A DOSE OF EXPERIMENTAL HORMESIS:
WHEN MILD STRESS PROTECTS AND IMPROVES ANIMAL PERFORMANCE

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ABSTRACT

The adaptive response characterized by a biphasic curve is known as hormesis. In a hormesis framework, exposure to low doses leads to protective and beneficial responses while exposures to high doses are damaging and detrimental. Comparative physiologists have studied hormesis for over a century, but our understanding of hormesis is fragmented due to rifts in consensus and taxonomic-specific terminology. Hormesis has been and is currently known by multiple names; preconditioning, conditioning, pretreatment, cross tolerance, adaptive homeostasis, and rapid stress hardening (mostly low temperature: rapid cold hardening). These are the most common names used to describe adaptive stress responses in animals. These responses are mechanistically similar, while having stress-specific responses, but they all can fall under the umbrella of hormesis. Here we review how hormesis studies have revealed animal performance benefits in response to changes in oxygen, temperature, ionizing radiation, heavy metals, pesticides, dehydration, gravity, and crowding. And how almost universally, hormetic responses are characterized by increases in performance that include either increases in reproduction, longevity, or both. And while the field can benefit from additional mechanistic work, we know that many of these responses are rooted in increases of antioxidants and oxidative stress protective mechanisms; including heat shock proteins. There is a clear, yet not fully elucidated, overlap between hormesis and the preparation for oxidative stress theory; which predicts part of the responses associated with hormesis. We discuss this, and the need for additional work into animal hormetic effects particularly focusing on the cost of hormesis.

Keywords: dose response, antioxidants, life history, trade-offs, POS hypothesis.

INTRODUCTION

The notion in the field of toxicology that the dose makes the poison, referencing dose responses and lethal effects, dates back to Paracelsus more than 500 years ago (Mattson and Calabrese 2010), and it is at the heart of the concept known as hormesis. The idea behind the term hormesis dates back to the 1800s when Hugo Schulz first described a low dose stimulatory response associated with a toxic agent; sodium hypochlorite (Calabrese and Baldwin 2000). But the term itself was first used to describe a low dose excitatory response of fungi to red cedar tree extracts (Southam and Ehrlich 1943, Calabrese 2014). As we use the term today, hormesis is defined as an adaptive biphasic dose response where low doses result in protective effects that can lead to improved organismal performance while high doses result in detrimental effects that lead to negative performance and fitness consequences (Calabrese and Baldwin 2001, Calabrese et al. 2007, Mattson 2008, Calabrese 2016a,b). In this framework, mild exposure to chemical, biotic, or abiotic stressors elicits an adaptive response that elevates cellular defenses and protects the organism. This protection is accompanied by a boost in performance that goes beyond that seen in untreated individuals as seen in our own hormetic dose response curve of *Drosophila melanogaster* exposed to x-ray irradiation (Fig. 1). Beyond toxicology and stress physiology, interest in this protective mechanism is growing in regard to human aging, the treatment of disease (Calabrese and Blain 2005, Calabrese et al. 2012), and exercise physiology (Ji et al. 2010, Ji et al. 2016). In this review, we will focus on how comparative animal physiologists have studied hormesis in non-human animals, and why there is additional need for mechanistic work elucidating how hormesis affects animal performance, as well as, the cost associated with this adaptive protective response.

In the comparative physiology literature, hormesis is known by different names and one of the biggest disconnects in stress physiology is the challenge that biological stress response terminology represents (Calabrese et al. 2007). Different subdisciplines focus on their own terminology to the disadvantage of an overall and universal term such as hormesis. Adaptive response and cross tolerance are the most common terms that displace hormesis in the literature. The term adaptive response refers to a plastic response, that occurs after exposure to mild doses of a toxic agent, aimed at the restoration of homeostasis (Samson and Cairns 1977). Similarly, adaptive homeostasis is used to explain the increased or decreased seen in the homeostatic range of an animal exposed to sub-lethal conditions (Davies 2016). While the term cross tolerance refers to the animal's ability to defend against damage caused by stressor B after a brief exposure to stressor A (Gruber and Keyser 1945). All four terms fall under the banner of hormesis, as hormesis is an adaptive response to stress that elevates cellular defenses targeting the restoration of homeostasis; those elevated defenses can then provide additional protection from a second, often more challenging stressor (Mattson 2008). The terms preconditioning or pretreatment are also occasionally encountered in the comparative physiology literature. Under pretreatment, preconditioning, or conditioning a mild/brief stressor is applied to the animal followed by a stronger stressor or cellular damaging event. The pretreatment is meant to elevate the cellular defenses to offer protection from the stronger stressor. The way we define and use pretreatment and preconditioning is functionally no different than the usage of cross tolerance. Catfish pretreated with hypoxia (low oxygen) being able to survive higher temperatures (Burleson and Silva 2011) and anoxia protecting flies from gamma radiation (Fig. 2; López-Martínez and Hahn 2012, 2014) are just a couple of examples of this type of hormesis. What clusters these approaches together is that they rely on the adaptive response to low doses that is hormesis.

One of the central tenets of hormesis is that it allows for cellular protection to build up during the mild dose exposure, and these defenses are present for some time following the end of the exposure (López-Martínez and Hahn 2012). It is these defenses that prevent the accumulation of stress-induced damage and therefore improve organismal performance over time. At the core of this mechanism of cellular protection is the mitochondria. It is hypothesized that reactive oxygen species (ROS) are disproportionately generated during bouts of stress (Halliwell and Gutteridge 1993), and during mild stress, these ROS serve as signaling molecules that promote normal functioning (Ristow and Schmeisser 2014, Sies 2017). In fact, it may be small amounts of ROS production that are a crucial part of the adaptive response we call hormesis (Huang et al. 2019). These ROS are thought to signal that the resumption of normal metabolism will be accompanied by higher and damaging levels of ROS production, and the ensuing oxidative damage, thus activating cellular defenses. It is this mechanism that is described as the Preparation for Oxidative Stress (POS) hypothesis (Hermes-Lima et al. 1998, Hermes-Lima and Zenteno-Savin 2002, Giraud-Billoud et al. 2019). And because there is ample evidence that animals prepare for oxidative stress when exposed to mild stress (Storey 1996, Giraud-Billoud et al. 2019), there is an overlap between the POS hypothesis and hormesis that is not yet currently fully understood.

OXYGEN HORMESIS

Low oxygen has been one of the leading stressors known to confer hormetic effects dating back at least 45 years (Robinson 1975). The benefits of low oxygen are mechanistically rooted in the POS hypothesis (Giraud-Billoud et al. 2019), where the mitochondria upon experiencing a decrease in oxygen, prepares for the ensuing oxygen reperfusion by elevating cellular defenses; primarily oxidative stress defenses (Hermes-Lima et al. 1998, 2001). These defenses often exceed what is required to compensate for oxygen reperfusion damage and the animal benefits from the additional protective boost. In addition to the multiple scenarios where an animal may encounter hypoxia in their habitat (under water, underground, inside hosts, high altitudes, etc.), many animals possess varying degrees of tolerance to a total lack of oxygen; anoxia (Storey 1996). The bulk of the low oxygen hormesis work has focused in anoxia and it is connected to the remarkable tolerance that some animals have to an oxygen free environment. Vertebrates do not have the robust tolerance for long periods in anoxia at physiological-relevant temperatures (above 20ºC) that invertebrates have (Storey 1996), still we find that poikilothermic vertebrates are able to survive prolonged periods of anoxia (i.e. more than 24hrs), if the exposure occurs at low temperatures normally associated with overwintering responses. When temperatures range from 3 to 5ºC, red-sided garter snakes, *Thamnophis sirtalis parietalis*, and leopard frogs, *Rana pipiens*, can survive several days of anoxia (Churchill and Storey 1992, Pinder et al., 1992), while red-eared slider turtles, *Trachemys scripta elegans*, experience anoxia for three to four months during their overwintering period (Hermes-Lima and Zenteno-Savín 2002). This tolerance to an oxygen free environment for such a long period is connected to increased activities of various antioxidant enzymes (Hermes-Lima and Zenteno-Savín 2002). An impressive anoxic response is seen in the goldfish, *Carassius auratus*, where individuals can survive an eight-hour exposure at physiologically relevant temperatures (20ºC; Lushchak et al. 2001). On the other hand, invertebrates encounter anoxia at higher degrees due to their soil-dwelling stages and their semi-aquatic ecologies, and they have evolved adaptations to prevent damage from oxygen deprivation (Storey 1996, Harrison et al. 2006). Because of this, invertebrates can tolerate longer periods of anoxia at physiological temperatures. The Caribbean fruit fly, *Anastrepha suspensa*, can survive upwards of 50 hours at 25ºC without negatively impacting flight performance (López-Martínez and Hahn 2012). On the more extreme side, larvae tiger beetles in the genus *Cicindela* can survive more than six days in anoxia, which is usually accompanied by being submerged in water (Hoback et al. 1998). For both invertebrates and vertebrates, survival of prolonged periods of anoxia is a remarkable physiological feat, nevertheless, most of this work has focused on extended anoxia tolerance and not on hormesis. While these animals may gain survival, longevity, and/or higher reproductive outputs from their anoxia exposure; whether these exposures lead to hormesis remains largely unexplored. In a hormetic framework, it is short bouts of anoxia exposure (minutes to a few hours) that will trigger protective mechanisms that confer defense and boost performance.

Most of what we know about anoxia hormesis comes from cross tolerance experiments where anoxia is used as a preconditioning treatment prior to exposure to sub-lethal or lethal doses of stress. In the locust, *Locusta migratoria*, thermotolerance increases after a short (1 hr.) exposure to anoxia (Wu et al. 2002). Locust that experienced anoxia can survive up to 1.5 hours at 53ºC; a benefit that is connected to the long flights experienced during migration that are accompanied by increased oxygen demand and high temperatures. On the other temperature extreme we have house flies, *Musca domestica*, that are able to survive at -7ºC after a short exposure (40 minutes) to anoxia (Coulson and Bale 1992). In this context, anoxia acts as the mild temperature pretreatment that triggers the adaptive hormetic response known as rapid stress hardening (RSH; described in the next section). Anoxia hormesis can also protect from additional exposure to anoxia. In *Anastrepha suspensa*, anoxia experienced during development triggers the reallocation of stored lipids and changes the dynamics of recovery by reducing the oxygen debt, without decreasing adult fecundity and longevity (Visser et al. 2018).

When it comes to anoxia hormesis, the most understood protective effects that boost animal performance come from work that examines the effects of anoxia when combined with a strong oxidizing event; ionizing radiation. Whether using gamma radiation or X-rays, short exposure (1 hr.) of anoxia prior to irradiation leads to significant improvements in organismal performance. The first recorded evidence of this was in codling moths, *Cydia pomonella*, where an oxygen free environment led to survival of normally lethal gamma radiation doses (Robinson 1975). This prompted an interest in exploring whether hypoxia could have similar protective effects and in fact low oxygen is results in improved treatment survivorship, flight performance, and mating competitiveness in Mediterranean fruit flies, *Ceratitis capitata* (Hooper 1971, Ohinata et al. 1977, Nestel 2007). In another fruit fly, *A. suspensa*, the mechanism of anoxia hormesis involves the upregulation of various antioxidant enzymes (mitochondrial and cytosolic SODs and glutathione peroxidase), which help lower radiation-induced oxidative stress and increase flight ability, starvation resistance, mating success, and longevity (López-Martínez and Hahn 2012, 2014). The increases in longevity were significant, allowing flies that would die in a matter of hours to live for weeks (Fig. 2). This mechanistic finding of how anoxia confers its hormetic benefits also provides additional support to the preparation for oxygen stress hypothesis as the same mitochondrial protection mechanisms are involved in both responses, providing further evidence for the link between hormesis and the POS hypothesis (Giraud-Billoud et al. 2019, Geihs et al. 2020). While most of these anoxia hormetic effects are male-specific, females survive higher doses of gamma radiation when combined with anoxia conditioning (López-Martínez and Hahn 2012). The benefits extend into mating, where male mating success is higher in anoxia-irradiated males at the peak of sexual maturity (10 days after treatment), but the males remain sexually competitive into old age (30 days); mating at a higher rate (19:1 at 30 days vs

3:1 at 10 days) than there non-hormetic counterparts (López-Martínez and Hahn 2014). Similar effects were recorded in the cactus moth, *Cactoblastis cactorum*, where anoxia prior to X-ray irradiation improves flight performance (López-Martínez et al. 2014). These male moths were more likely to fly, flew for longer periods of time, and for further distances. Additionally, the male moths had increased antioxidant capacity, which was linked with increased mating success, living longer, and higher F_1 progeny hatching. In *Trichoplusia ni* moths, anoxia hormesis rescues gamma radiation induced mortality (López-Martínez et al. 2016b).

Most of these anoxia hormetic effects were male-specific and the likely reason is that females bear a strong cost of hormesis that has been harder to quantify in males. Dating back to Robinson's work, he found that the anoxia-mediated survival of lethal gamma radiation doses experienced by the moths came with a reduction of F_1 offspring (~10% less; Robinson 1975). Even though he did not present his findings in a hormesis context, this represents the first recorded cost of anoxia hormesis and one of the first ever recorded costs associated with any type of hormesis. Since then, other groups have found that the cost of anoxia hormesis is connected to reproduction. In the cabbage looper moth *T. ni*, females receiving anoxia hormesis (in the absence of cross tolerance), experienced a significant decreased in the number of eggs laid (~60%), and an additional decrease in the number of laid eggs that hatched (~70%; López-Martínez et al. 2016b). This reduction in fecundity and fertility associated with anoxia hormesis was also recently found in the mealworm beetle, *Tenebrio molitor*. Female beetles that lived longer and were more active during old age as a result of anoxia hormesis, experience a total reproduction output decline of 40% (De La Torre and López-Martínez unpublished). This suggests that the anoxia hormesis protective response operates under a classic life history trade-off response (Stearns 1989). Quantifying the costs of hormesis is crucial given the increased performance experienced by the animals that received it, and these benefits extend beyond the parental generation and provide transgenerational protection. In cactus moths, there was higher pupation and adult emergence of F_1 offspring (López-Martínez et al. 2014). And the offspring that experienced higher rates of survival were more readily able to build protective webs upon hatching, in the absence of food (López-Martínez et al. 2016a). An additional and usual protective effect of anoxia was the rescue of X-ray and gamma radiation-induced sterility in cactus and cabbage looper moths (López-Martínez et al. 2016a,b); indicating that at least a fraction of the radiation-induced sterility normally associated with ionizing radiation sterilization is related to oxidative damage to DNA that can be prevented with anoxia hormesis.

TEMPERATURE HORMESIS

Given the connection between seasonality and temperature, we find a lot of the work on temperature hormesis primarily focusing on low temperature as it relates to overwintering survival strategies (Storey and Storey 1988, Denlinger and Lee 2010). A common type of animal temperature hormesis is rapid cold hardening (RCH). First studied in flies (Lee et al. 1987), under RCH a brief (1 to 2hr) exposure to non-freezing low temperatures (2 to 5ºC) triggers an adaptive response that provides protection from lower temperatures and freezing injury (Chen et al. 1987). The literature on RCH is extensive with at least 120 papers that carry rapid cold hardening in the title and at least an additional 100 that deal, in part, with the phenomena. The known short-term effects of RCH include increased survival of sub-zero exposures (Denlinger and Lee 2010), decreased chill-coma inducing temperature (Kelty and Lee 1999), decreased lower freeze tolerance limit (Lee et al. 2006), decreased water loss rates (Yoder et al. 2006, Wada and Matsukura 2011), and increased cell viability protecting against apoptosis (Yi et al.

2007). Investigations into RCH have mostly focused on the short-term survival benefits of this type of hormesis, but studies into the long-term benefits have revealed that this type of hormesis is not just about short-term stress survival. Some of the long-term effects are connected to fitness as RCH preserves courtship and mating performance in flies (Shreve et al. 2004), increases fecundity and longevity in aphids (Powell and Bale 2005), prevents the disruption of learning/conditioning in flies (Kim et al. 2005), and preserves flight ability in butterflies (Larsen and Lee 1994). Mechanistically, sugar alcohols and trehalose are involved in this type of hormesis, alongside heat shock proteins and membrane restructuring genes. Transcriptomic analysis have revealed that redox signaling is also a part of this type of hormesis response (MacMillan et al. 2016).

As in most types of hormesis, very little work exists quantifying the cost of RCH. It has been found that in certain species, like *Musca domestica*, the increased cold tolerance associated with RCH can lead to shorter lifespan, reduced oviposition, and lower F_1 emergence rates (Coulson and Bale 1992), but this was not the case in *Drosophila melanogaster* (Kelty and Lee 1999) or *Sarcophaga crassipalpis* were RCH rescues cold-shock induced losses in fecundity in both sexes (Rinehart et al. 2000). It is conceivable that the reduction in reproduction could be masked by the increased performance in the short term, but it would be made apparent in the long-term. It stands to reason that a certain tradeoff between performance and reproduction must be present in this hormetic scenario (Stearns 1989), and previous studies have focused more on the strong and broad short-term response than the less visible long-term effects, including the cost of RCH. Rapid stress hardening (RSH) responses are mostly studied in low temperature (RCH), but there is some work indicating that rapid heat hardening (RHH) also confers protection from lethal high temperatures. A brief (1 hr.) pretreatment at 37ºC showed improved survival at 43ºC in the codling moth, *Cydia pomonella* (Chidawanyika and Terblanche 2011) but this was not the case in hematophagous bed bugs, *Cimex lectularius* (Benoit et al. 2009a). In other systems, like the channel catfish *Ictalurus punctatus*, a preconditioning low oxygen treatment triggers an RHH response that increases their CT_{max} (Burleson and Silva 2011). This indicates that hormetic responses to temperature, just like those to anoxia, are widespread among animals and may be triggered by short/sublethal temperature exposures or other stressors such as variations in oxygen concentration.

IONIZING RADIATION HORMESIS

Ionizing radiation (ultraviolet, gamma rays, and X-rays) may be one of the first non-chemical stressors used to investigate low dose protective effects. This work dates back over one hundred years when low dose X-ray experiments first showed lifespan extension in animals (Davey 1917, 1919). At a time that predates much of our understanding of oxidative stress, antioxidant biology, and hormesis; it was clear that X-rays had a protective effect in flour beetles, *Tribolium confusum*, with potential to stimulate cell proliferation and immune function (Calabrese 2013). Much of what we know about X-ray hormesis comes from insects, and a large proportion of the work relates to radiation-based control strategies like the sterile insect technique (SIT; Klassen and Curtis 2005) and phytosanitary irradiation (IPT; Hallman 2011); where high doses are used to achieve pest control through sterility or death. However, in a hormesis context, we are interested in the effect that low doses have at providing protection and ionizing radiation-induced lifespan extensions have been observed in flies, mosquitoes, moths, crickets, beetles, and wasps (Calabrese 2013). In addition to finding the appropriate hormetic dose that can induce protection and boost performance, the age of the individual at the time of treatment can have dramatic effects on hormesis. A few days can strongly influence response outcomes between a treatment being hormetic, and extending lifespan, or becoming lethal in beetles (Ducoff 1975) and caterpillars (López-Martínez et al. 2016b). Exposure to low dose gamma radiation shortened embryogenesis time and led to heavier larvae that produced more silk in their pupal cocoons in the silkworm, *Bombyx mori* (Yusifov et al. 1990, Shibamoto et al. 2017). This represents a strong hormetic effect that improved multiple life history traits in these animals, but without lifespan information we do not know the full extent of the benefit to the silkworms. These hormetic effects of low dose ionizing radiation are not limited to invertebrates, and low dose gamma radiation is known to extend the lifespan of laboratory mice by about 20% (Caratero et al. 1998).

While the mechanism behind low dose X-rays life extension has not been completely elucidated (see Table 1), there seems to be a connection to the mitochondria and energetics as seen in *Drosophila* where starvation plays a role in their increase in longevity (Lamb 1964). These flies live longer when low dose radiation is combined with starvation. The redox signaling connection is deepened when *Drosophila* mutants for antioxidant genes, heat shock proteins, and DNA repair genes had reduced activity and shorter lifespans when exposed to low dose X-rays (Moskalev et al. 2006, Moskalev et al. 2009). Whether looking at males or females, low dose gamma radiation extends lifespan in *Drosophila,* and that hormetic effect is connected with the elevated expression of stress genes including heat shock proteins (Zhikrevetskaya et al. 2015). Additionally, expression of antioxidant enzymes and oxidative stress response genes seem to be a crucial component of a proposed mechanism for lifespan extension in response to X-ray hormesis (Seong et al. 2011). This mechanistic explanation has gathered support from a vertebrate model where low-dose X-rays trigger increases in glutathione, catalase, and glutathione-S-transferase in the tissues and blood of female Wistar rats (Sharma et al. 2019). This increase in antioxidants led to a decrease of oxidative damage to the liver, kidney, and brain in these animals. And while oxidative damage in the blood was not decreased as it was in tissues, there was an increase of immune cells (lymphocytes and eosinophils) in response to low dose radiation indicating a level of protection being activated in the blood (Sharma et al. 2019).

Another type of ionizing radiation, ultraviolet radiation (UVR), is more pervasive across animal taxa because it reaches the earth's surface and penetrates deeply into the waters (UVA-320-400nm; Misra et al. 2002), some of it penetrates through regions with thinner ozone layers (UVB-(290-320nm; McKenzie et al. 2011), but fortunately its most energetic and damaging form is absorbed by the atmosphere (UVC-200 -290nm; Schuch et al. 2017). UVA incidence is naturally much higher than UVB in any given latitude in the northern and southern hemispheres (Schuch et al. 2017), but UVB represents a challenge for polar animals (López-Martínez et al. 2008). Increases in ROS production and the ensuing oxidative stress are associated with increases in the activity of various antioxidant enzymes (Hermes-Lima et al. 1998, Agnez-Lima et al. 2012, Won et al. 2014). This response is mediated in part by transcription factors that stimulate antioxidant gene expression (Cadet et al. 2005, Agnez-Lima et al. 2012). The combination of the production of ROS and the increase in antioxidant enzymes suggests that UVR hormesis would likely occur given the appropriate level of exposure, nonetheless, little experimental data exists. Most data on UVR hormesis comes from plants, where UVR promotes growth (Tezuka et al. 1993). There is recent data showing that marine copepods exposed to non-lethal UVR doses, will have larger first clutches of eggs (Heine et al. 2019) and we have data indicating that UVR exposure in *Drosophila* flies improves performance (Berry III and López-Martínez unpublished data). These are the first steps in trying to quantify UVR hormetic effects, and if these effects follow the same pattern as other types of ionizing radiation then they would also be rooted in redox signaling and the POS hypothesis.

CHEMICAL HORMESIS

Although comparative physiology hormesis work largely focuses on environmental abiotic factors and how they lead to hormetic effects on animals, studies featuring environmental toxins and pesticides continue to increase in prevalence because of the potential exposure of animals to low doses of these chemicals and the effect of those exposures can have on their performance and survival. Heavy metals like arsenic and cadmium are dangerous soil contaminants but low doses of arsenite prolongs lifespan in nematodes (Schmeisser et al. 2013). Arsenite triggers the production of ROS which in turn activates antioxidant defenses. These defenses are linked to the longer life experienced by the worms, similarly to other types of hormesis (Fig. 3). Low doses of cadmium increase growth rate in American toads (*Bufo americanus*, James and Little 2003) and leopard frogs (*Rana pipiens,* Gross et al. 2007), and result in body size gains and faster metamorphic rates in Chinese toads (*Bufo gargarizans*, Ya et al. 2019). Additionally, low doses of cadmium elicit a protective heat shock protein response in mouse cells (Damelin 2000).

Another big class of environmental contaminants where hormesis has been found is pesticides. At low doses, pesticides can increase fecundity and reproductive output by increasing the net reproductive rate (NRR); a measure of how well females are being replaced in subsequent generations. Neonicotinoids, like clothianidin, increase fecundity and NRR in black cutworm moths (*Agrotis ipsilon*, Ding et al. 2018). Imidacloprid, another neonicotinoid, leads to faster development and higher NRR in thrips (*Frankliniella occidentalis*, Cao et al. 2019), increased fecundity in *Paederus fuscipes* beetles (Feng et al. 2019), increased reproductive output and survival under stress in green peach aphids (*Myzus persicae*, Rix et al. 2016), and increased survival when challenged with higher doses in *M. persicae* (Rix and Cutler 2018). The pyrethroid Lambda-cyhalothrin increases fecundity and NRR in *Mythimna separata* moths (Li et at. 2019), and the pyrethroid deltamethrin increases NRR in maize weevils (*Sitophilus zeamais*, Guedes et al. 2010), while cyantraniliprole, a ryanoid insecticide, increases fecundity in *Drosophila suzukii* (Shaw et al. 2019). Even glyphosate, known to have hormetic effects in plants (Brito et al. 2018), was found to make earthworms grow heavier and live longer when exposure was combined with warmer soil temperatures; possibly a type of cross tolerance hormesis (Pochron et al. 2019). Because of concerns over off-target effects of insecticides and colony collapse disorder, Cutler and Rix (2015) reviewed whether pesticide hormesis was recorded in honeybees. Caffeine and nicotine were shown to positively impact long-term memory and retention (Wright et al. 2013), and improved olfactory learning (Thany and Gauthier 2005). The effects of chemical hormesis in honeybees were not originally reported as hormesis but it was the efforts of additional investigation that revealed them as such (Cutler and Rix 2015). It is likely that there are more pesticide hormetic effects in the literature, as these effects are not reported as hormetic but rather as failures of pesticide efficacy. Pesticide hormesis is not just limited to invertebrates, wood frogs survive exposure from certain insecticides after experiencing prior exposure to different insecticides (Hua et al. 2013). Thus, it is foreseeable that pesticides trigger a type of rapid stress hardening hormetic response that allows for additional protection.

OTHER TYPES OF HORMESIS

Hormesis has also been investigated in a variety of systems and in response to stressors that are far less understood than oxygen, temperature, and chemicals. Mild (small amounts of water loss) or slow (water loss over a longer period) dehydration is associated with hormetic effects in a cross-tolerance framework. Dehydration (mild or slow) leads to increased pupariation, increased cell viability, and faster recovery from low temperature exposure in flesh flies, *Sarcophaga bullata* (Yi et al. 2017). In a polar insect, *Belgica antarctica*, slow mild dehydration leads to greater survival when exposed to -14ºC (Benoit et al. 2009b, Kawarasaki et al. 2019); and that slow rate of dehydration also increases survival to lethal high temperatures (30 ºC; Benoit et al. 2009b). There seems to be a type of pathway cross talk between low doses of anoxia, temperature, and dehydration which allows these stressors to induce rapid stress hardening responses and protect against lethal temperatures. Additionally, these three stressors are linked through shared metabolomic profile responses (Michaud et al. 2008). Slow dehydration is associated with increased expression of heat shock proteins, antioxidant enzymes, and membrane remodeling genes (López-Martínez et al. 2009). Even when that dehydration comes about from exposure to hyperosmotic sea water, it results in higher freezing tolerance survival in *B. antarctica* (Elnitsky et al. 2009). This effect of dehydration extends to *D. melanogaster* flies that can recover faster from chill coma if they were previously selected for dehydration resistance, although there is evidence that this hormetic effect might not be entirely related to the selection event (Sinclair et al. 2007).

A stressor that is far less understood than most is gravity and there is strong evidence that hypergravity (3 or 5 g) has hormetic effects in *Drosophila* flies (Le Bourg et al. 2004). Two weeks at hypergravity at a young age increased longevity in males (Le Bourg et al. 2004). Hypergravity also had additional protective effects as it triggered a rapid stress hardening response that increased survival time at 37ºC. But these improvements in longevity and survival are not related to the antioxidants enzymes (Le Bourg and Fournier 2004), which indicates that this type of hormesis does not align with the POS hypothesis and likely results from a completely different mechanism. Hypergravity also has an effect in cichlid fish, *Oreochromis mossambicus*, were otoliths growth was decreased but overall size was not (Anken et al. 2001). It is unclear whether this otolith growth response represents a hormetic effect and if so what the magnitude of that effect might be, but responses to hypergravity are not restricted to invertebrates and lower vertebrates and have also been recorded in pregnant Sprague-Dawley rats (Plaut et al. 2003). In the mammary tissues of these rats, the metabolic rate was decreased as a response of gravity manipulation as gravitational load increased; an effect similar to the response of flies to repeated anoxia (Visser et al. 2018).

A recent hormetic effect has been associated with crowding during development. In the larvae of *Drosophila*, crowding increases survival time at low (-3ºC) and high (38ºC) temperatures (Youn et al. 2018). Higher density crowded larvae emerged as adults faster and lived longer (Lushchak et al. 2019). It is clear from these two studies that certain stress genes (i.e. HSP70) are involved in this adaptive stress response but the role of antioxidant enzymes and oxidative stress protection genes is not as clear. Larval crowing leads to competition and the reduction of high-quality food, and in addition to the stresses associated from a larger group of animals present (i.e. temperature and water balance), starvation leads to mitochondrial efficiency differences. These mitochondrial differences are linked to ROS production and likely play a role in this rather unique type of hormesis.

THE MECHANISM OF HORMESIS

In the last ten years, we have seen nearly a doubling in the number of hormesis publications that aim at understanding the mechanisms of these adaptive responses (Calabrese et al. 2016A). The mechanistic underpinnings for the extensive performance effects seen in animals as a result of hormesis range from the quantification of specific polyols to genome-wide analysis highlighting specific pathways (Table 1). There are specific key players that are consistently linked to hormetic responses throughout the animal kingdom. Genes related to redox signaling, such as antioxidant enzymes and non-enzymatic antioxidants, whose role is the prevention/reduction of oxidative damage are involved nearly everywhere they are investigated (Table 1). Whether it is gene expression (Moskalev et al. 2009, Seong et al. 2012), protein expression (Yi et al. 2007), or enzyme activity (Hermes-Lima et al. 1998, Hermes-Lima and Zenteno-Savín 2002, López-Martínez and Hahn 2012), antioxidant mechanisms that reduce oxidative damage play a pervasive role in hormesis. Stress genes, like heat shock proteins, are also involved along with genes involved in DNA repair and apoptosis. To date, the data published indicates that redox and stress signaling play a central role in most hormetic responses, indicating a potential universal mechanism for hormesis. However, given the wide array of stressors that animals endure, there are stress-specific responses that widen the mechanistic targets of hormesis. Such are the cases of RCH and hypergravity. Under rapid cold hardening, multiple low temperature mechanisms (homeoviscous adaptation, increase polyol concentration, and freezing resistance) are activated in this response; in addition to the expected hormesis genes. Hypergravity exposure seems to be independent of the involvement of the main antioxidant enzymes, which challenges the notion of a universal hormetic mechanism. Our own ongoing *Drosophila* transcriptomic work comparing different hormesis treatments (low oxygen, low temperature, x-ray irradiation, UV irradiation) points toward general and unique responses for each condition. We are emboldened by recent research in this area and we encourage more mechanism studies that compare contextual factors in stress response to further our understanding of the role hormesis plays in animals.

FUTURE DIRECTIONS

We highlighted studies that show some of the remarkable protective effects that hormesis can have in animals across the spectrum from tiny invertebrates to mammals. These effects can be short and long-term, and whenever a broad time scale is used, hormesis is found to even have protective transgenerational effects. Short-term effects include treatment survival, improved performance, and increased mating success while long-term effects range from increasing longevity and performance at old age to improved offspring performance and starvation resistance. While we are hopeful and encouraged by the recent uptake in the study of hormesis in animals, we want to highlight the need for studies that deal with the mechanism of different types of hormesis, as well as, the cost of this adaptive response. Hormesis is rooted in the preparation for oxygen stress hypothesis, but there is evidence that hormetic benefits extend beyond antioxidant and oxidative stress, and into membrane remodeling and other aspects of animal physiology. The cost of hormesis to the parental and subsequent generations remains elusive with just a handful of studies aiming to quantify it. Understanding the cost of hormesis will allow us to elucidate how hormetic mechanisms evolved, which selection pressures drove and continue to drive these responses, and what might be the full range of these adaptive responses. There is a strong link between hormetic effects and the age of application, and a clear connection exists when these hormetic treatments are applied early in life leading to long-lasting effects present at advanced age (Le Bourg 2005, López-Martínez and Hahn 2014, López-

Martínez et al. 2014, López-Martínez et al. 2016a,b, Visser et al. 2018). The studies outlined in this review reinforce the need for additional hormesis investigation into how small doses of chemical, biotic, and abiotic stressors can dramatically improve organismal performance in non-human animals.

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FIGURE CAPTIONS

Figure 1. X-ray irradiation dose response curve showing the effect of increasing doses on the flight ability of *Drosophila melanogaster.* The increased performance as a result of hormesis is seen at the lowest doses as a hormesis model would predict. Three quantitative effects of hormesis (zero equivalent point, maximum response, and no observed adverse effect level) are labelled based on Calabrese et al. 2012, and show the control effect, the hormetic effect, and the point where negative effects being, respectively.

Figure 2. Anoxia hormesis protects from irradiation-induced oxidative damage in a cross tolerance hormetic framework. The strong protective effect of anoxia was evident at all doses (B, C, & D) tested but was more dramatic at 400 Gy (D), where the irradiated control flies die within 24 hrs. but anoxia hormesis flies live more than two months. Sex differences can be seen at all doses and females tend to live longer at higher doses (F; Figure 2 from López-Martínez and Hahn 2014).

Figure 3. Chemical hormesis (arsenite) triggers an increase in the production of reactive oxygen species (ROS; A & B). The presence of ROS triggers an increase in antioxidants, both enzymes (D & E) and non-enzymatic ones (F). That strong protective response leads to an increase in longevity (I & J) for the arsenite treated worms (Figure 3 from Schmeisser et al. 2013).

Figure 1

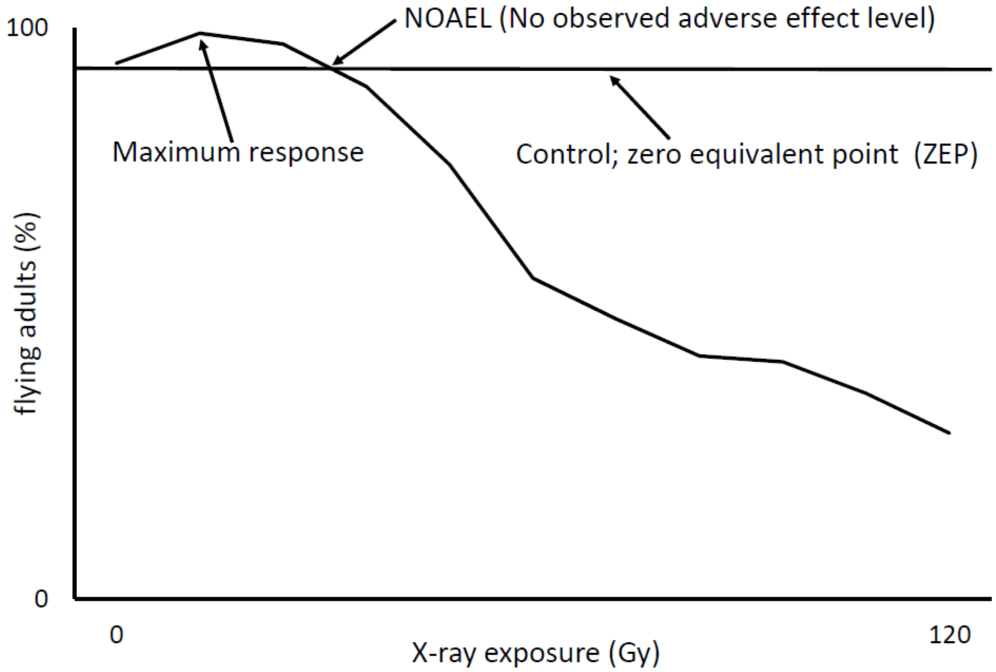
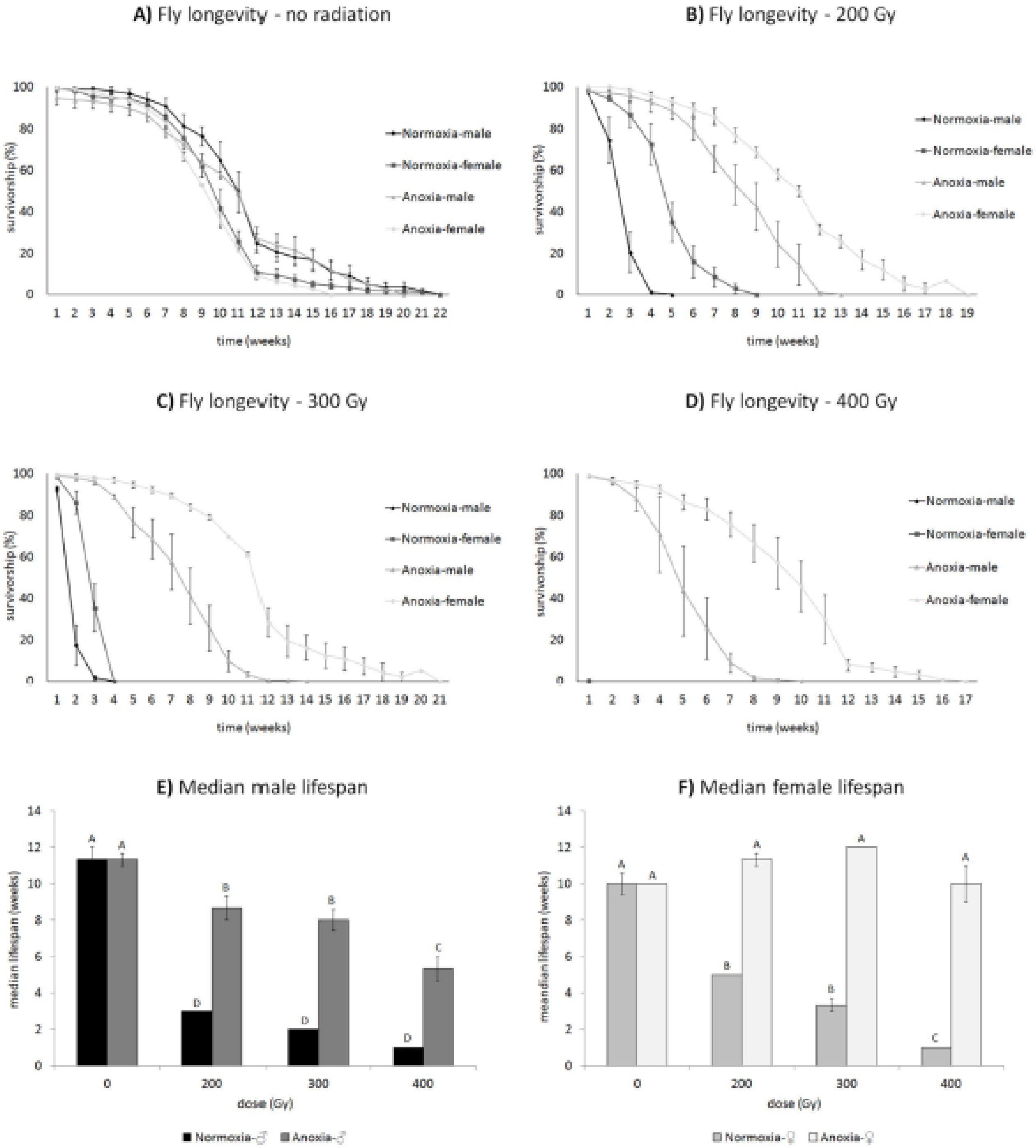

100
NOAEL (No observed adverse effect level)
Maximum response
Control; zero equivalent point (ZEP)
flying adults (%)
0
0
120
X-ray exposure (Gy)

Figure 2



A) Fly longevity - no radiation

B) Fly longevity - 200 Gy

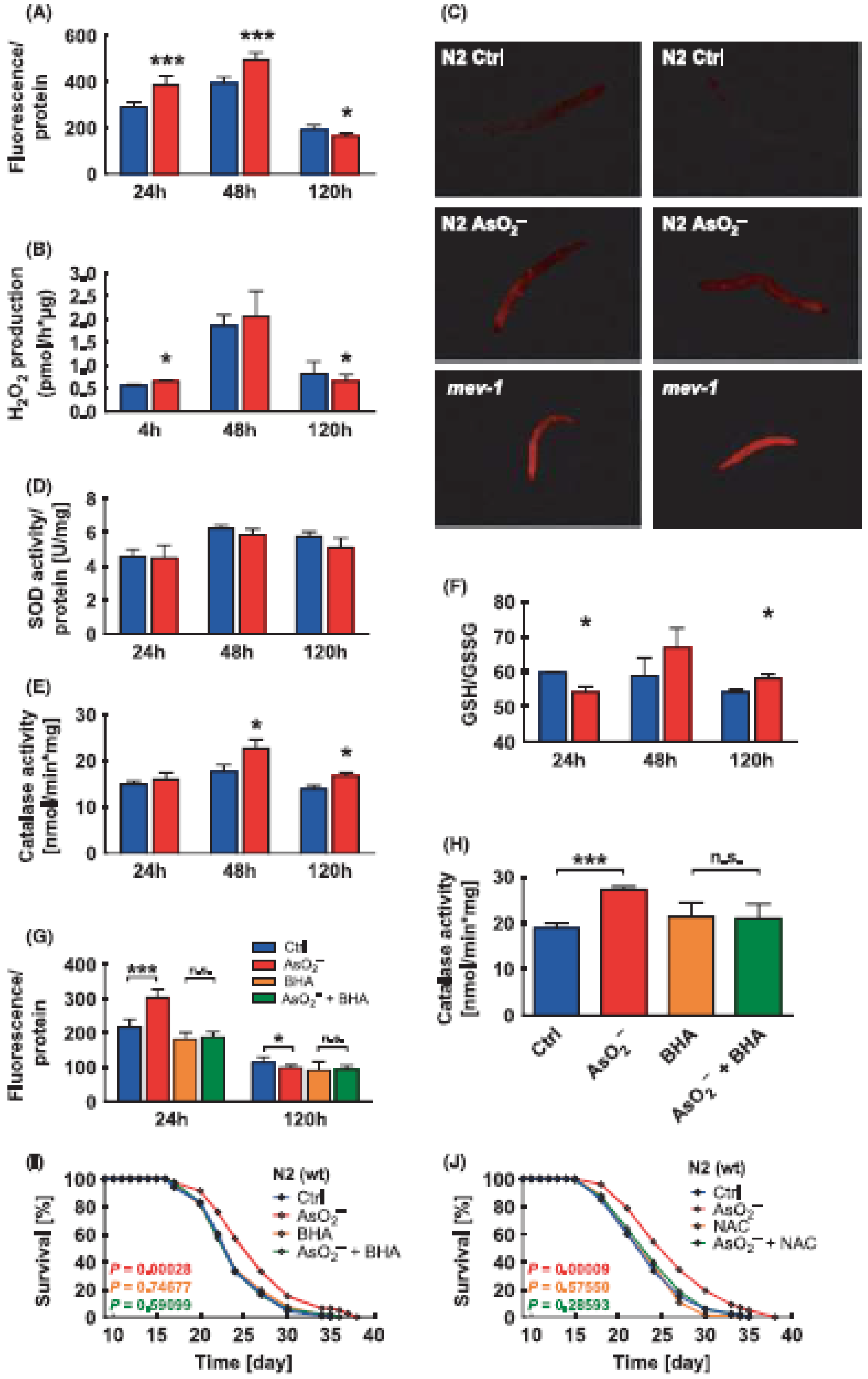
C) Fly longevity - 300 Gy

D) Fly longevity - 400 Gy

E) Median male lifespan

F) Median female lifespan

Figure 3



(A)
Fluorescence/ protein
600
400
200
0
***
***
*
24h
48h
120h
(B)
H_2O_2 production (pmol/h*µg)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
*
4h
48h
120h
(C)
N2 Ctrl
N2 Ctrl
N2 AsO_2^-
N2 AsO_2^-
mev-1
mev-1
(D)
SOD activity/ protein [U/mg]
8
6
4
2
0
24h
48h
120h
(E)
Catalase activity [nmol/min*mg]
30
20
10
0
*
*
24h
48h
120h
(F)
GSH/GSSG
80
70
60
50
40
*
*
24h
48h
120h
(G)
Fluorescence/ protein
400
300
200
100
0
***
n.s.
*
n.s.
24h
120h
Ctrl
AsO_2^-
BHA
AsO_2^- + BHA
(H)
Catalase activity [nmol/min*mg]
30
20
10
0
***
n.s.
Ctrl
AsO_2^-
BHA
AsO_2^- + BHA
(I)
Survival [%]
100
80
60
40
20
0
N2 (wt)
Ctrl
AsO_2^-
BHA
AsO_2^- + BHA
P = 0.00028
P = 0.74677
P = 0.59099
10
15
20
25
30
35
40
Time [day]
(J)
Survival [%]
100
80
60
40
20
0
N2 (wt)
Ctrl
AsO_2^-
NAC
AsO_2^- + NAC
P = 0.00009
P = 0.57550
P = 0.28593
10
15
20
25
30
35
40
Time [day]

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