth processes such as protein

synthesis and cell growth. Thus, through the modulation of AMPK, FoxO has an indirect inhibitory effect on TOR, and this has been specifically documented to occur in insects under conditions of oxidative stress (Lee *et al.* 2010).

Across diverse animal taxa, FoxO activation is tightly linked with enhanced stress-resistance responses (i.e., increased lifespan and ROS resistance) and reduced growth (Greer *et al.* 2007). Thus, it seems possible that the inhibitory effect of ionizing radiation on adult body size could be attributed to growth deficits during development as an indirect consequence of ROS-mediated FoxO activation. Future research should further explore how activation of these cellular stress response pathways translates to life-history plasticity at the organismal level.

Effects on age-specific fecundity

Despite the significant inhibitory effect of developmental radiation exposure on adult female body mass throughout the dose range tested, fecundity in both early and late life remained stable throughout the 0 – 2 Gy dose range and were not significantly inhibited until 4 Gy (**Figure 3.2, 3.3**). This inconsistency between the inhibition of fecundity and adult body mass is surprising given that female body size tends be a strong predictor of reproductive output in Orthoptera (Whitman 2008). It is possible that this body mass deficit could be attributed to the underdevelopment of features other than egg production (e.g., lipid storage or muscle). However, it is also possible that the reproductive biology of *A. domesticus* might allow for females to maintain their targeted reproductive output despite reductions in total egg production. For instance, mature females continuously develop and store eggs in their ovaries throughout their adult life and retain many more eggs than will be laid at any given time. This means that there is likely a range in which females can modify their reproductive output independent of changes in total egg provisioning (Adamo 1999). The fact that females produce more eggs than will be laid

at any given period could buffer the fitness deficits imposed by developmental constraints on body size or egg production.

Another possible reason for the lack of observed decrease in fecundity throughout the 0 – 2 Gy dose range is that radiation stress in development triggered females to alter their reproductive schedule. For instance, it is possible that developmentally stressed females might favor early reproductive output at the expense of later reproduction if their total reproductive lifespan is shortened. Life-history theory predicts that iteroparous animals should adopt this strategy under circumstances where their future reproduction or survival is jeopardized (Williams 1966; Stearns 1992).

Under this scenario, it is possible that developmental radiation exposure could accelerate senescence by increasing free radical damage, causing the organism to favor investment in earlier reproduction. Although we measured reproductive rate over two periods in our study, both periods occurred within the first 24 days of adulthood, and neither residual reproductive output nor survival were monitored. Given that adult females live 60 days in the laboratory on average (Adamo 1999), it is possible that "late life" reproduction was not accounted for by these measurements. To test the hypothesis that developmental radiation exposure caused crickets to prioritize current reproductive output at the expense of future reproduction, studies of lifetime reproductive output and survival would be required.

Effects on offspring investment and offspring fitness

Remarkably, we found that crickets exposed to moderate levels of ionizing radiation (1 – 2 Gy) in early life produced larger eggs in adulthood (**Figure 3.4**). This suggests that early life radiation stress had a hormetic effect on offspring investment. Consistent with this, hatching success of eggs from irradiated mothers increased in the 0.5 – 2 Gy range (**Figure 3.5**),

indicating that there was also a hormetic impact on offspring performance Many insects, including crickets (Fox & Czesak 2000), have been shown to adaptively increase their egg size in response to environmental stressors, such as food deprivation (Stahlschmidt & Adamo 2015). However, this is the first study we are aware of demonstrating offspring size plasticity in response ionizing radiation stress. Future work should examine the potentially important role that oxidative stress mechanisms might have in mediating this type of plasticity.

Previous research, albeit in vertebrates, has shown that stress in early life can have hormetic effects on traits related to reproductive investment in adulthood. For instance, male zebra finches fed a corticosterone diet during the nestling period sired more offspring and reared nestlings that were in greater condition compared to those reared by control males (Crino *et al.* 2014). Additionally, cichlid fish subjected to food restriction during early development produced larger, faster growing offspring in adulthood (Taborsky 2006).

The ultimate reasons for why early life stressful conditions can result in hormetic-like responses in the adult phenotype are unclear; however, several hypotheses have been proposed (reviewed in Chaby 2016). For instance, the environmental matching hypothesis suggests that organisms experiencing stress during early life may undergo developmental changes that adjust the adult phenotype to maximize fitness in stressful environments (Love & Williams 2008; Costantini *et al.* 2012; Costantini 2013). This environmental priming effect may influence reproductive decisions in that parents experiencing stressful juvenile conditions may increase offspring investment to "prepare" their offspring for adverse environments (Mousseau & Fox 1998; Qvarnström & Price 2001). It is thought that the adaptiveness of this priming response crucially depends on the degree to which early and late environments are positively correlated, as it has been shown that this response can be costly under environmental mismatch (Costantini,

Monaghan & Metcalfe 2014). Thus, we might expect this type of response to evolve in animals with short generation times, such as insects, where there may be a relatively higher degree of overlap between the developmental environment of the mother and her offspring.

Despite the evidence that crickets exposed to moderate levels of early life radiation stress produced larger eggs with greater hatching success, there was no evidence for a hometic effect on hatchling starvation resistance (**Figure 6**). However, we did find that hatchlings of the crickets irradiated at the 4 and 5.5 Gy doses did perform worse than controls when subjected to starvation. This suggests that developmental radiation stress had transgenerational effects on offspring performance, at least in the relatively high dose range where stress is inhibitory.

Previous work demonstrating transgenerational effects of ionizing radiation has been performed in plants from the radio-contaminated areas of Chernobyl. For instance, progeny of plants from the Arabidopsis genus from Chernobyl were shown to have greater resistance to various forms of mutagens, and this was associated with increased expression of DNA repair and free radical scavenging genes, as well as increased genome methylation (Kovalchuk *et al.* 2004). These results suggest that transgenerational protective effects of radiation stress can have an epigenetic basis. Future work should test the possibility that ionizing radiation can induce transgenerational epigenetic effects in animals.

Effects on adult antioxidant capacity and hydrogen peroxide levels

Across all doses tested, we found that there were no strong impacts of developmental radiation exposure on total antioxidant capacity (**Figure 3.8**), non-enzymatic antioxidant capacity (**Figure 3.9**), or hydrogen peroxide levels in adulthood (**Figure 3.7**). Given that one of the primary biological effects of ionizing radiation is ROS production (Riley 1994), changes in these variables should have been expected. For instance, the environmental matching hypothesis

for hormetic responses would predict that organisms experiencing oxidative stress in early life may be expected to upregulate their antioxidant defences in anticipation of stressful environments (Costantini 2014). Alternatively, high levels of developmental radiation stress may have been expected to deplete antioxidants or increase hydrogen peroxide levels (i.e., oxidative stress). Since total antioxidant capacity is a comprehensive measure of both enzymatic and non-enzymatic (i.e., dietary) antioxidant levels, our results cannot exclude the possibility that there were differences in specific antioxidant enzymes (e.g., glutathione, superoxide dismutase or catalase) that were not revealed by this assay.

It is also possible that any increases in enzymatic antioxidant or hydrogen peroxide levels following juvenile radiation exposure were transient and therefore were no longer present in adulthood when tissues were sampled. This could be possible if the radiation doses tested in our experiment were not severe enough to induce sustained changes in antioxidants or hydrogen peroxide production throughout the lifespan. While previous work in invertebrates has shown that radiation exposure increases oxidative stress and antioxidant enzyme production, this was associated with extremely high radiation doses (50 – 250 Gy) and tissue sampling occurred soon after exposure (Won & Lee 2014).

We also cannot rule out the possibility that there were changes in levels of oxidative damage among treatment groups that were not revealed by our analyses. Oxidative damage (e.g., lipid peroxidation, DNA damage, or protein degradation) occurs when ROS levels outweigh antioxidant capacity (Dotan *et al.* 2004). Hydrogen peroxide was the only ROS molecule examined in our study, so we are unable to determine whether there were changes in other ROS molecules, which could potentially contribute to oxidative damage. Given that oxidative damage accumulation can have important fitness consequences (Selman *et al.* 2012; Speakman & Garratt

2014), future studies should explore the potentially important role oxidative damage might have in mediating phenotypic responses to early life radiation stress.

Another possible explanation for the lack of observed effect of radiation on antioxidant capacity or hydrogen peroxide levels in our study could be that stress-induced changes in oxidative statues are tissue-specific. Given that we only measured antioxidant capacity and hydrogen peroxide concentrations in head tissues, these measurements may not be representative of the organism's true oxidative physiology. Indeed, previous work has shown that oxidative damage can vary across tissues (Meitern *et al.* 2013; Speakman & Garratt 2014), so it is possible that antioxidants can be employed in a tissue-specific manner to meet these competing demands.

Another possibility is that due to the importance of the brain, oxidative stress physiology may be under tight homeostatic control in this organ, and this could potentially explain why no significant changes in these responses were observed throughout the dose range tested. To address these possibilities, future research should assess diverse measures of oxidative statues (e.g., oxidative stress, enzymatic and non-enzymatic antioxidants, and oxidative damage markers) in multiple tissues.

Conclusion

In summary, this work provides evidence that early developmental stress can affect the adult phenotype in a complex, dose-dependent manner. These results support the idea that the developmental outcome of early life stress varies depending on the trait measured, and responses can range from inhibitory, to sustained, to stimulatory (i.e., hormesis). While hypotheses regarding potential adaptive responses to early stress continue to be formulated (Monaghan 2008; Monaghan & Haussmann 2015; Chaby 2016), there is currently no predictive framework

for developmental outcomes of stress in early life. However, such outcomes likely depend on complex interactions between the magnitude of the stressor, the developmental stage at which the stress is received, as well as the life-history strategy of the organism (Monaghan & Haussmann 2015). Future work should adopt multi-trait approaches such as those employed in this study to assess the developmental outcomes of early life stress in diverse species.

This work focused specifically on developmental responses to early life ionizing radiation stress. From these experiments, we are unable to determine the specific phenotypic changes responsible for producing the developmental outcomes at the organismal level. However, it is possible that these outcomes can be attributed to a broad range of direct and indirect effects that ionizing radiation could have on host physiology or gene expression across development. Many of these outcomes are likely to by driven by oxidative stress physiology, since ROS production is the primary effector of ionizing radiation (Riley 1994). Given the important role of oxidative stress in influencing life-histories (Monaghan, Metcalfe & Torres 2009; Selman *et al.* 2012; Costantini 2014), future work should consider oxidative stress physiology as a proximate mechanism for linking environmental stress to life-history outcomes.

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Table 3.1. Summary of linear and quadratic regression models for radiation dose-response relationships measured for each life-history trait in adult female crickets.

Trait	Equation	R^2	F (DFn, DFd)	p	Preferred model
Adult body mass	$y = -0.02723x + 0.6491$	0.97	176.2 (1, 5)	< 0.0001	Linear
	$y = -1E\text{-}04x^2 - 0.0121x + 0.6592$	0.97	0.09248 (1, 4)	0.78	
Early adulthood oviposition rate	$y = -11.55x + 94.16$	0.95	116.6 (1, 5)	0.0001	Linear
	$y = -0.2534x^2 - 1.8176x + 91.052$	0.98	5.006 (1, 4)	0.089	
Late adulthood oviposition rate	$y = -10.84x + 82.46$	0.95	97.9 (1, 5)	0.0002	Linear
	$y = 0.0587x^2 - 6.4997x + 89.266$	0.97	0.236 (1, 4)	0.65	
Average egg size	$y = -0.007644x + 1.125$	0.08	0.35 (1, 4)	0.58	Neither
	$y = -0.0037x^2 + 0.0437x + 1.0362$	0.65	4.879 (1, 3)	0.11	
Hatching success	$y = -0.0235x + 0.7353$	0.49	3.913 (1, 4)	0.12	Quadratic
	$y = -0.0042x^2 + 0.0429x + 0.641$	0.98	92.95 (1, 3)	0.0024	
Hatchling starvation resistance	$y = -0.1501x + 4.264$	0.91	43.64 (1, 4)	0.0027	Linear
	$y = 0.0011x^2 - 0.0888x + 4.3656$	0.92	0.05073 (1, 3)	0.84	

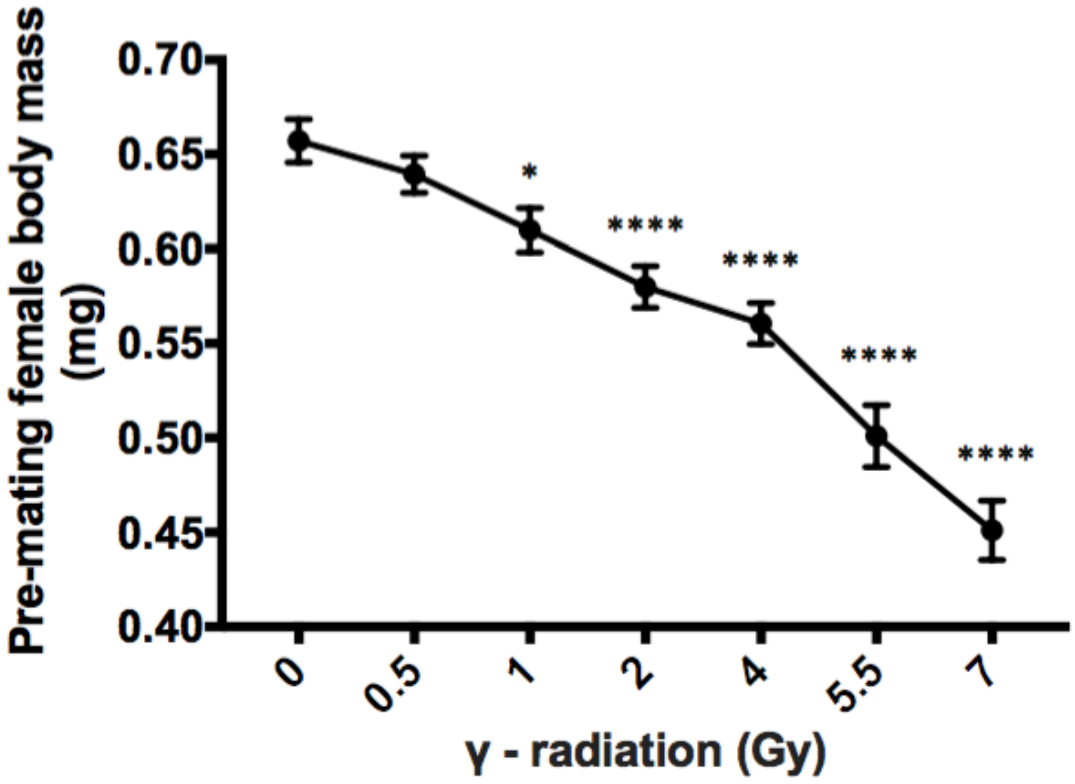

Figure 3.1: Dose-response effects of early life gamma-radiation exposure on pre-mating adult body mass of female crickets (mg). All mass measurements were taken on day 13 post maturation. All females were irradiated at 14 d of age at the following radiation doses: 0 (n = 69), 0.5 (n = 67), 1 (n = 60), 2 (n = 61), 4 (n = 50), 5.5 (n = 31), and 7 Gray (Gy) (n = 17) (dose rate = 0.25 Gy/min). Values are reported as means ± SEM (* $p < 0.05$, **** $p < 0.0001$). All significant differences reported are in comparison to the 0 Gy treatment group as revealed by Tukey's HSD test.

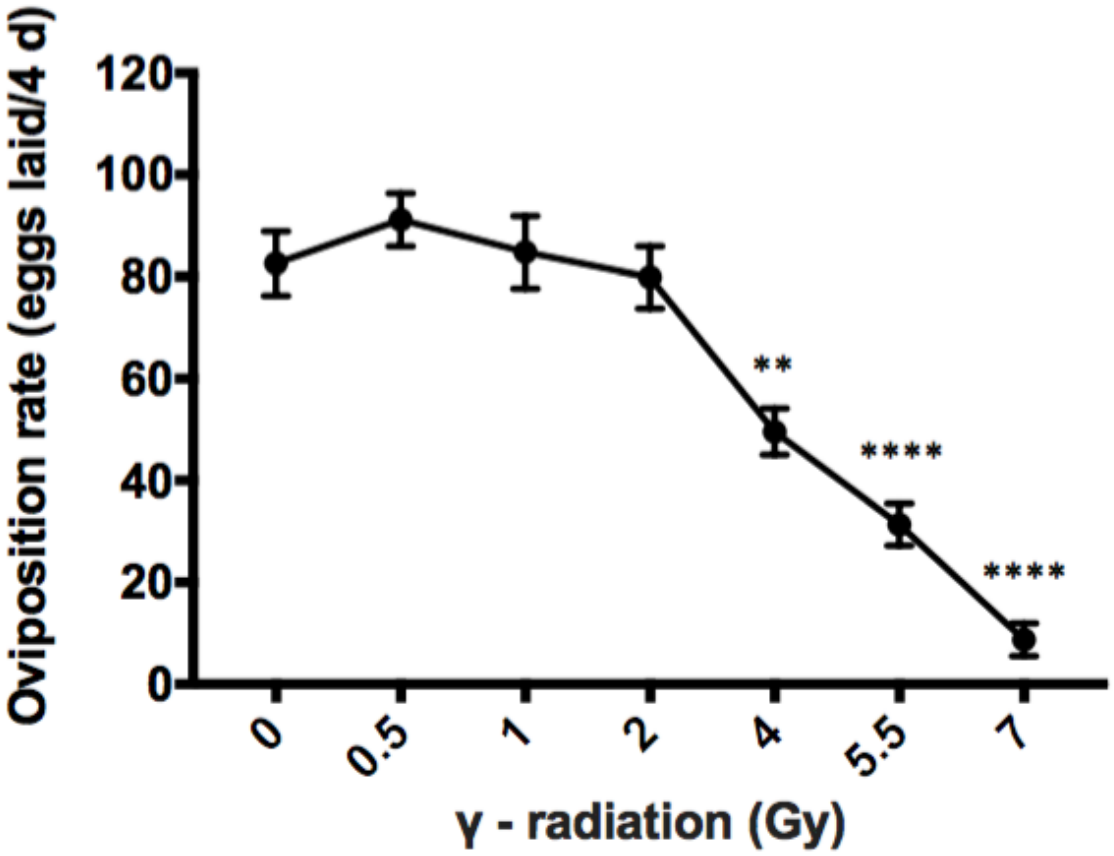

Figure 3.2: Dose-response effects of early life gamma-radiation exposure on 4-day oviposition rate of female crickets in early adulthood. Oviposition was recorded 14 – 18 d post maturation. All females were irradiated at 14 d of age at the following radiation doses: 0 (n = 33), 0.5 (n = 25), 1 (n = 32), 2 (n = 30), 4 (n = 24), 5.5 (n = 19), and 7 Gray (Gy) (n = 10) (dose rate = 0.25 Gy/min). Values are reported as means ± SEM (** $p < 0.01$, **** $p < 0.0001$). All significant differences reported are in comparison to the 0 Gy treatment group as revealed by Tukey's HSD test.

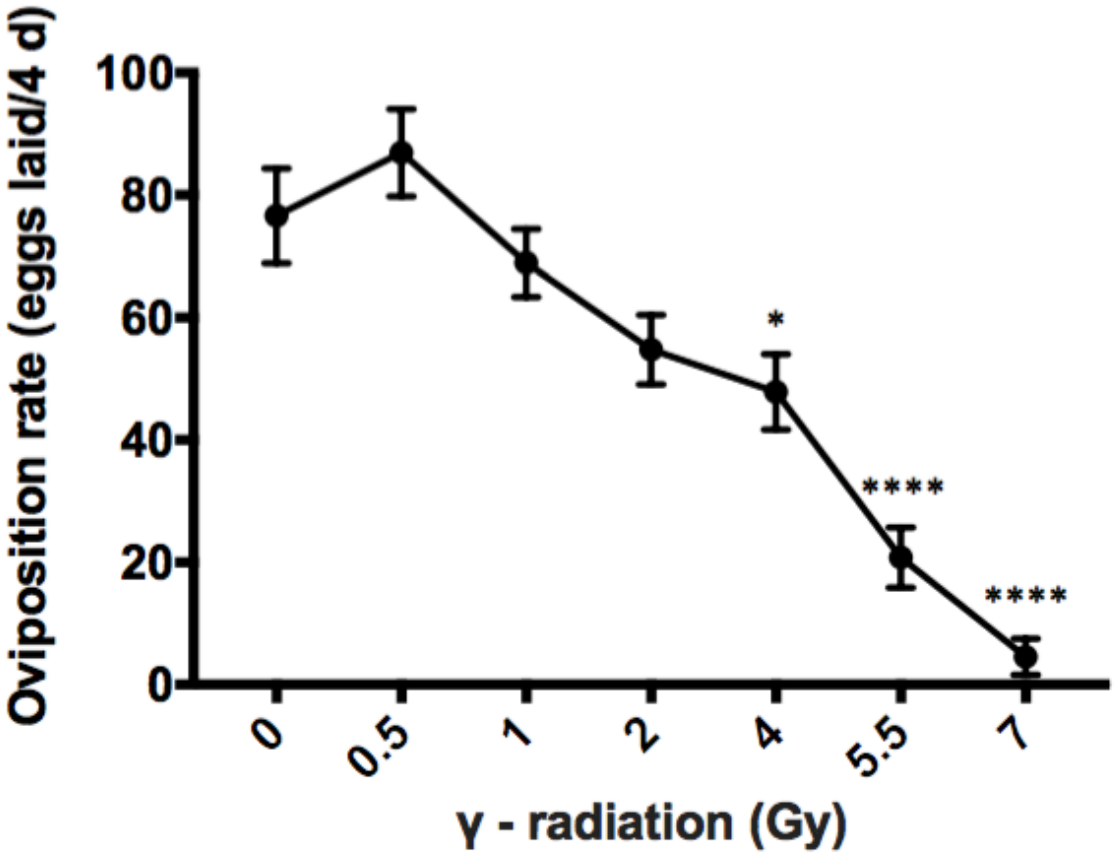

Figure 3.3: Dose-response effects of early life gamma-radiation exposure on 4-day oviposition rate of female crickets in late adulthood. Oviposition was recorded 20 – 24 d post maturation. All females were irradiated at 14 d of age at the following radiation doses: 0 (n = 24), 0.5 (n = 18), 1 (n = 25), 2 (n = 26), 4 (n = 18), 5.5 (n = 15), and 7 Gray (Gy) (n = 9) (dose rate = 0.25 Gy/min). Values are reported as means ± SEM (* $p < 0.05$, **** $p < 0.0001$). All significant differences reported are in comparison to the 0 Gy treatment group as revealed by Tukey's HSD test.

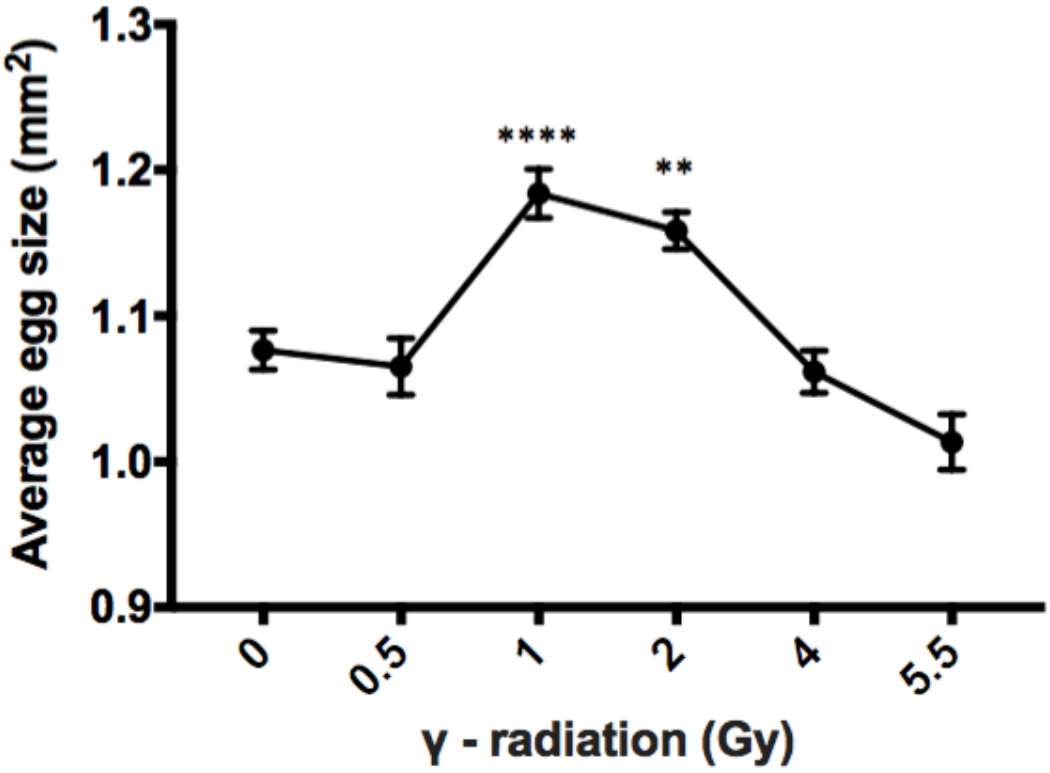

Figure 3.4: Average size of eggs (length x width) laid by adult female crickets exposed to gamma-radiation in early life. For each female, 5 – 11 eggs were measured (average = 6.7) for eggs laid 14 d post maturation. All females were irradiated at 14 d of age at the following radiation doses: 0 (n = 20), 0.5 (n = 14), 1 (n = 17), 2 (n = 17), 4 (n = 12), 5.5 Gray (Gy) (n = 10) (dose rate = 0.25 Gy/min). Values are reported as means ± SEM (** $p < 0.01$, **** $p < 0.0001$). All significant differences reported are in comparison to the 0 Gy treatment group as revealed by Tukey's HSD test.

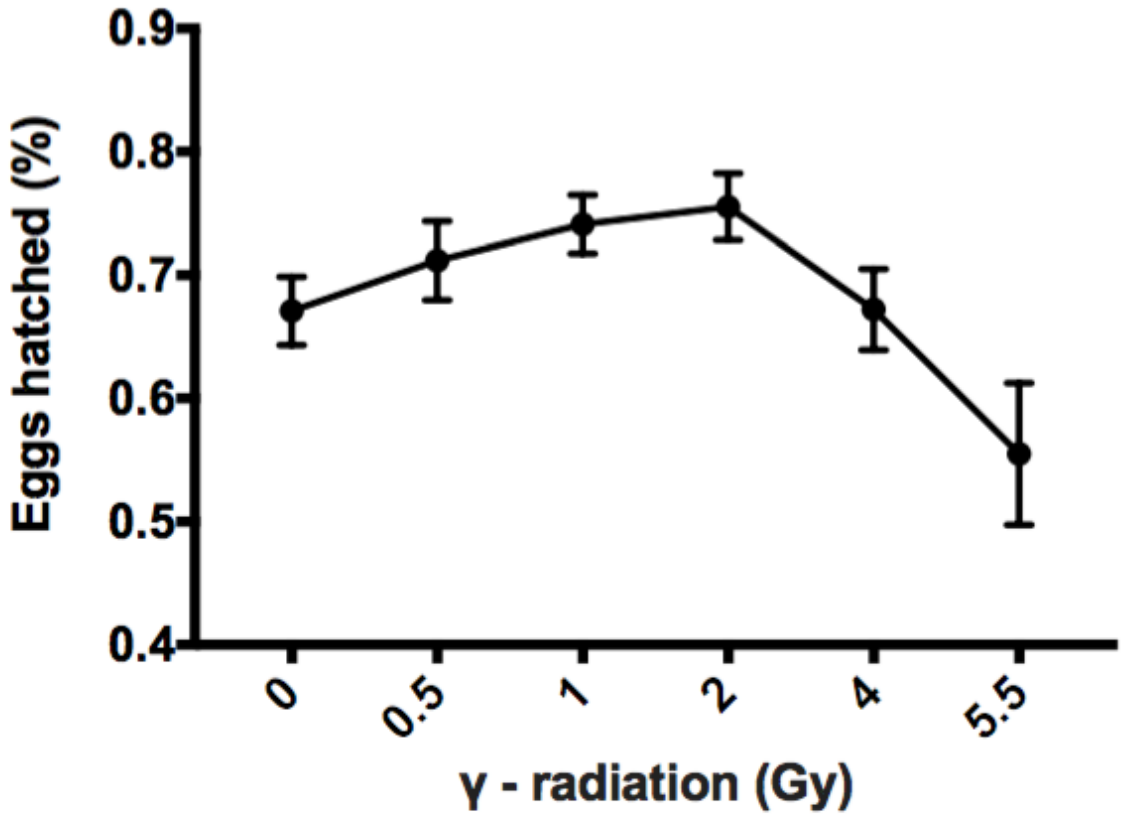

Figure 3.5: Hatching success of eggs laid by adult female crickets exposed to gamma-radiation in early life. For each female, hatching success of 20 eggs was recorded for eggs laid 14 – 18 d post maturation. For each female, hatching success of 20 eggs was recorded for eggs laid 14 – 18 d post maturation. All females were irradiated at 14 d of age at the following radiation doses: 0 (n = 19), 0.5 (n = 17), 1 (n = 17), 2 (n = 19), 4 (n = 18), 5.5 Gray (Gy) (n = 10) (dose rate = 0.25 Gy/min). Values are reported as means ± SEM. Significant differences were tested in comparison to the 0 Gy treatment group with Tukey's HSD test.

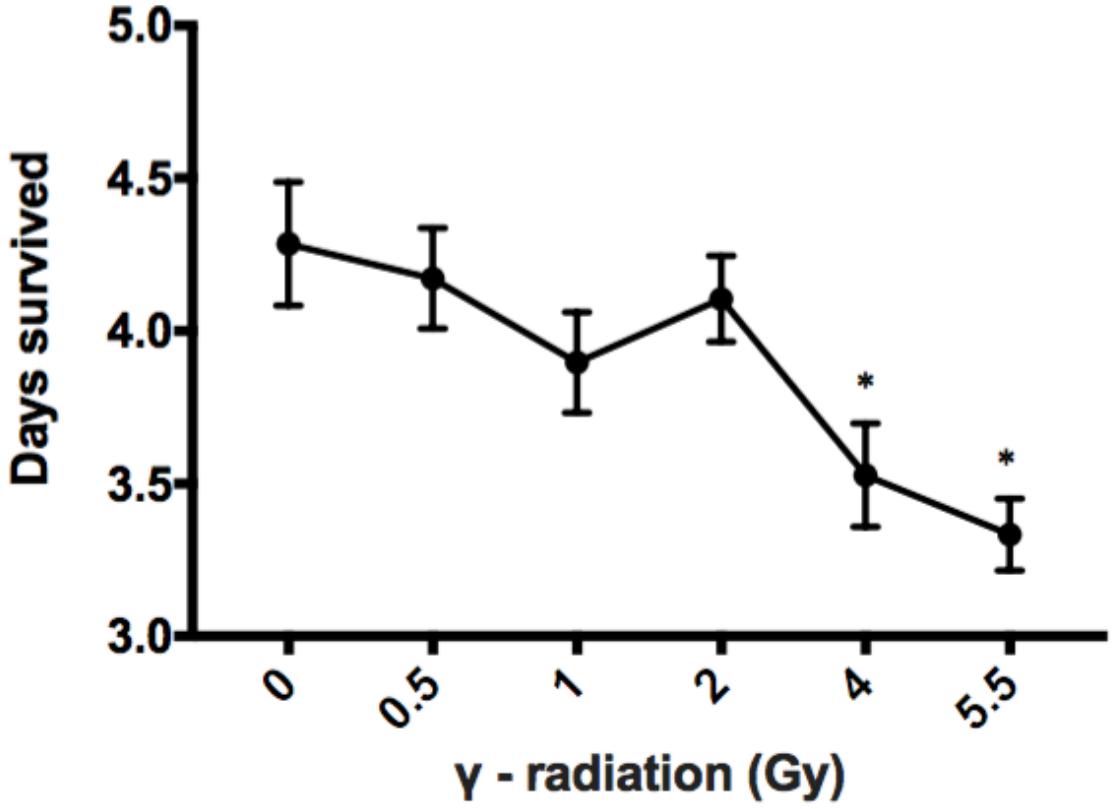

Figure 3.6: Starvation resistance (days survived without food) for hatchlings from eggs laid by adult female crickets exposed to gamma-radiation in early life. For each female, the average starvation resistance of three of her hatchlings was recorded from eggs laid 14 – 18 d post maturation. All females were irradiated at 14 d of age at the following radiation doses: 0 (n = 19), 0.5 (n = 16), 1 (n = 17), 2 (n = 19), 4 (n = 18), 5.5 Gray (Gy) (n = 9) (dose rate = 0.25 Gy/min). Values are reported as means ± SEM (* $p < 0.05$). All significant differences reported are in comparison to the 0 Gy treatment group as revealed by Tukey's HSD test.

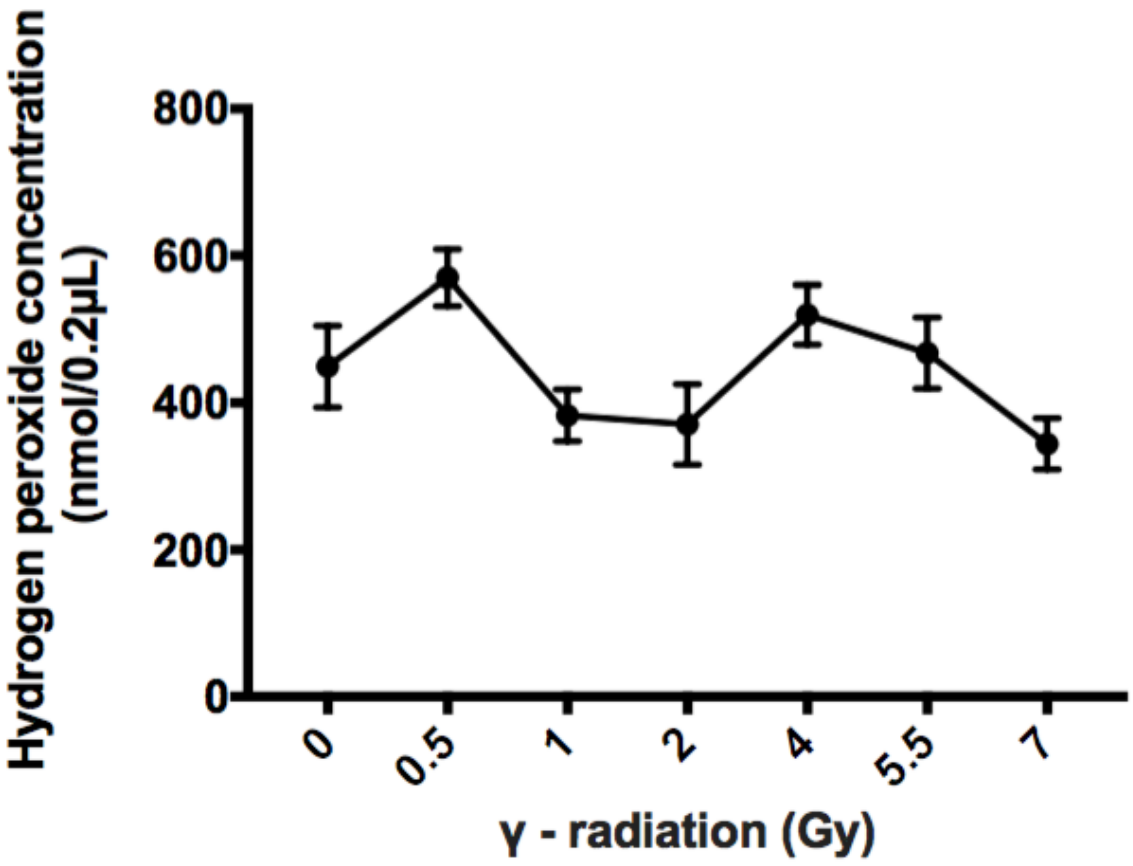

Figure 3.7
Dose-response effects of early life gamma-radiation exposure on hydrogen peroxide concentration (nmol/0.2 µL) in head homogenates from adult female crickets. Heads were removed at 14 d of age post maturation. All females were irradiated at 14 d of age at the following radiation doses: 0 (n = 8), 0.5 (n = 8), 1 (n = 8), 2 (n = 8), 4 (n = 8), 5.5 (n = 7), and 7 Gray (Gy) (n = 7) (dose rate = 0.25 Gy/min). Values are reported as non-transformed means ± SEM following ANCOVA with adult body mass as a covariate. Significant differences were tested in comparison to the 0 Gy treatment group with Tukey's HSD test.

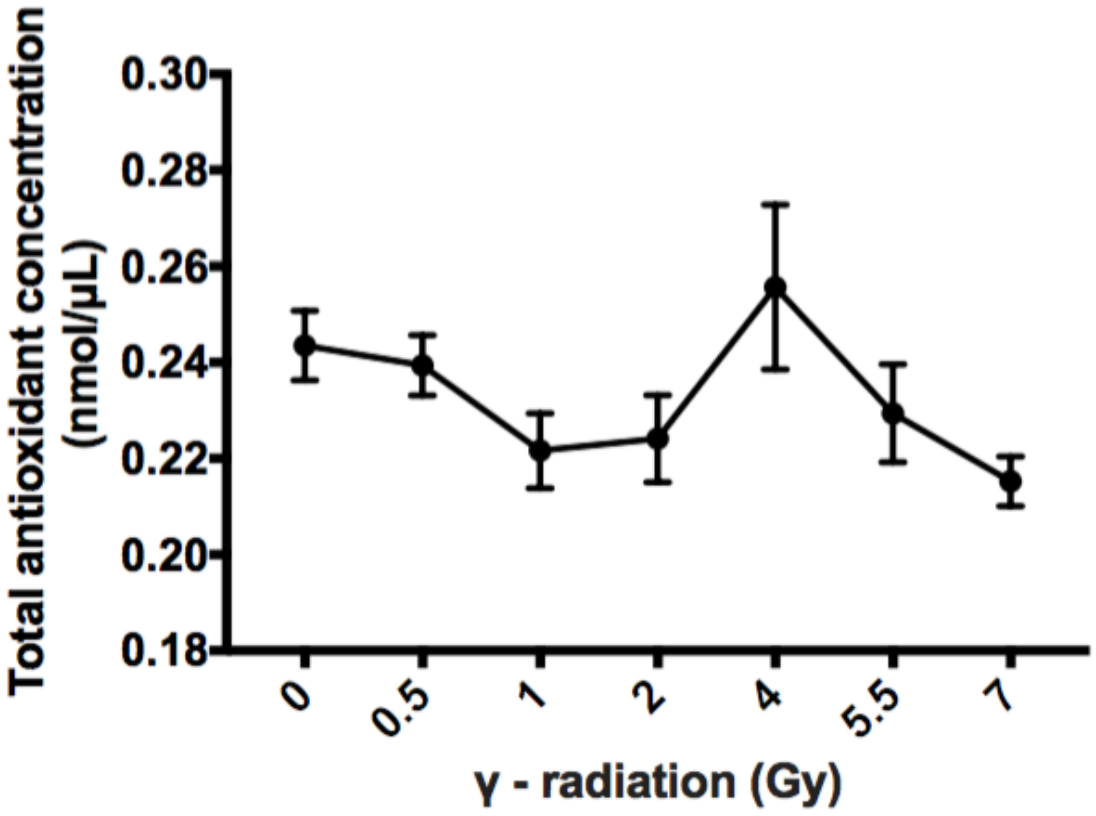

Figure 3.8
Dose-response effects of early life gamma-radiation exposure on total antioxidant capacity (nmol/µL) of head homogenates from adult female crickets. Heads were removed at 14 d of age post maturation. All females were irradiated at 14 d of age at the following radiation doses: 0 (n = 8), 0.5 (n = 8), 1 (n = 8), 2 (n = 8), 4 (n = 8), 5.5 (n = 7), and 7 Gray (Gy) (n = 7) (dose rate = 0.25 Gy/min). Values are reported as non-transformed means ± SEM following ANCOVA with adult body mass as a covariate. Significant differences were tested in comparison to the 0 Gy treatment group with Tukey's HSD test.

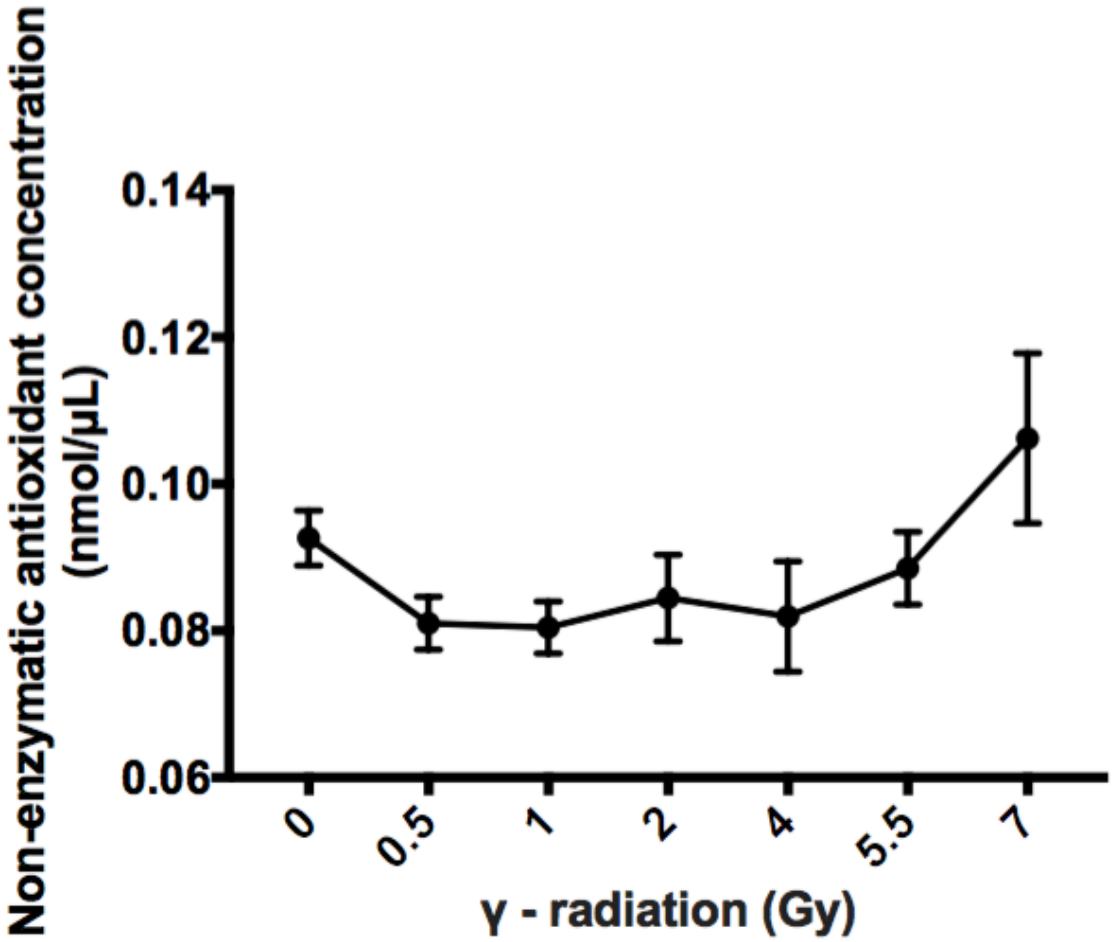

Figure 3.9: Dose-response effects of early life gamma-radiation exposure on non-enzymatic antioxidant capacity (nmol/µL) of head homogenates from adult female crickets. Heads were removed at 14 d of age post maturation. All females were irradiated at 14 d of age at the following radiation doses: 0 (n = 8), 0.5 (n = 8), 1 (n = 8), 2 (n = 8), 4 (n = 8), 5.5 (n = 7), and 7 Gray (Gy) (n = 7) (dose rate = 0.25 Gy/min). Values are reported as non-transformed means ± SEM following ANCOVA with adult body mass as a covariate. Significant differences were tested in comparison to the 0 Gy treatment group with Tukey's HSD test.

Conclusion

Summary, Perspectives, Limitations, and Future Directions

The idea that low levels of stressors or toxins can have stimulatory or hormetic biological effects is well known in toxicology and medicine. However, from an ecological and evolutionary perspective, hormesis has received far less attention (Costantini, Metcalfe & Monaghan 2010). Given that organisms in today's world are increasingly exposed to low levels of stressors or pollutants from human activities, understanding the potential fitness impacts of low-level stress exposures is an important goal. Early development is a life-history stage where hormetic responses are thought to be particularly robust and is also a time when organisms are highly susceptible to stress (Costantini 2014). In attempt to integrate early life hormesis into a life-history paradigm, this thesis aimed to explore the life-history effects of early development ionizing radiation exposure using the house cricket (*Acheta domesticus*) as a model system. This work focused on two central questions:

1. Does early life radiation hormesis reflect a tradeoff between growth and self-maintenance?
2. What are the dose-response effects of early life ionizing radiation exposure on adult life-history traits?

In this concluding section, I will briefly summarize the main results of this thesis, as well as their implications and applications. I will also discuss limitations to the work performed and suggest directions for future research.

Chapter 2: Evidence for a tradeoff between growth and self-maintenance

The research performed in Chapter 2 addressed the question of whether early life ionizing radiation hormesis reflects a tradeoff between growth and self-maintenance. This question was inspired by one of the predictions made by classical life-history theory that developing organisms ultimately face a tradeoff between investment in growth-related traits and self-maintenance (e.g., survival, repair or defense systems) (Stearns 1992). I hypothesized that a hormetic response to early life stress might reflect increased investment in one of these broad fitness components and might therefore be accompanied by decreased investment in the other (i.e., a life-history tradeoff). The results showed that crickets exposed to moderate doses of early life radiation stress exhibited a higher mean survivorship but lower growth rate relative to controls. This suggests that early life radiation hormesis reflected prioritization of self-maintenance over growth.

The idea that hormetic responses can be associated with life-history tradeoffs has important evolutionary and ecological implications. For instance, this suggests that hormetic responses to early life stress are associated with shifts in life-history allocation. This hypothesis has been argued theoretically by several authors (Forbes 2000; Costantini *et al.* 2010; Jager, Barsi & Ducrot 2013) and also has some empirical support (Saul *et al.* 2013; McClure *et al.* 2014). Theoretically, organisms should make such shifts in life-history allocation to maximize fitness under varying environmental conditions. For instance, a shift in life-history in which self-maintenance traits are prioritized over growth should ultimately maximize fitness in harsh or stressful environmental conditions. However, our finding that hormesis of survival is associated with reduced growth rate (i.e., a cost) is an indication of why this strategy requires a priming process to occur and is not constitutively expressed. Reduced growth rate can be expected to

carry several fitness costs that might be realized under natural ecological settings (Dmitriew 2011). For instance, organisms that grow slower may have a greater risk of mortality prior to first reproduction (e.g., due to predation or disease). Additionally, slower growth may limit access to mates and resources in adulthood relative to faster growing conspecifics. It is important to realize that the actual fitness consequences of this shift in life-history strategy were not examined in our study and can only be speculated. An important goal for future research should be to examine the degree to which such hormetic shifts in life-history allocation are adaptive in real ecological settings (i.e., in stressful vs. non-stressful environments).

The realization that hormetic responses may carry fitness costs also has important practical implications. For instance, risk models for low-dose radiation exposure tend to be based on testing whether single trait responses are best described by the LNT (i.e., radiation is harmful at all doses) or the hormetic model (i.e., radiation may be beneficial in the low dose range) (Vaiserman 2010). From a fitness perspective, however, evaluating the risks of low-dose exposures is more complex given that fitness is not a singular concept but consists of multiple interacting components (e.g., growth, reproduction, maintenance, behavior). This suggests that even hormetic responses in single traits may not reflect "risk-free" scenarios given that they can be associated with liniear responses in other fitness components (e.g., tradeoffs). This complicates our understanding of what can be considered a "safe" or "beneficial" effect of low-dose stress. I argue that future research addressing the fitness and environmental risks of low-dose stress and hormesis should adopt a multiple-trait approach similar to the one employed in this study to account for the possibility that hormesis can have underlying fitness costs. Additionally, future work should add environmental relevance to evaluating the risks of low-

dose stress exposures, since hormetic shifts in life-history strategies may improve fitness in certain environments but decrease fitness in others.

Chapter 3: Mixed effects on adult life-history traits

The research performed in Chapter 3 aimed to understand the dose-response effects of early life ionizing radiation exposure on fitness-related traits in adulthood. There is an emerging body of literature suggesting an important role for early life stress in shaping the adult phenotype (Crino & Breuner 2015; Monaghan & Haussmann 2015; Chaby 2016). While it is often assumed that stressful conditions in early life should result in negative fitness outcomes, sustained and stimulatory effects of early life stress are increasingly reported. In this work, we examined the dose-response effects of early life radiation exposure on measures of adult body size (a proxy for lifetime fecundity), age-specific fecundity, oxidative stress physiology (hydrogen peroxide levels and total and non-enzymatic antioxidant capacity) offspring investment (egg size), and proxies of offspring fitness (hatching success and hatchling starvation resistance) in adult female *A. domesticus*. It was found that trait performance varied with respect to the magnitude of radiation stress received in early life and that responses were trait-specific. For instance, adult body size showed a strongly linear response to early life radiation exposure, while age-specific fecundity measured in early and late adulthood both showed threshold responses (i.e., both were relatively unaffected by low to moderate radiation doses but were significantly impaired by high radiation doses). In contrast, egg size showed a hormetic response to low radiation doses, whereas measures of oxidative stress physiology and offspring fitness were unaffected throughout the dose range tested.

The main significance of this work is that it shows that early life stress can have diverse and unpredictable impacts on adult fitness-related traits and that rather than being purely detrimental, adult responses can be sustained or hormetic. We did not examine the phenotypic changes responsible for producing these diverse effects, but they can be hypothesized to involve altered patterns of gene expression or physiology during development. As discussed in Chapter 3, oxidative stress physiology likely plays a key role in determining developmental responses to early life radiation exposure, given the direct effect of ionizing radiation on ROS production (Riley 1994; Koch & Hill 2017). Future work should explore the potentially important role of oxidative stress in mediating life-history effects of radiation exposure, as well as differentiating adaptive developmental responses from general susceptibility to stress (i.e., dysfunction). This work also has implications for the emerging field of ecological evolutionary developmental biology (eco-evo-devo), which considers the important role of environmental factors in shaping the phenotypic variation on which natural selection acts (Sultan 2007; Gilbert, Bosch & Ledón-Rettig 2015). Future research integrating concepts from toxicology (including radiation biology research) and eco-evo-devo will be extremely important for understanding ecological and evolutionary responses to human-induced environmental change.

There are several limitations to the research presented in this chapter that should be discussed. For instance, it would have been ideal to measure adult survival and longevity in these adult females to determine how early life radiation stress impacts the hypothesized life-history tradeoff between reproduction and longevity. However, measurements of longevity were not possible in this experiment given that adult females had to be sacrificed for the oxidative stress assays. For testing such questions, vertebrates may be a more feasible model given that non-terminal measures of oxidative stress endpoints (e.g., blood samples) are easier to obtain.

Another important point is that although *A. domesticus* offers several advantages for studying adult female reproductive allocation strategies (e.g., there are no complexities associated with parental care or multiple breeding seasons), this species is capable of laying thousands of eggs over its adult lifetime, making measurements of true reproductive output unfeasible. Although we accounted for two measures of age-specific fecundity and approximated lifetime fecundity by measuring pre-mating adult body mass, potential complexities of early life stress on reproductive output could have been missed due to our inability to monitor females over their entire reproductive lifespan.

An additional limitation to using an insect model like *A. domesticus* to examine the effects of early life stress on adult phenotypes is that this species naturally has a high background juvenile mortality rate. Therefore, it is possible that the range of phenotypic effects observed in this study could be biased by the relatively "high quality" individuals that make it to adulthood. A more general limitation to the work in this thesis is that the Chapter 2 and 3 studies were each performed on separate populations of animals. Using the same population for both studies would have been ideal to avoid potential confounding factors that could make the study populations less comparable (e.g., genetic differences or environmental or rearing conditions). However, this was hardly possible given the nature of the experimental design as well as the space and resources available.

Lastly, it should be realized that even though this work could have implications for the fitness impacts of ionizing radiation pollution in natural ecosystems, all work was performed under controlled laboratory conditions, meaning that there are limitations to the degree to which these results are relevant to the real world. The fact that all studies described here involved acute delivery of radiation when organisms in radio-contaminated areas are likely to experience

chronic low-dose exposures is one factor that could limit the applicability of these results to field scenarios.

Concluding remarks

The empirical work presented in this thesis was largely exploratory in nature and was part of an effort to develop *A. domesticus* as a model system for testing between LNT and hormetic life-history trait responses to ionizing radiation. This work barely scratches the surface of the vast range of topics that can be explored under this paradigm. For instance, the direct relationship between ionizing radiation and ROS physiology provides a unique opportunity for testing the role of oxidative stress in mediating life-history tradeoffs (Monaghan, Metcalfe & Torres 2009; Costantini 2014). Additionally, all work described here focuses on the effects of radiation exposure at one stage of development (i.e., 2 weeks of age). An interesting direction for future work would be to see how life-history responses to radiation vary with age or stage of exposure (e.g., juveniles vs. adults). The relevance of early life hormetic responses to other aspects of life-history, such as mate choice, cognition, and immunity, also present interesting avenues for future inquiry.

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Supplementary Figures

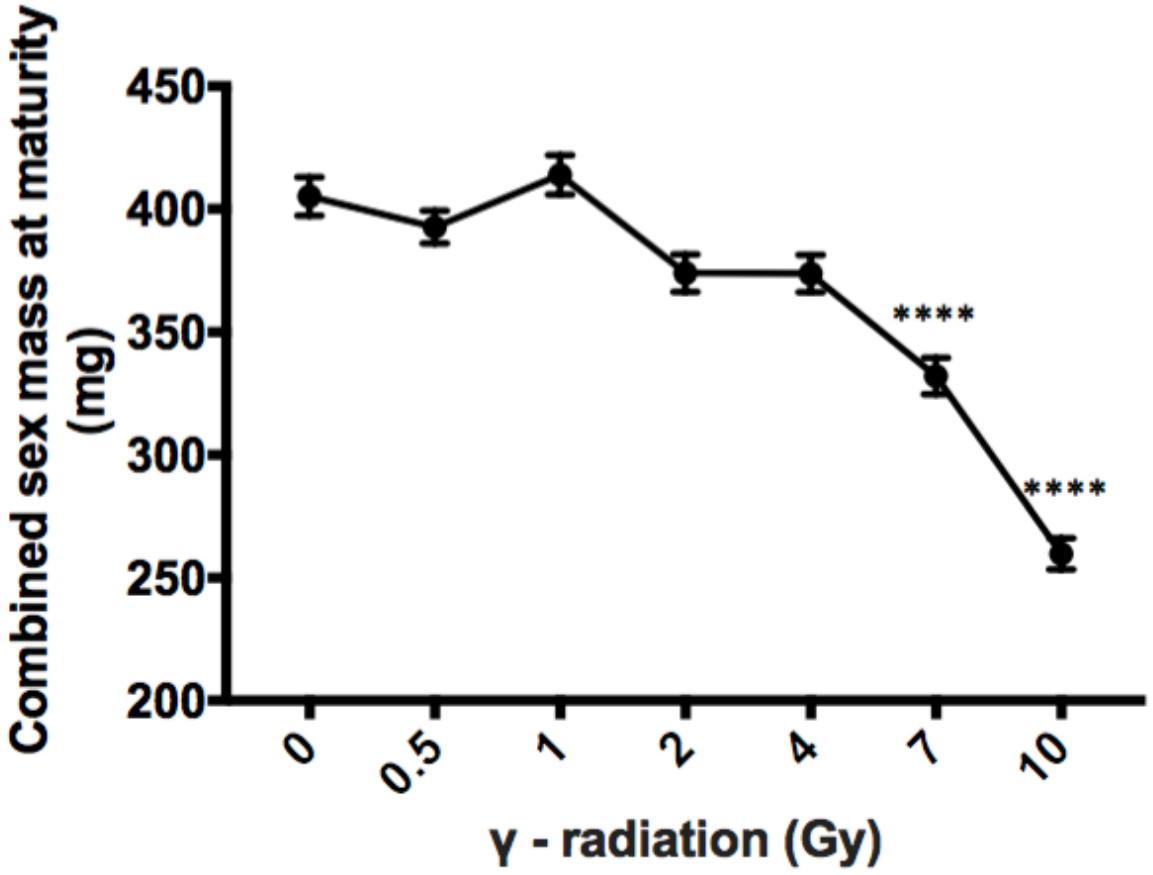

Figure S2.1: Dose-response effects of early life gamma-radiation exposure on combined male and female cricket body mass on the day of maturity (mg). All crickets were irradiated at 14 d of age at the following radiation doses: 0 (n = 42), 0.5 (n = 50), 1 (n = 47), 2 (n = 51), 4 (n = 53), 7 (n = 51), and 10 Gray (Gy) (n = 48) (dose rate = 0.25 Gy/min). Values are reported as means ± SEM (**** $p < 0.0001$). All significant differences reported are in comparison to the 0 Gy treatment group as revealed by Tukey's HSD test.

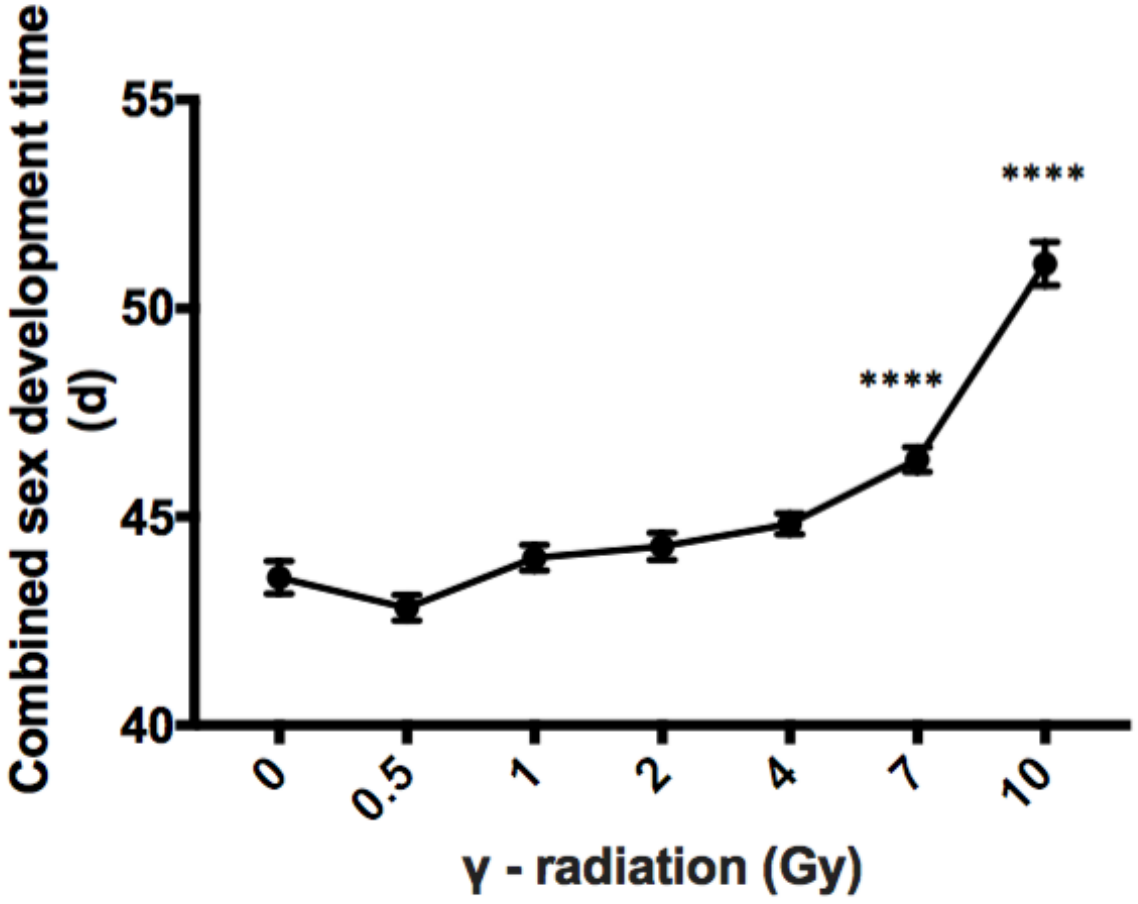

Figure S2.2: Dose-response effects of early life gamma-radiation exposure on combined male and female cricket juvenile development time (d). All crickets were irradiated at 14 d of age at the following radiation doses: 0 (n = 42), 0.5 (n = 50), 1 (n = 47), 2 (n = 51), 4 (n = 53), 7 (n = 51), and 10 Gray (Gy) (n = 48) (dose rate = 0.25 Gy/min). Values are reported as means ± SEM (**** $p < 0.0001$). All significant differences reported are in comparison to the 0 Gy treatment group as revealed by Tukey's HSD test.

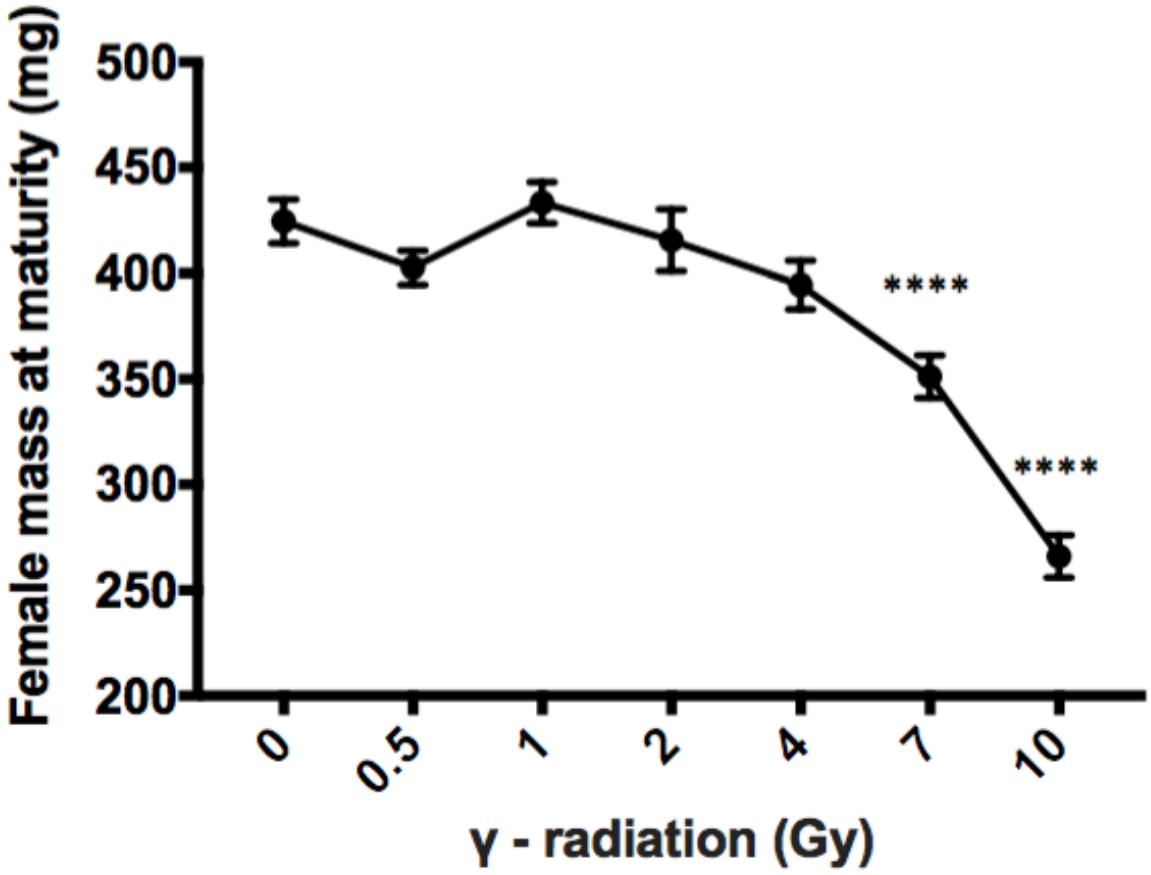

Figure S2.3: Dose-response effects of early life gamma-radiation exposure on female cricket body mass on the day of maturity (mg). All crickets were irradiated at 14 d of age at the following radiation doses: 0 (n = 22), 0.5 (n = 31), 1 (n = 26), 2 (n = 14), 4 (n = 25), 7 (n = 28), and 10 Gray (Gy) (n = 23) (dose rate = 0.25 Gy/min). Values are reported as means ± SEM (**** $p < 0.0001$). All significant differences reported are in comparison to the 0 Gy treatment group as revealed by Tukey's HSD test.

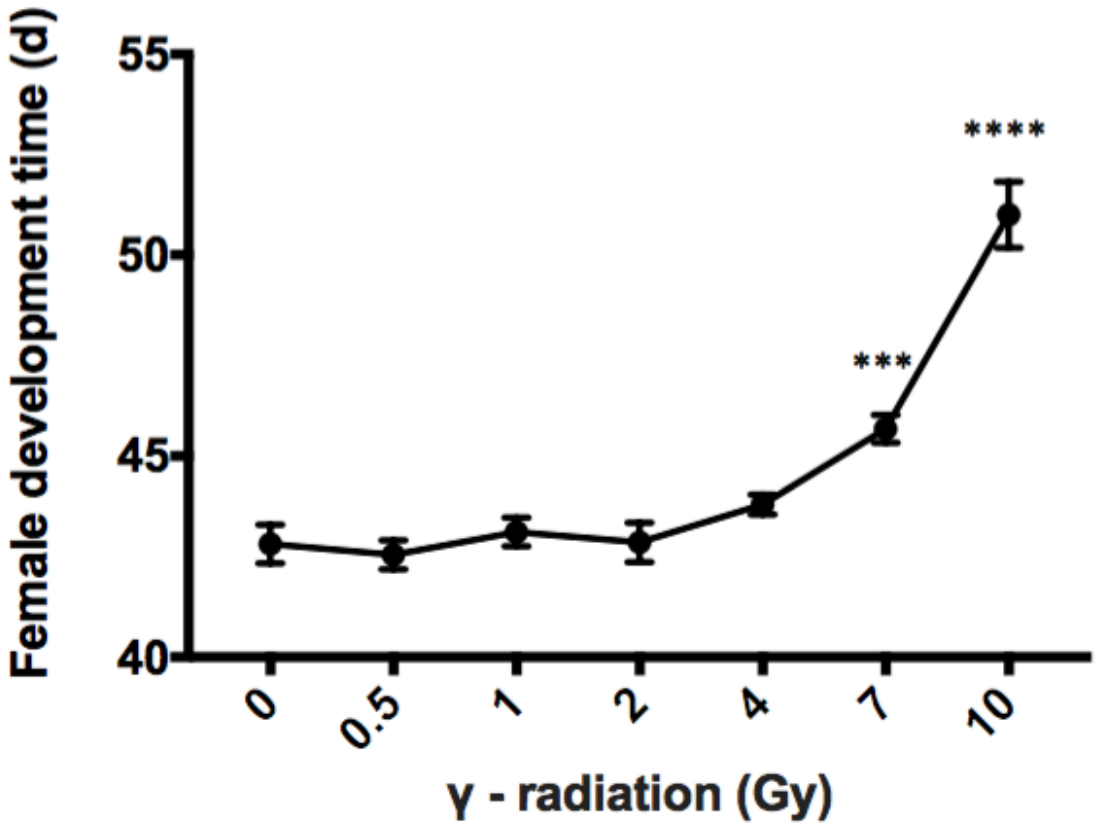

Figure S2.4: Dose-response effects of early life gamma-radiation exposure on female cricket juvenile development time (d). All crickets were irradiated at 14 d of age at the following radiation doses: 0 (n = 22), 0.5 (n = 31), 1 (n = 26), 2 (n = 14), 4 (n = 25), 7 (n = 28), and 10 Gray (Gy) (n = 23) (dose rate = 0.25 Gy/min). Values are reported as means ± SEM (*** $p < 0.001$, **** $p < 0.0001$). All significant differences reported are in comparison to the 0 Gy treatment group as revealed by Tukey's HSD test.

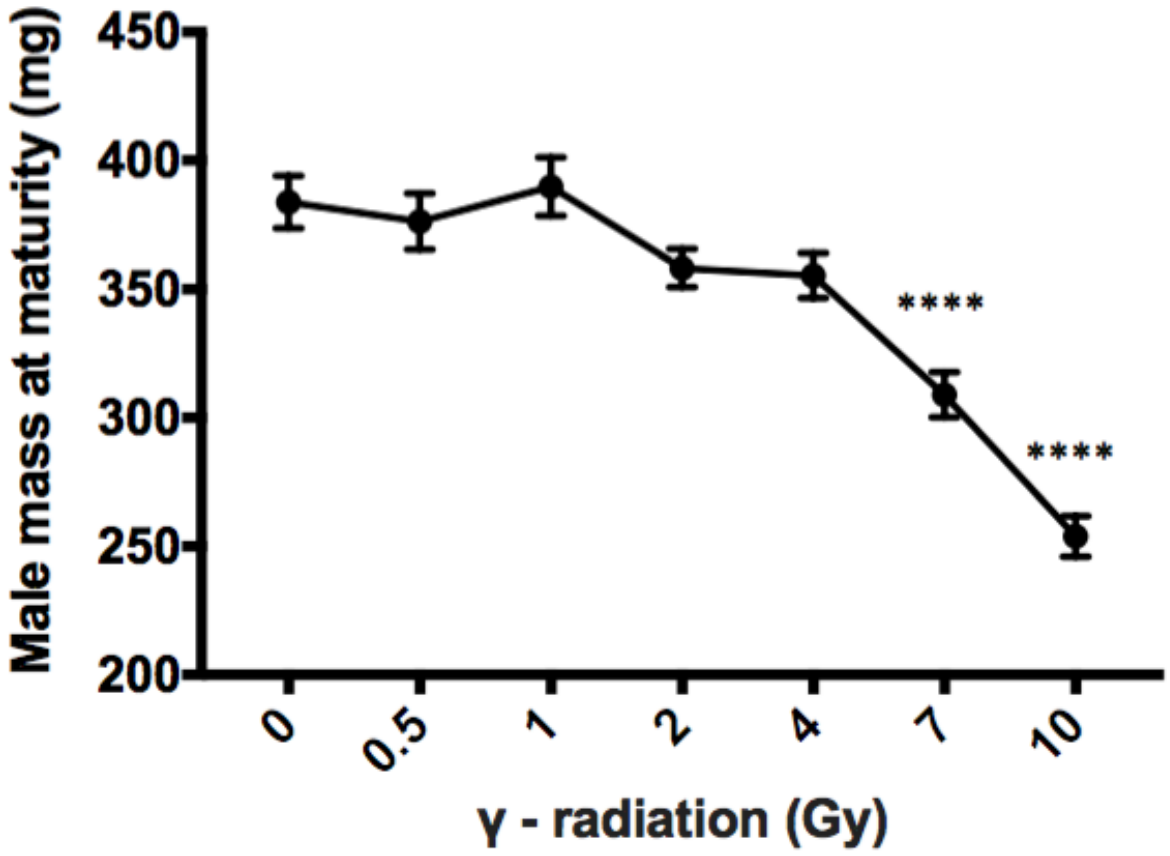

Figure S2.5: Dose-response effects of early life gamma-radiation exposure on male cricket body mass on the day of maturity (mg). All crickets were irradiated at 14 d of age at the following radiation doses: 0 (n = 20), 0.5 (n = 19), 1 (n = 21), 2 (n = 37), 4 (n = 28), 7 (n = 23), and 10 Gray (Gy) (n = 25) (dose rate = 0.25 Gy/min). Values are reported as means ± SEM (**** p <0.0001). All significant differences reported are in comparison to the 0 Gy treatment group as revealed by Tukey's HSD test.

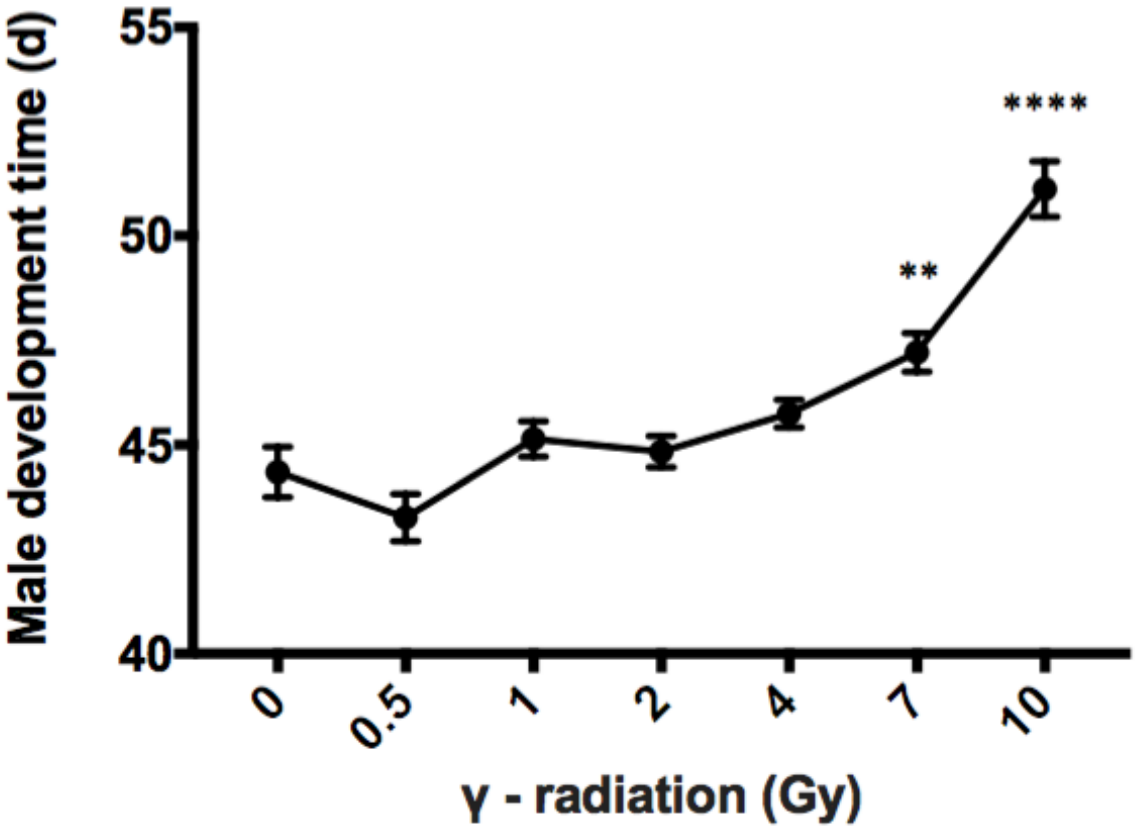

Figure S2.6: Dose-response effects of early life gamma-radiation exposure on male cricket juvenile development time (d). All crickets were irradiated at 14 d of age at the following radiation doses: 0 (n = 20), 0.5 (n = 19), 1 (n = 21), 2 (n = 37), 4 (n = 28), 7 (n = 23), and 10 Gray (Gy) (n = 25) (dose rate = 0.25 Gy/min). Values are reported as means ± SEM (** $p < 0.01$, **** $p < 0.0001$). All significant differences reported are in comparison to the 0 Gy treatment group as revealed by Tukey's HSD test.

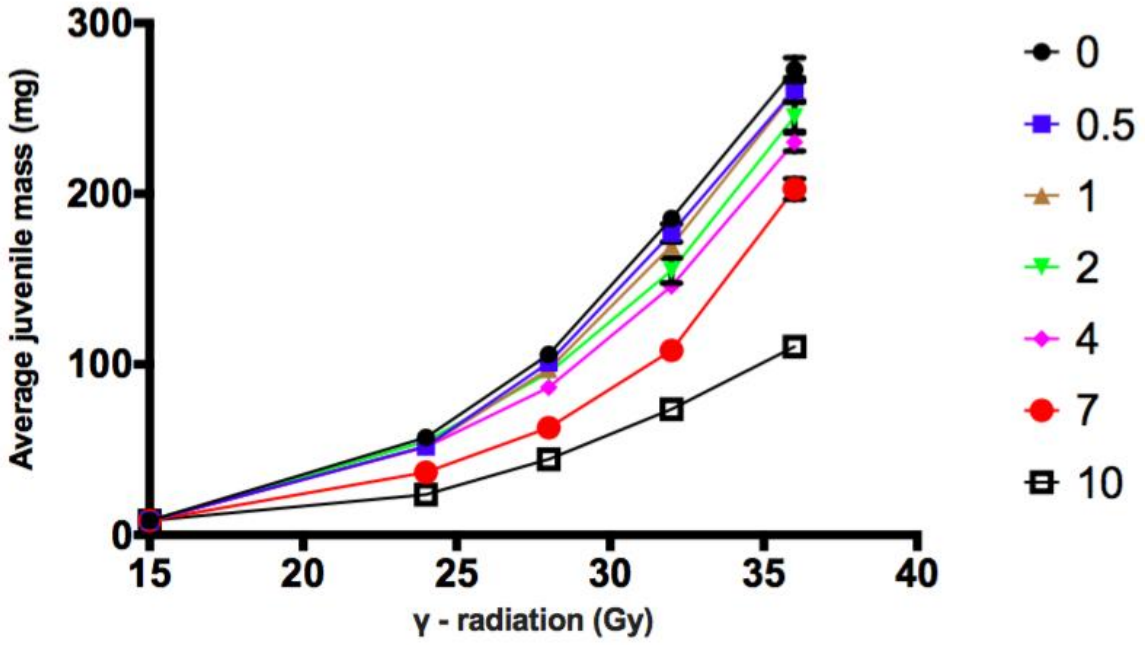

Figure S2.7: Dose-response effects of early life gamma-radiation exposure on juvenile mass (mg) measured at 24, 28, 32, and 36d of age. Repeated mass measurements in each radiation treatment group were recorded for groups of 6 crickets. All crickets were irradiated at 14 d of age at the following radiation doses: 0, 0.5, 1, 2, 4, 7, and 10 Gray (Gy) (dose rate = 0.25 Gy/min). Values are reported as means ± SEM. At each time point, 7 Gy and 10 Gy treatments are significantly smaller than 0 Gy ($p < 0.0001$). The 4 Gy treatment is significantly smaller than 0 Gy at 28 ($p < 0.01$), 32 ($p < 0.001$), and 36 d ($p < 0.001$). The 2 Gy treatment is significantly smaller than 0 Gy at 32 d ($p < 0.001$). At each time point, all significant differences reported are in comparison to the 0 Gy treatment group as revealed by Tukey's HSD test.