THE IMPACTS OF ENVIORNMENTAL PERTUBATIONS ON LIFE HISTORY TRAJECTORIES IN *CAENORHABDITIS ELEGANS*.

by

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A DISSERTATION

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DISSERTATION ABSTRACT

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Title: The Impacts of Environmental Perturbations on Life-History Trajectories in *Caenorhabditis elegans*.

Environmental fluctuations are ubiquitous in nature and can serve to drive phenotypic differences among individuals in an environment-specific manner, a phenomenon known as phenotypic plasticity. Phenotypic plasticity can have implications for an organism's fitness. Here, I address how two distinct environmental perturbations (acute nutrient deprivation and treatment with a compound known to extend lifespan) impact individual life-history traits within and across generations using the *Caenorhabditis elegans* nematode model system.

To quantitively assess the impacts of acute maternal starvation, I engineered a novel microfluidic device to starve adults and simultaneously collect progeny with fine-scale temporal resolution. I found no evidence for changes in maternal provisioning of embryos (egg size) laid under acute maternal starvation, highlighting that, even in the face of limited nutrient availability, equal investment is provided to embryos in-utero. This consistency in provisioning is further evidenced by the fact that I also found no significant changes to life history traits such as lifespan and reproductive output in offspring produced by starved parents.

To quantitively assess the role that timing of application of a compound

previously identified lifespan extension (Thioflavin T) might play in shifting longevity responses, I contrasted early intervention responses from treatment early in development to those observed after treatment as adults. Here, I identify a novel, recoverable, developmental delay state induced by Thioflavin T treatment in a dose-dependent manner after larval treatment. These effects include disruption of normal development and increased early life mortality while on the compound, as well as decreased reproductive output and, importantly, increased longevity, after recovery following removal from the compound. Using mutants in known stress response pathways to assess specificity of response, it appears that developmental exposure results in a general hormetic stress response acting across multiple stress response systems.

Overall my dissertation explores the way environmental perturbations affect life history trajectories within and across generations. Using a combination of novel experimental approaches and high throughput techniques, I find that the major drivers of phenotypic plasticity in my experiments to be the type of environmental stressor and the age of treatment onset.

This work includes unpublished coauthored material.

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S-q Shi, MJ white, HM Borsetti, JS Pendergast, A Hida, CM Ciarleglio, **PA de Verteuil**, AG Cadar, C Cala, DG McMahon, RC Shelton, SM Williams, and CH Johnson. Molecular analyses of circadian gene variants reveal sex-dependent links between depression and clocks.Translational Psychiatry Open Access 2016. doi:10.1038/tp.2016.9

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To my Hero Rory Anthony John de Verteuil

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CHAPTER I

INTRODUCTION

Scientists have grappled to understand life history theory and aging since the early 20th century. While we have come a long way since the works of theorist August Weismann (Weismann et al. 1904) and naturalist Alfred Russel Wallace (Bulmer 2005), known most for their contributions to the theory of natural selection, in 2020 we are still discovering new paradigms and empirical evidence to better understand the biological foundations and ecological context of senescence (Gladyshev 2016). Historically, senescence has presented itself as an evolutionary paradox because it is assumed that under favorable conditions evolution should prevent aging so as to maximize survival and reproductive success over the lifetime of an individual. This led to the assumption that natural selection favors a "death mechanism" that allows room for younger and more reproductively prolific individuals to enter the population, thereby ensuring the long-term survival of the species (Moorad 2016). While this assumption seems plausible, it has been proven incorrect. J.B.S. Haldane and Peter B Medawar demonstrated that under equivalent reproductive output, longer lived individuals produce more offspring than short lived individuals and that the cost of death exceeds the benefit of the group species. The force or strength of natural selection (a measure of how strongly selection acts on survival and/or reproduction) declines as a function of age is a major theoretical insight that was later mathematically formalized by William D. Hamilton (Akazawa 2016; Flatt & Partridge 2018). In his proof Hamilton describes what is known as the "Selection Shadow" that represents the part of the lifespan post birth and reproductive maturity in

which the force of natural selection declines rapidly with age prior to death. The selection shadow prevents natural selection from acting on deleterious mutations that are constrained to late life stages(Kirkwood & Austad 2000). In addition, there is the potential for various mutations to have pleiotropic effects such as being early life beneficial and late life deleterious for the same trait(WELLS 2003). It is likely that such mutations will be passed on to offspring of the individuals bearing it and that selection will thus be inefficient at eliminating such mutations from the population. This concept of the declining force of selection is the fundamental basis for the evolutionary theories of aging we study today.

Even in this new era of scientific research with advanced genomics and CRISPR-Cas-9 gene editing technologies(Doudna 2017) there is still some debate about the evolutionary theory of aging and weather numerous small-effect genes, or few large-effect genes are responsible for the biological aging process (Flatt & Partridge 2018). The notion of mutation accumulation was first proposed by Medawar in 1952, suggesting that deleterious late life constrained mutations can rise in population frequency due to weakening selection. Huntington's Disease is one classical example of said phenomena, in addition to Alzheimer's and Parkinson's Disease. There is also empirical evidence in support of manipulations that result in biological fitness tradeoffs among various life history traits (Gladyshev 2016). Significant environmental perturbations experienced early in life can shift the allocation of resources between somatic maintenance and reproduction, this can drive adverse fitness consequences to occur later. Nutrient deprivation (caloric restriction) *in-utero* is the most consistent non-genetic mechanism

that can extend lifespan; however, it comes at a cost to growth rates, obesity, diabetes, cardiovascular disease and schizophrenia.

These and other types of biological fitness tradeoffs have been studied by scientists for centuries, in fact some of the first empirical evidence to support biological fitness tradeoffs was gathered during the Dutch Famine of 1944. Multiple longitudinal studies have examined the relationship between nutrient deficiency and progeny fitness (Lumey et al. 2012; Stein et al. 2009). However, even using cohort sizes of 900+ individuals it is hard to uncouple correlation and causation with respect to fitness tradeoffs. Furthermore, the results of most studies do not account for any genetic predisposition to adverse fitness consequences which we now know to be a potential mitigating factor in population fitness trajectories (Kogenaru et al. 2009; Orr 2009). Alternative animal models for understanding the role of genetic variation and how it results in alternative fitness trajectories include but are not limited to *Saccharomyces Cerevisiae*, *Drosophila melanogaster*, and *Caenorhabditis elegans (C. elegans)*.

In comparison to *Saccharomyces Cerevisiae* and *Drosophila melanogaster*, *C. elegans* has a well annotated stress response network and its insulin signaling pathway is homologous to the FOXO pathway in humans. Thanks to Sydney Brenner establishing *C. elegans* as a model, the scientific community has been using *C. elegans* to examine the effects of nutrient deprivation and environmental perturbations for centuries (Lee et al. 2013; Baugh 2013) Experimental findings within the last decade highlight alternative gene expression profiles associated with L1 arrest (Maxwell et al. 2012). Furthermore, empirical evidence has shown that nutrient deprivation can lead to a maximal lifespan extension in *C. elegans* through the induction of *daf-16* (Ching & Hsu 2011). Additional

studies have also demonstrated that alternative developmentally programmed arrest states like dauer can also impact life history traits (Ludewig et al. 2013).

Apart from nutrient deprivation, compound interventions and genetic mutations can also affect population lifespan trajectories. Within the last few years, studies supported through the National Institute of Aging have uncovered various compounds that under sustained treatment have the potential to reverse the aging process (Phillips et al. 2018). Screening various compounds for their effects on aging has advanced our understanding about genes and fundamental pathways involved in the aging process that are conserved across species. While there are various model systems that are applicable to understanding senescence of aging, few allow for rapid high throughput population-based screening coupled with advanced genomics.

C. elegans is a great model system to explore biological fitness tradeoffs, and population wide lifespan extension fitness trajectories (Tissenbaum 2014). In addition to its robust life cycle (embryo – adult within 4 days), *C. elegans* also has well characterized stress response pathways and an insulin signaling pathway homologous to humans (Kim et al. 2014). In addition to all the genomic resources, *C. elegans* also has an average lifespan of 25-30 days, making it an ideal candidate for understand the aging process within and across generations.

One outstanding question that is now starting to be addressed in the study of senescence is how epigenetic changes via nutrient deprivation or compound intervention can impact fitness trajectories within and across multiple generations of offspring. *C. elegans* is the best aging model system to screen for multigenerational effects in a rapid, high throughput manner. However, recapitulating some specific senescence paradigms,

such as maternal starvation, are more of a challenge than others due to physiological and biological constraints of the organism.

Dissertation Outline

For my dissertation I strengthen our understanding of how environmental perturbations impact life history trajectories in within two distinct paradigms (nutrient deprivation and compound intervention): through the development of a novel microfluidic device (which allows for high throughput population based starvation and progeny collection simultaneously), and through developmental exposure to a lifespan extension compound Thioflavin T (ThT).

In Chapter I, I describe how I used microfluidic technology to engineer a novel maternal starvation chip to measure the impact of maternal starvation on progeny fitness trajectories. In this chapter, I highlight how, unlike mammalian systems, *C. elegans* under acute adult starvation do not alter their allocation of resources but rather appear to invest equally in embryos laid. This results in no overall changes to progeny lifespan trajectories or fecundity as adults, despite their parent experiencing acute starvation as adults.

In Chapter II, I describe how early life exposure to the compound ThT induces a novel, recoverable, population wide, larval stage three developmental delay. Coupled with a high early life mortality and late life extension in adulthood. Furthermore, based on the novelty of my early life treatment paradigm, I am also able to measure the impact of lifespan extension on reproduction, and I find that it significantly reduces fecundity in adults post exposure. Lastly, I describe how early life compound exposure acts through a generalized hormetic stress response system, as opposed to the previously described *HSF-1* and *SKN-1* pathway in adulthood treatment.

Together, both chapters of my dissertation enhance our fundamental understanding of senescence by uncovering novelties that have the potential to constrain *C. elegans* ability to significantly restructure the allocation of resources post specific developmental timepoints, as well as discovering a novel developmental delay state induced through compound intervention that has the power to bypass previously characterized arrest response states.

CHAPTER II

THE IMPACT OF MATERNAL STARVATION ON PROGENY FITNESS IN CAENORHABDITIS ELEGANS

The microfluidic device described in this chapter was developed with this assistance of lab member Stephen Banse. My undergraduate mentee Juliana Rantisi contributed substantially to this work by participating in the experimental approach. Patrick C. Phillips and I developed the approaches. I wrote the manuscript and I am the primary investigator for this work.

INTRODUCTION

Encountering environmental perturbations are a universal feature of the life for all organisms. During periods of high nutrient availability (boom) there remains a positive association among life-history traits such as mating efficiency, lifetime reproductive success, and overall lifespan trajectories. During periods of low nutrient availability (bust) this association can turn negative, generating a trade-off of overall energy investment [1]. For example, in humans low nutrient availability *in-utero* is associated with health-related impacts later in life, including an increased risk of type-two diabetes, obesity, cardiovascular disease and cancer [2]. This mismatch between early-life and late life environments can result in a "thrifty phenotype" [3], leading to long-term fitness impacts in progeny even after later shifts to high nutrient availability and beneficial environmental conditions. The thrifty phenotype hypothesis postulates that this environmental mismatch is a result of epigenetic inheritance of adverse early-life conditions resulting in increased fat storage and nutrient rationing in subsequent generations [4]. Some early evidence regarding the long-term consequences of starvation

and nutrient deprivation on progeny fitness was gathered during the Dutch Famine of 1944-45. During WWII, limited food rationing had significant implications for pregnant mothers, resulting in major health implications for children such as stunted growth, increased rates of obesity, diabetes, cardiovascular disease and schizophrenia [5]. Today, we still find that malnutrition and caloric restriction *in-utero* is linked to adverse health conditions, however, we also find caloric restriction to be the most consistent non-genetic mechanism that can extend lifespan in mammals [6].

Indeed, dietary restriction in the absence of malnutrition can extend the median and maximal lifespan across a wide variety of organisms, including yeast, worms and flies [6]. The Insulin Signaling Pathway (IIS) is conserved across these species, and its activation in known to accelerate aging [7]. For example, the conserved Forkhead box O (FOXO) pathway within the IIS from animals as diverse as humans and *Caenorhabditis elegans* (*C. elegans*) roundworms has been shown to mediate these caloric restriction lifespan extension effects [8]. While the mechanisms for the role that this pathway may play in regulating lifespan in humans is still poorly understood, understanding the impact of caloric restriction in the *C. elegans* is likely to point the way towards understanding the underlying mechanisms of life-span effects in general [9].

Multiple studies have demonstrated that the strength of caloric restriction, starvation, or malnutrition, as well as the temporal pattern of the onset of nutrient limitation, are linked to the severity of downstream consequences [10]. Furthermore, there is evidence that epigenetic changes in gene expression profiles persist for multiple generations following the initial environmental perturbation [11]. These changes are independent of the current environmental conditions experienced by the individual.

Here, we sought to examine if the effects observed during nutrient deprivation early in an individual's life are also observed when that individual's parent is starved, rather than the individual itself (i.e., intergenerational embryonic effects). Within the *C. elegans* model system, traditional methods used to evaluate adult starvation are highly problematic because of the high loss of hermaphrodites from desiccation, burrowing, and facultative vivipary. Facultative vivipary is somewhat unique to nematodes, in which unlaid embryos hatch, grow and develop within the gonad of the hermaphrodite, killing the parent/mother [12]. In order to maintain normal reproductive patterns while achieving complete, acute adult starvation, we developed a novel microfluidic device ("chip"), the Embryo Gathering Gadget (EGG), to induce adult starvation in *C. elegans* while simultaneously collecting progeny in the form of laid eggs. Our new device allows for the separation of brood at specific time intervals, giving us temporal resolution of egg laying behavior under starvation conditions in real time. Overall, we find that eggs produced from starved parents display few ill effects of the treatment, indicating that parents are apparently able to compensate for shifting nutrient availability (likely via changes in total reproductive output) to compensate for this deprivation. Beyond dietary responses, our new microfluidic design provides the basis for addressing questions that depend on the clean separation of embryos from adults under tightly controlled environmental conditions.

Methods

Nematode strains and maintenance

Caenorhabditis elegans strain N2-PD1073 [25] was obtained from the *Caenorhabditis* Genetics Center CGC, and maintained using standard culture protocols [26]) unless otherwise noted. All worm cultures were maintained at 20°C in a biological incubator on NGM. Live *streptomycin* resistant *E. coli* strain OP50-1 was used as the nematode food source. Every 2 days, a subset of animals from the growing population is transferred to a new plate by excising out a portion of the agar and placing it onto a freshly seeded plate. Approximately 75 animals (at various stages) are transferred to start each new population. Once a week, when gravid hermaphrodites are present, animals are aspirated off the plate and dispensed into a 15 ml conical tube. At this time, a 5% solution of 4M NaOH and bleach is added to extract embryos from gravid adults. The surviving embryos are immediately placed onto freshly seeded plates to mature. Progeny synchronization is independent of L1 arrest, as embryos hatch in the presence of food.

Microfluidic device fabrication

Microfluidic devices were designed and fabricated using standard soft photolithography techniques[27,28]. In brief, the chip design was drafted in Vectorworks 2013 Fundamentals (Nemetschek SE, Munich, DE) and photomask transparencies were printed at 20k resolution (CAD/Art Services Inc, Bandon OR, United States). The corresponding CAD file is available as Supporting Information (S1). To manufacture the chips, SU-8 (MicroChem Corp.) photoresist masters were made and treated with (Tridecafluoro-1,1,2,2-Tetrahydrooctyl) Trichlorosilane (Gelest Inc., Product # SIT8174.0). These masters served as molds into which polydimethylsiloxane (PDMS)

(Sylgard 184, Dow Corning) mixed at a PDMS:developer ratio of 1:10 was poured and cured at 60 °C. Cured PDMS chips were cut out and appropriate holes were punched using 1.5 mm biopsy punches. The cut and punched PDMS chips were then exposed to air plasma (PDC-32G Plasma Cleaner, Harrick Plasma Inc.) and bonded to 50x75 mm glass slides.

Microfluidic perfusate

The EGG-platform uses a flow through system to maintain environmental consistency and to provide a constant food level when food is provided. The perfusate was driven at ~2 psi. Under constant pressure of 2 psi, total volume of liquid in the chip is replaced every 30 seconds. A second microfluidic chip with narrow channels was used upstream in serial to provide additional resistance. This enables the system to be run a higher PSI while minimizing the variability in the flow rate as previously described [13].

The perfusate used in this study was either S-basal or S-basal supplemented with *E. coli* OP50-1. To prepare the *E. coli* for feeding, 1 Liter of Terrific Broth is autoclaved in a wide-bottom 2-liter flask, pre inoculation of one OP50-1 CFU. OP50-1 is incubated at 37°C while rotating at 180RPM. Post 24H of incubation, OP50-1 is pelleted (6,000 RPM for 5 mins) and re-suspended into a 50 mls total volume S-basal Solution. For each µ-flux run, the final volume of OP50-1 must be at a minimum concentration of 10^8 CFU per ml to ensure a positive control. Aliquots of OP50-1 in S-basal Complete can last in 4°C for several weeks and maintain a 10^8 CFU per ml concentration.

Loading the microfluidic device

To ensure proper animal synchronization without L1 arrest, three days prior to loading in the EGG-Chip, populations are bleached. At 48H post bleaching, animals at larval stages four (L4) are picked to a freshly seeded plate for the duration of development. After 24H, this results in a synchronized population of day 1 adults, ready for microfluidic set-up 700 Day 1 adult hermaphrodites are aspirated off each plate and suspended into a 1.7 ml microcentrifuge tube. A 5 ml syringe pump is used to load animals through the top port, down the worm loading and distribution network, and into the Arena portion of each EGG-chip (Fig 1).

C. elegans husbandry in the microfluidic device

Adult hermaphrodites remain within the arena portion (Fig 1A, region ii) of the microfluidic chip for the duration of the experiment. Under starvation conditions, the left port and bottom port are closed, forcing liquid to exit the chip through the right port. As adult hermaphrodites in the arena portion of the chip experience starvation, all progeny laid pass through the egg capture chamber and exit the chip through the right port due to the laminar flow of buffer through the chip (Fig 1). Under fed conditions the procedure is the same with one exception: OP50-1 is added to the buffer of fed hermaphrodites at a density of 10^8 CFUs/ml. A magnetic stir bar and stir plate is used to ensure that OP50-1 is continuously evenly distributed within the buffer.

Progeny collection

Post the 24H adult starvation, a 5 ml syringe is used to flush buffer through the left port, clearing the egg capture chamber. Post flush, the left and right ports are closed and the bottom port is open. This changes in the direction of laminar flow, allowing fluid to travel in from the top port and out through the bottom port (Fig 1).

Once the left and right ports are closed, all laid progeny are collected in the egg capture chamber. The bottom micron filter prevents them from traveling through the waste channels and exiting the chip (Fig 1). Progeny are collected at the 2.5H interval post starvation. A 5 ml syringe is used to collect all eggs from the egg capture chamber into a 1.7 ml microcentrifuge tube.

Progeny maintenance post microfluidic capture

Following collection in the EGG-chip, embryos were plated onto large, seeded agar plates and left to develop at 20°C (normal developmental conditions). All animals remained under normal developmental conditions until adulthood. Once progeny reached adulthood, a subset of the population was evaluated for lifespan and fecundity.

Lifespan Measurements

Lifespan was evaluated using Automated Lifespan Machine technology [29]. Plate preparation and worm handling for the Lifespan Machine assays were performed as previously described [30]. Survivorship curves and statistical analyses were all generated using JMP PRO 13 software package [31].

Reproduction measurements

Brood size was quantified by isolating 30 adult hermaphrodites at L4 stage to individual plates and transferring daily per treatment. Assays were performed at 20°C and using OP50-1 as a nematode food source using standard agar. The total brood size was measured as hatched larvae for each individual over 4 days or until a 24H period with <5 embryos. Embryos that did not hatch were not included in brood size measures for that individual.

Embryo size measurements

To determine embryo size, collected embryos were mounted on 2% agar pads and imaged on an inverted microscope (Olympus IX73P1F) at 60X (PLAN APO 60X - NA 1.42). Collected images were analyzed using ImageJ software [32]. Calibration of the pixel/size ratio was performed using images collected with a micrometer and the ImageJ measure function was used to measure the circumference of ten embryos per treatment to generate size measurements.

Results

Design of the Embryo Gathering Gadget

To determine the effects of maternal starvation on offspring life-history traits we developed a microfluidic device (Fig 1 and S1) that enables (1) experimental control of food levels, (2) retention of animals in low-food environments, and (3) collection of embryos with complete temporal flexibility. Our new device, the Embryo Gathering Gadget (EGG) consists of four features. The first is a worm loading and distribution network (Fig 1A, region i) that enables introduction of animals at the start of the experiment and the delivery of perfusate during normal operation. The second is a large

arena (Fig 1A, region ii), which houses the adult animals during the experiment. The inside of the arena is patterned with a series of pillars to form an "artificial dirt" environment (see Fig 1B) that elicits normal crawling motion and plate-like behavior [13] (see S2). The pillars are 200 microns in diameter and spaced 300 microns on center, an arrangement that has been published as optimal for normal crawling behavior in *C. elegans* adults [14]. The animals that reside in the arena experience a constant flow of perfusate that maintains environmental consistency, as well as food levels under feeding conditions, while removing eggs from the arena. Eggs that leave the arena pass through a filter made up of 40-micron wide channels (see Fig 1B) that are sized to allow passage of eggs while retaining adults. Following this, they enter the third chip feature, the embryo capture chamber (Fig 1A, region iii), which sits between the upstream 40-micron filter that separates it from the arena and a downstream 20-micron filter that is sized to retain eggs within the chamber. The chamber also has an independent inlet and outlet that enables extraction of captured eggs from the device at will. The fourth feature of the device is a series of waste removal channels (Fig 1A, region iv) downstream from the 20-micron egg retention filter, which allows for exit of the perfusate during normal operation.

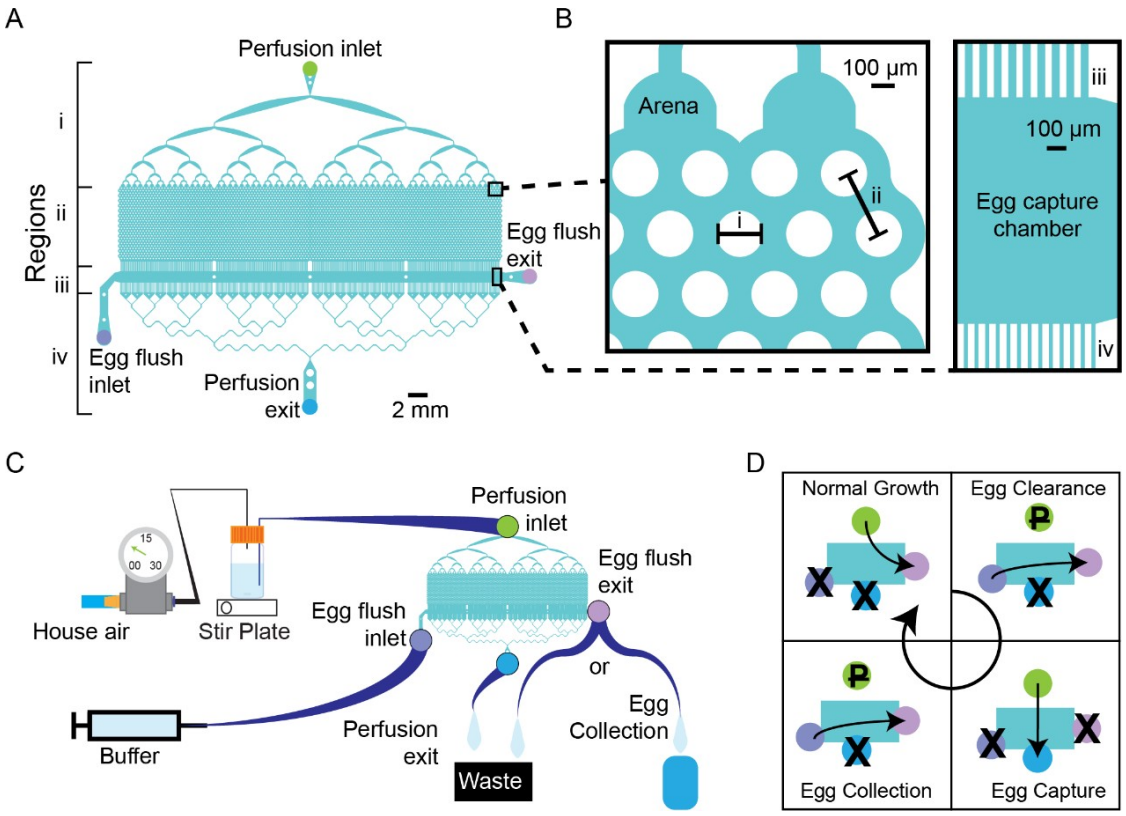

Fig 1. The Egg Gathering Gadget (EGG) platform (A) The EGG-chip is a custom microfluidic device consisting of four regions; i - a worm loading and distribution network, ii- a large worm husbandry arena, iii – an egg capture chamber, and iv – a waste removal network. (B) Zoom of the arena and egg capture chamber. The pillars in the arena are 200 microns in diameter (i) and spaced 300 microns apart on center (ii). The egg capture arena sits between an upstream filter made up of 40-micron wide channels (iii) and a downstream 20-micron wide filter (iv). (C) Perfusion of buffer (+/- food) is driven by pressurized air. The egg-flush inlet is connected to a syringe with buffer for removal of eggs. (D) Under normal operation the EGG-platform cycles through four states. Under normal growth the egg flush inlet and the profusion exit are blocked which causes flow from the profusion to travel through the arena which collects the eggs, passes through the 40-micron filter, then exits the egg flush exit into waste. To start a timed egg collection the air pressure that drives the perfusate is turned off and the perfusion exit is blocked. Egg clearance is then accomplished by driving buffer through the egg collection chamber from a syringe attached to the egg flush inlet. After egg clearance the egg flush inlet and exit are blocked, the perfusion exit is opened, and pressure driven flow through the perfusion inlet is resumed to begin egg capture. At the end of the timed egg capture eggs are collected by again turning off the pressure, blocked the perfusion exit, unblocking the egg flush inlet and exit.

Starvation in the EGG-Chip alters egg laying

To ensure that egg laying behavior was maintained in the EGG-Chip, embryos were collected and quantified from each chip at various timepoints (1H, 3H, 5H, 24H, 31H, and 48H) (Fig 2). The 1H timepoint shows the largest number of embryos laid per treatment group (mean of 684 for fed and 698.55 for starved). This is not surprising as previous literature suggests that the sudden change in environment elicits a "dumping" response in which adults immediately lay all fertilized embryos, presumably to enhance survival under changing conditions [15]. Samples at 3, 5 and 24 hours all trended toward fewer embryos laid (means of 529.5 [fed], 314.55 [starved], 484.5 [fed], 394.05 [starved], 334.05 [fed], 244.05 [starved], respectively) compared to the 1H timepoint. These slight differences are not statistically significant, and the decrease is consistent among starved and fed environments. Furthermore, this confirms that at least within the first 24H starved adult hermaphrodites will continue to lay embryos despite poor environmental conditions. At 31H post starvation, there is a significant decrease in the number of embryos laid from adults in starved versus fed conditions (mean of 293.55 versus 134.55, p=0.0423). Lastly at 48H there is another significant decrease in the number of laid embryos, with starved adults laying no embryos (mean of 0 versus 64.05, p=0.0457). In both starved and fed environmental conditions one major contributing factor for decreased embryo production is the increasing percentage of adults who experience facultative vivipary.

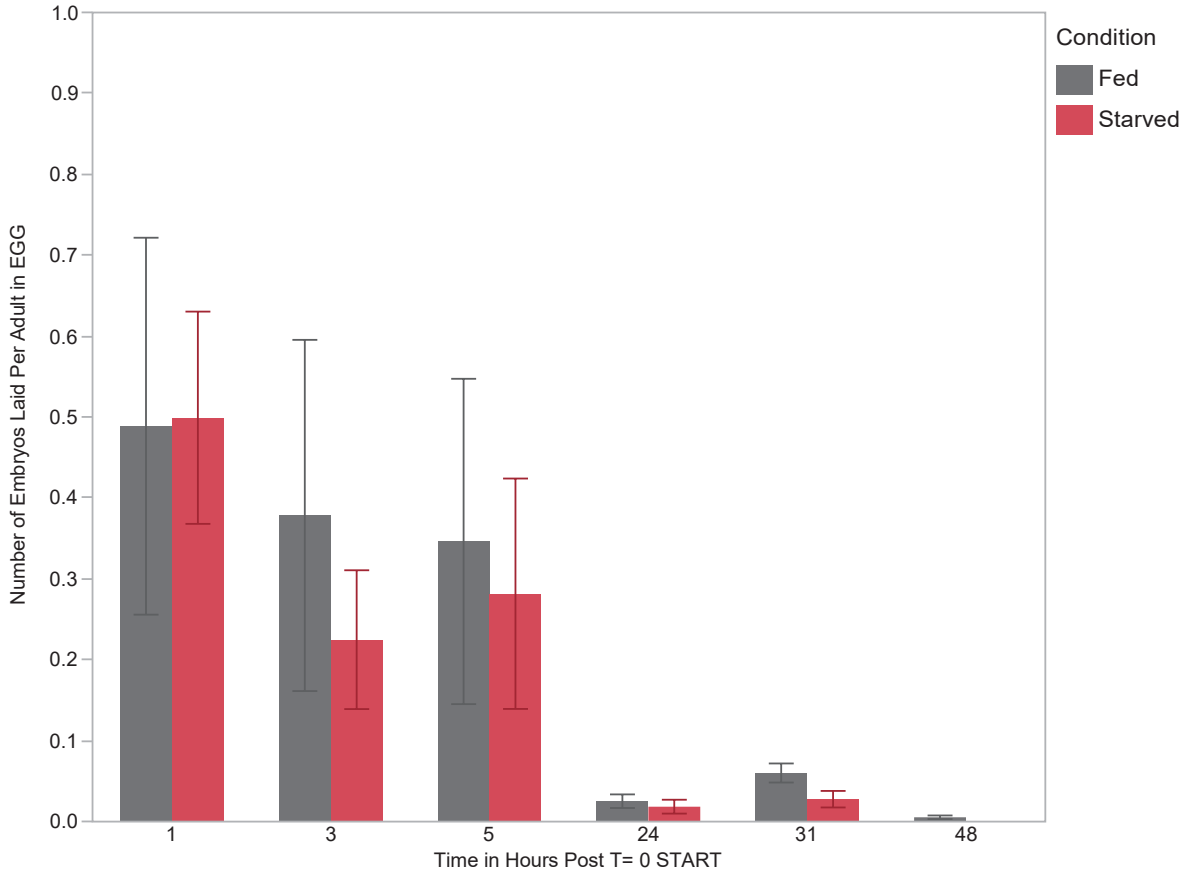

Fig 2. Egg Laying Behavior of Adults in EGG *C. elegans* embryos are captured and counted from each EGG chip under fed and starved conditions respectively. For each time interval per treatment condition we collected embryos and calculated the total number of embryos per adult per hour through liquid density calculations. Bars represent the mean per treatment and error bars represent +/- 1 SEM. Fig 2. shows that for the first 24 hours, both starved and fed animals continue to lay progeny in EGG. Only at hours 31 and 48 is there a significant difference between the number of embryos laid under fed versus starved treatment conditions.

Maternal starvation in the EGG-chip does not alter progeny lifespan

To assesses the effects of the maternal environment on progeny fitness trajectories in the following generation (maternal effects), we isolated embryos laid after 24H of adult starvation and examined their lifespan (Fig 3). To control for environmental changes, we also isolated embryos laid in an EGG-Chip at the same time period from adults who were continuously exposed to food. Our findings show that the maternal environment has no significant effects on progeny lifespan (mean lifespan 19.721[Fed] vs

19.76 [Starved]). Embryos laid from fed or starved mothers experienced no changes to overall lifespan expectancy.

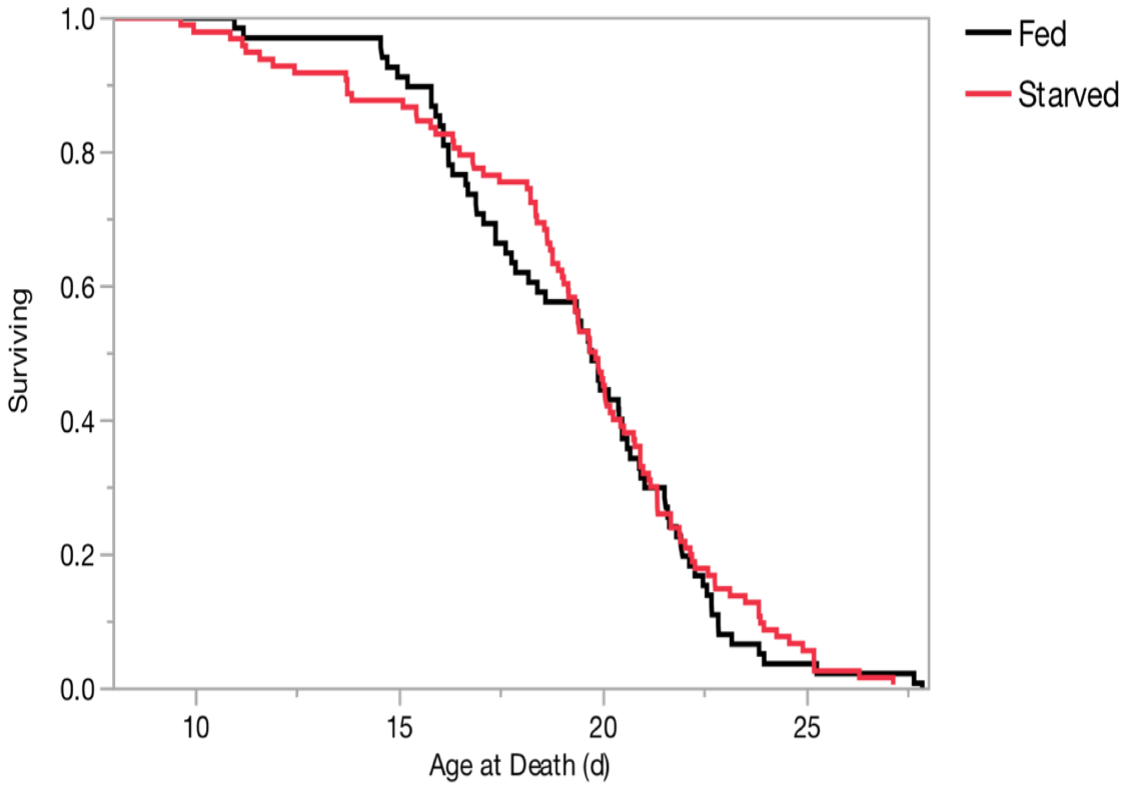

Fig 3. C. elegans Adult Lifespan Post Being Laid in EGG We examined the lifespan of C. elegans adults post being laid in EGG under each treatment condition respectively. We observe no significant difference in adult lifespan (log-rank p value = 0.8964, n=69 [fed], n=96 [starved], combined 2 experimental replicates) trajectories in progeny post maternal environment under fed nor starved conditions.

Maternal starvation in the EGG-chip does not alter progeny fecundity

To further evaluate the effects of maternal starvation on progeny fitness we examined the fecundity of 30 individuals from each microfluidic treatment group respectively in addition to the adult hermaphrodites who never experienced a microfluidic environment. Among all three treatment groups our results show no significant differences in reproduction (p=0.8614).

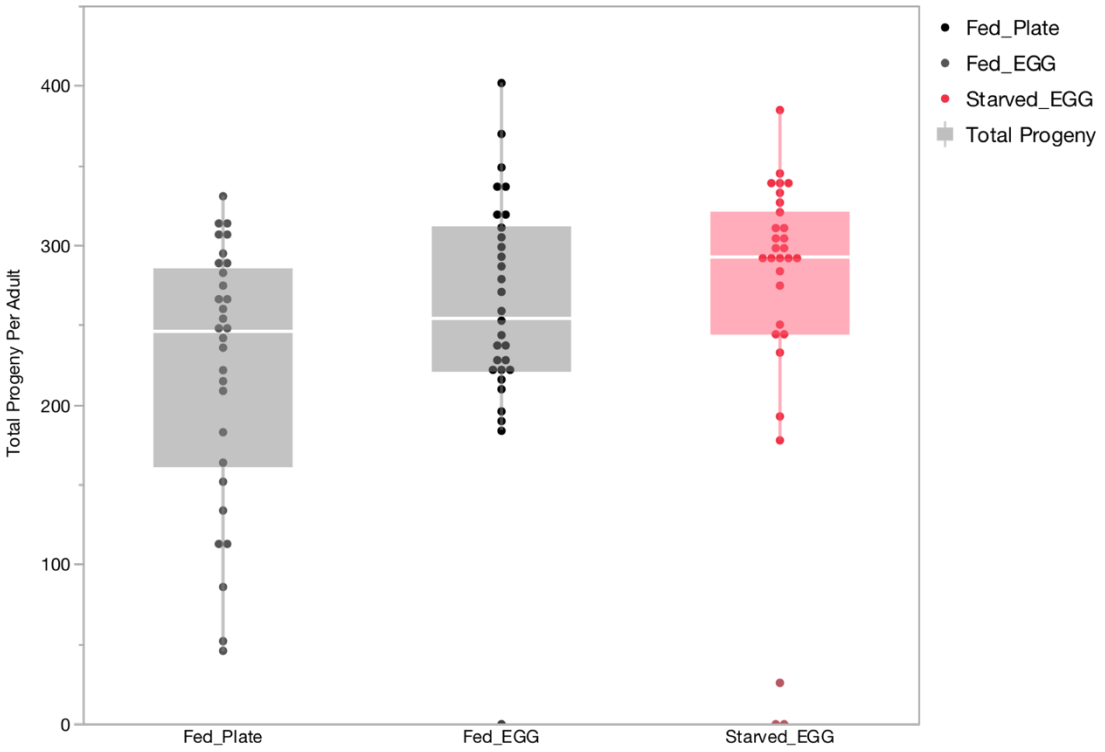

Fig 4. Fecundity of Adults Post Being Laid in EGG Animals were isolated as L4's and transferred each day for 4 days or until the total number of embryos laid was </= 5. To control for EGG environment, the fecundity for fed individuals was also assayed on plates. We observed no significant difference in the number of progeny laid by adults for each treatment condition respectively (n=30 per treatment, Fed_Plate mean 222.667 SEM +/- 14.7702, Fed_EGG mean 260.3 SEM +/- 13.6872, Starved_EGG mean 264.133 SEM +/- 17.692. Starved_EGG vs Fed_EGG P=0.8614.

Embryo size is not influenced by parental adult starvation

Lastly, we assessed if any changes in embryo size were observed following maternal starvation. To do this we imaged embryos collected within 24 and 31 hours of adults in microfluidic chips under starved and fed conditions. Using a micrometer and Image J we measured the circumference of ten embryos from each treatment group to determine if there was a significant variation in size (Fig 5). Our results show that there is no significant difference in progeny embryo size based on maternal environmental conditions (mean size 4.2 microns [fed] versus 4.3 [starved], p=0.7817).

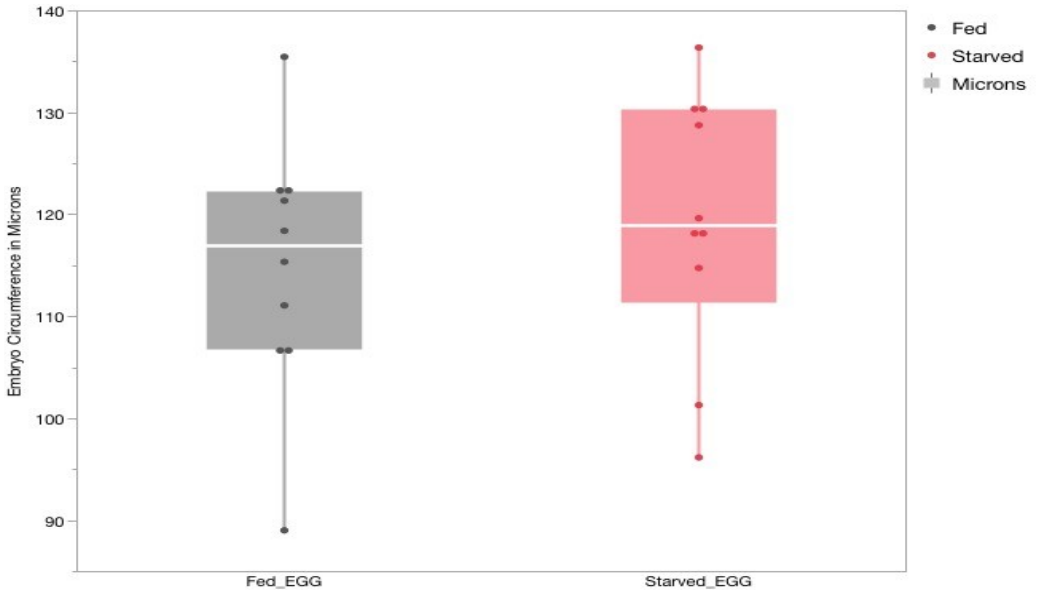

Fig 5. Embryo Circumference To examine changes to embryo size under EGG treatment conditions we measured the circumference of ten embryos collected per treatment group. We found no significant difference in the size of embryos from starved versus fed treatment conditions respectively (Mean = 4.1613 SEM +/- 0.4132647 [Fed], mean = 4.3253 SEM +/- 0.4182128 [Starved], P= 0.7817.

Discussion

Previous findings from multiple sources examining starvation during *C. elegans* development have demonstrated major consequences for progeny both within and across multiple generations [15–17]. Starvation during development has a variety of effects, including but not limited to, significant changes in RNA expression levels [18], extended time in various developmental stages, decreased fecundity, and even changes in feeding behavior [19]. These effects have also been linked to transgenerational effects in progeny, such as changes in embryo size and quantity [20].

The aim of our study was to examine the impact of maternal starvation post larval development and determine if it had the same consequences to progeny fitness. Unlike starvation during development, the effects of maternal starvation in *C. elegans* is poorly understood and the multigenerational effects of adverse environmental conditions post development is unknown. Furthermore, because adult starvation does not induce developmental arrest, we are able to decouple correlation and causation of the impact of starvation within a generation and examine its effects in a subsequent generation. We sought to examine if acute adult starvation resulted in previously identified embryonic yolk provisioning [21- 22].

Our findings show that individuals that have experienced less than 30H of starvation to produce progeny at the same rate as un-starved control populations. However, we observe an increased incidence of facultative vivipary when starvation conditions exceed 35H, which itself results in a decrease in embryo production. Upon examination of embryo size from starved and un-starved adults, we see no changes in embryo circumference under starvation conditions.

Our findings, along with additional published work, demonstrate that the temporal changes in the onset of starvation is responsible for transgenerational consequences in fitness effects [23] [24]. Our study highlights the implications for temporal variation of stressors and their impacts on progeny fitness. Under our *C. elegans* maternal starvation paradigm we do not observe a "thrifty phenotype" in progeny with respect to embryo size, lifespan, or overall fecundity, although we do observe a decrease in total embryo production over time during maternal starvation. We interpret this to mean that *C. elegans* compensate for reduced nutrient availability by producing fewer offspring of equivalent quality. Unlike previous studies, under our maternal starvation paradigm starved mothers are not mated post starvation, nor are they starved for a period of time and then given food to stimulate egg laying. Thus, what we observe in our study represents how maternal starvation directly effects progeny fitness in the absence of external stimuli such as late life mating and nutrient availability.

Furthermore, we have developed a novel experimental approach for the starvation of adult *C. elegans* hermaphrodite populations, with the potential to segment broods based on precise discrete changes to environmental inputs. This approach can be used to evolve populations over multiple generations to a specific environmental stressor. It can also be applied to high throughput studies to generate quantitative data on the efficiency and onset of phenotypic consequences for various environmental perturbations. The EGG is valuable a *C. elegans* resource as it makes it possible to design high throughput quantitative studies in a consistent and reliable way, previously inaccessible.

Bridge

In Chapter II I address the influence of maternal starvation and subsequent changes in resource allocation during embryogenesis on reproduction and longevity using the nematode *C. elegans* as a model. I found that starved parents produce far fewer offspring, but that those offspring did not suffer from decreased longevity or reproductive success in the next generation, suggesting that *C. elegans* maintains the level of per-egg investment in each offspring as overall resources decline. In Chapter III I will address the influence of a different stressor (Thioflavin T) during development and examine its effects on life history trajectories using *C. elegans* as a model.

CHAPTER III

LARVAL EXPOSURE TO LIFESPAN EXTENDING COMPOUND THIOFLAVINT DELAYS DEVELOPMENT AND ALTERS ADULT LIFESPAN IN *CAENORHABDITIS ELEGANS*

The experimental design for this chapter was developed by me with input from my advisor Patrick C. Phillips. Co-author Christine Sedore contributed substantially to this work by participating in the experimental approach and figure design. I wrote the manuscript and co-author Stephen A. Banse contributed substantially to the edits. I am the primary investigator for this work.

Introduction

Biological aging results in decreased cellular ability to maintain protein structure, function and overall integrity (Gladyshev 2016). The maintenance of these protein and homeostatic networks (proteostasisis) late in life has been shown to benefit normal aging and slow the progression of some types of late life of neurodegenerative disorders such as Alzheimer's and Parkinson's disease (Roux et al. 2016) (Li & Casanueva 2016). In neurons, the ubiquitin–proteasome system and the autophagy–lysosomal system control proteostasis and both decline with respect to age (Baptista et al. 2012).

Screening compounds for their effects on aging has advanced our understanding about genes and fundamental pathways involved in the aging process that are conserved across species. While there are various model systems that are applicable to the study of aging, few allow for rapid high throughput screening coupled with advanced genetic analysis of the *Caenorhabditis elegans* model system (Tissenbaum 2015). Within *C.*

elegans, while many compounds have been identified as potential lifespan extending interventions using the canonical wildtype strain N2, few have been shown to be robust in their ability to extend lifespan across genetic backgrounds (Lucanic et al. 2017). One of the most reliable extenders of lifespan is Thioflavin T (ThT). ThT also been shown to induce a 60% lifespan extension effect when *C. elegans* are when treatment is initiated and continuously applied during adulthood (Alavez et al. 2011; Lucanic et al. 2017; Banse, Lucanic, Sedore, Coleman-Hulbert, Plummer, et al. 2019). ThT is a widely used amyloid binding dye known for its green fluorescence upon binding to amyloid proteins, insoluble protein aggregates, DNA and RNA (Biancalana & Koide 2010). ThT has a wide variety of applications and has most recently has been used in studies examining neurodegeneration in models for Alzheimer's and Parkinson's disease (Gamir-Morralla et al. 2019) (Sciacca et al. 2017). While current studies to date do not explore the lifespan extending effects of ThT in other organisms, they do examine ThT's ability to maintain protein homeostasis in vivo and in vitro (Jagota & Rajadas 2012)(Xue et al. 2017).

Although the mechanism for ThT induced lifespan extension is not fully understood, it is known to require two stress-related transcription factors, HSF-1 and SKN-1(Alavez et al. 2011). Consistent with the dependency on HSF-1 to extend life, ThT treatment induces an upregulation of several molecular chaperones, with HSP-16.2 particularly notable among them (Alavez et al. 2011). Previous work on HSP-16.2 demonstrates that populations of genetically identical animals can be divided into short-lived and long-lived subpopulations after a stress application based on *hsp-16.2* reporter levels in young adulthood (Rea et al. 2005). In essence, *hsp-16.2* can be used as a post-stress lifespan biomarker, "a parameter that, at a young age, predicts viability at some

later age: a biomarker of life span" (Mendenhall et al. 2012). Given that *hsp-16.2* promoter activity can be used as a proxy for biological states that are predictive of lifespan, ThT's induction of *hsp-16.2* may suggest that exposure to ThT elicits a change in lifespan by a similar stress-inducing change in biological state, and chronic exposure is unnecessary for lifespan extension.

Here, we sought to examine the effects of initiating ThT exposure early in development. In particular, we were interested in whether developmental exposure to ThT early in life would generate a similar, if not more profound effect on lifespan than that seen via treatment in adults. Instead, we discovered that ThT has rather profound effects on the developmental trajectories of treated individuals. First, we show that early life exposure to ThT results in a population wide developmental delay. Second, we show that larval exposure to 100 μM ThT recapitulate the WT N2 adult lifespan extension to a lesser degree (early life mortality, coupled with a late life extension). Finally, we show that unlike the adulthood lifespan extension, developmental exposure to ThT likely results in a widespread hormetic effect, as opposed to operating solely through the SKN-1 and HSF-1 pathways. Overall, our findings demonstrate that application of longevity-promoting compounds can have a dramatically different effects depending on age of treatment.

Methods

Caenorhabditis elegans strains and culture

Caenorhabditis elegans strains N2-PD1073 (Yoshimura et al. 2019), GR1307 *daf-16(mgDf50) I*, CB1370 *daf-2(e1370) III*, MY16, JU775, and CB4856, were obtained from the *Caenorhabditis* Genetics Center CGC, and maintained using standard culture

protocols (Stiernagle 2006) unless otherwise noted. All worm cultures were maintained at 20 °C in a biological incubator. Live streptomycin resistant E. coli strain OP50-1 was used as the nematode food source.

ThioflavinT (ThT)

NGM plates were treated as previously published (Coleman-Hulbert et al. 2019; Lucanic et al. 2017; Plummer et al. 2017). In brief, ThioflavinT powder (Sigma-Aldrich, Product T3516) was dissolved in deionized water to generate working stocks ranging from 1.6 mM to 10 mM. Each stock solution was briefly vortexed, and filter sterilized through a 0.2 micron filter syringe. Caution was taken at all times to mitigate ThT exposure to light. Working stock concentrations were selected such that treatment of 3 mL assay plates with 125 µl, and 25 mL assay plates with 1 mL, of working stock solution would generate the desired final ThT exposure concentrations. For example, for a final concentration of 50 µM on a 4 mL plate, the working solution was 1.6 mM Aqueous ThT solutions were added to NGM plates previously seeded with OP50-1. ThT was distributed evenly across the surface of each plate and left to dry under aluminum foil for 24H prior to experimental use. Plates not used immediately were stored at 4°C in a light protective box for up to three weeks. To minimize contamination and maintain reproducibility, aqueous solutions of ThT were made fresh prior to treating plates.

Larval Exposure to ThT

Populations of embryos were obtained by bleaching gravid day 1 adult hermaphrodites (Stiernagle 2006). Larvae were exposed to ThT on plates at a density of 50-100 eggs per small plate and 300-500 eggs per large plate and left to develop at 20°C

in the dark for three days. The same assay has been done using L1 synchronized populations.

Larval Recovery from ThT

Following three days of ThT treatment for each plate, animals were screened for developmental delays by light microscopy. “Leaky” animals that develop past the L3 stage are excluded (incomplete penetrance). All L3 developmentally delayed progeny are then gently aspirated off the treated plate into 200 - 500 µl of S-basal and liquid transferred to a fresh NGM plate with no ThT. Animals are then left to develop at 20°C unless otherwise noted.

Length Measurements

Length measurements were calculated for individual worms using light microscopy images and Image J software. Developmentally synchronized populations were staggered at treatment intervals to observe developmental delays between working hours. Larvae on plates were imaged using a stereo microscope and a Dino-light eyepiece camera at 40x magnification. The Image J freehand line tool was used to annotate the length of the worm, from nose to tail, for individual replicates across treatments. The pixel length of the line drawn was converted, to microns to determine the true size measurements for each worm individually. The conversion from pixels to microns was done using a micrometer imaged in the same felid of view as the worm under the microscope. Statistical significance between treatment groups was done using MANOVA in JMP PRO 13 software. Logistic regression curves were made using RStudio.

Lifespan Analysis

For all ThT treated individuals, lifespan was measured post recovery from larval ThT exposure. To control for developmental delay for all lifespan measurements, timepoint 0 represents day 1 of adulthood. For ThT treated and untreated individuals at day 1 of adulthood, nematodes are transferred to 50 μM FUdR treated NGM plates for two days to inhibit reproduction. Lifespan assays were conducted using the *Caenorhabditis elegans* Lifespan Machine (Stroustrup et al. 2013) using our previously published protocol (Banse, Lucanic, Sedore, Coleman-Hulbert, Plummer, et al. 2019). Each condition maintained a minimum of four biological replicates with a minimum of 100 nematodes per treatment and two experimental replicates per lifespan assay. Survivorship curves and statistical significance (Wilcox Rank Statistic) were generated using JMP PRO 13 software package. (Lucanic et al. 2017)

Pharyngeal Pump Rate

Pharyngeal pump rate of animals on ThT was measured by visual inspection under light microscopy. Per treatment, we tracked individual larvae on ThT and counted pharyngeal pumps for 60 seconds as animals were moving. 10 individuals were chosen at random per treatment group. There was a minimum of two experimental replicates and ten biological replicates per treatment group. Each biological replicate was an individual worm. Statistical differences between groups was done in Excel using a t-test.

Fecundity

Post ThT recovery, at day 1 of adulthood 20 - 30 L4s were isolated individually across treatments per experimental replicate. Individual hermaphrodites were transferred every 24H for four days or until the number of embryos laid on the plate was 5 or less.

Progeny plates were scored 48H later to count the number of L4's. Statistical differences between groups was done in Excel using a t-test.

RNAi

RNAi bacterial strains that express double stranded RNA to inactivate *SKN-1* were cultured and used as previously described in (Timmons et al. 2001) Succinctly, *SKN-1* and empty vector HT115 strains were received from the Ahringer library (Kamath & Ahringer 2003). Prior to use in treatment, a single colony was sequenced to confirm double stranded RNA and plasmid identification. Post sequence confirmation, an individual colony was isolated and grown up in an overnight (16 hours) LB broth with carbenicillin at 37°C. The following day a 1:100 dilution of the overnight culture with the addition of 0.01mM IPTG was added and the new culture was inoculated at 37°C for 6 hours. Following the 6-hour inoculation, the bacteria was plated onto RNAi plates and left to grow overnight at room temperature before use. RNAi plates were made using standard NGM lite agar with the addition of 100µg per mL of carbenicillin and 0.01mM IPTG at room temperature overnight (or at 4°C post 24H) in the dark. RNAi plates were stored at 4°C in the dark for a maximum of 3 weeks.

Results

Larval Exposure to ThT Delays Development

Chronic exposure to ThT during adulthood increases lifespan (Alavez et al. 2011; Plummer et al. 2017; Banse, Lucanic, Sedore, Coleman-Hulbert, Plummer, et al. 2019), but the effect of larval exposure to ThT is unknown. We therefore sought to determine if ThT exposure during development could similarly prolong lifespan. Somewhat surprisingly, we were unable to test this effect directly because ThT disrupted larval development. To quantify this disruption, the developmental progression of synchronized populations exposed to 0, 50 and 100 μM ThT was measured. The developmental rate of *C. elegans* at 20°C is well characterized and *C. elegans* are expected to reach day 1 of adulthood approximately 56 hours post hatching (Byerly et al. 1976). Using size and developmental features to classify larval stages (Woodruff et al. 2019; Byerly et al. 1976). We determined the developmental stage of WT animals four days after placing embryos on ThT containing plates (~96 hours of exposure in total). As expected, animals hatching on control plates progressed through larval development normally, with 100% of the population reaching adulthood by day four (Fig 2.1A). In contrast, when raised on 50 μM of ThT, only ~20% of the population matured to adulthood, with the remaining 80% remaining in an L4-like stage (Fig 2.1A). In the presence of 100 μM ThT the developmental disruption is even stronger, with 100% of the population remaining at an L3-like stage three days after the embryos were plated (Fig 2.1A). This ThT induced developmental delay is not unique to the laboratory adapted N2 strain and was replicated in three additional wild isolates, although with some degree of dose-dependent differences.

To further characterize development under ThT exposure we observed synchronized L1 populations of wildtype animals over six days of treatment (Figure 2.1B). Specifically, we imaged animals at six different time points during the first 48 hours of exposure and then continued to image animals once every 24 hours afterward until the end of the 6 days of treatment. As expected, wildtype L1 animals plated on 0 µM ThT plates reach adulthood within 48 hours (Figure 2.1B). In contrast, wildtype L1 animals exposed to 100 µM ThT develop more slowly until they reach an L3-like stage, after which growth appears to stop (Figure 2.1B). Furthermore, if left on 100 µM ThT roughly 75% of the population animals will eventually die within 3 weeks (data not shown).

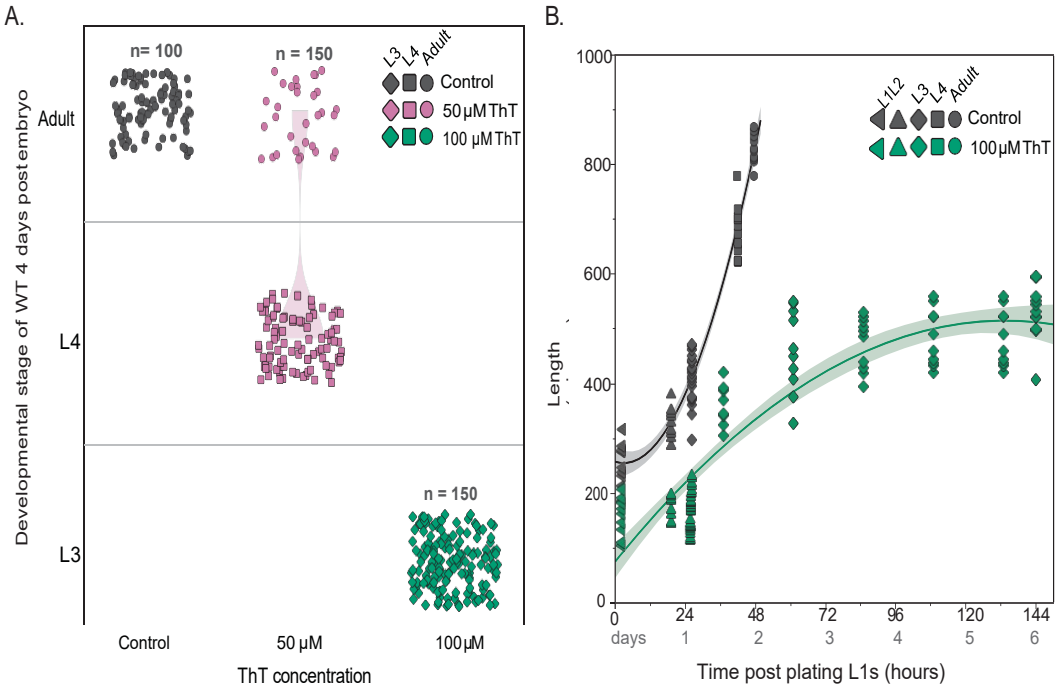

Figure 2.1 Larval Exposure to ThT Delays Development Panel A shows the developmental delay in N2 WT as a factor or [ThT] 4 days post embryonic exposure. Each dot, square and diamond represent a single individual. n=50 per replicate. Total of three replicates for ThT exposure and 2 replicates for control. **Panel B** shows the ThT population length trajectories over 6 consecutive days with and without ThT exposure. Each icon represents a single individual at a specific point in time. n=10 individuals per timepoint.

ThT Exposure Reduces Larval Pharyngeal Pump Rates

One possible explanation for the observed developmental delay is ThT modulation of feeding rates. *C. elegans* developmental rate is affected by food concentration, and at low enough concentrations development is expected to slow to the point where it appears to stop altogether (Uppaluri & Brangwynne 2015). If larval ThT exposure effects pharyngeal pump rate it could reduce the amount of food ingested by the animal, effectively phenocopying the slow growth caused by low food concentrations. To test this hypothesis, we measured the pharyngeal pump rates of N2 WT animals following 3 days of treatment on 50 and 100 μM of ThT. For ThT induced L3-like larvae, pharyngeal pump rates were significantly decreased (mean = 36.87 and 16.95 for 50 μM and 100 μM ThT respectively) relative to the control group (mean = 203.48) (Figure 2.2). When comparing the 50 μM and 100 μM ThT exposed groups there were no statistically significant differences in pharyngeal pump rates (One way ANOVA Lower CL [0,50] = 160.220, Upper CL [0,50] 270.1491, Lower CL [0,100] 142.855, Upper CL [0,100] 263.1446 p=0.7183).

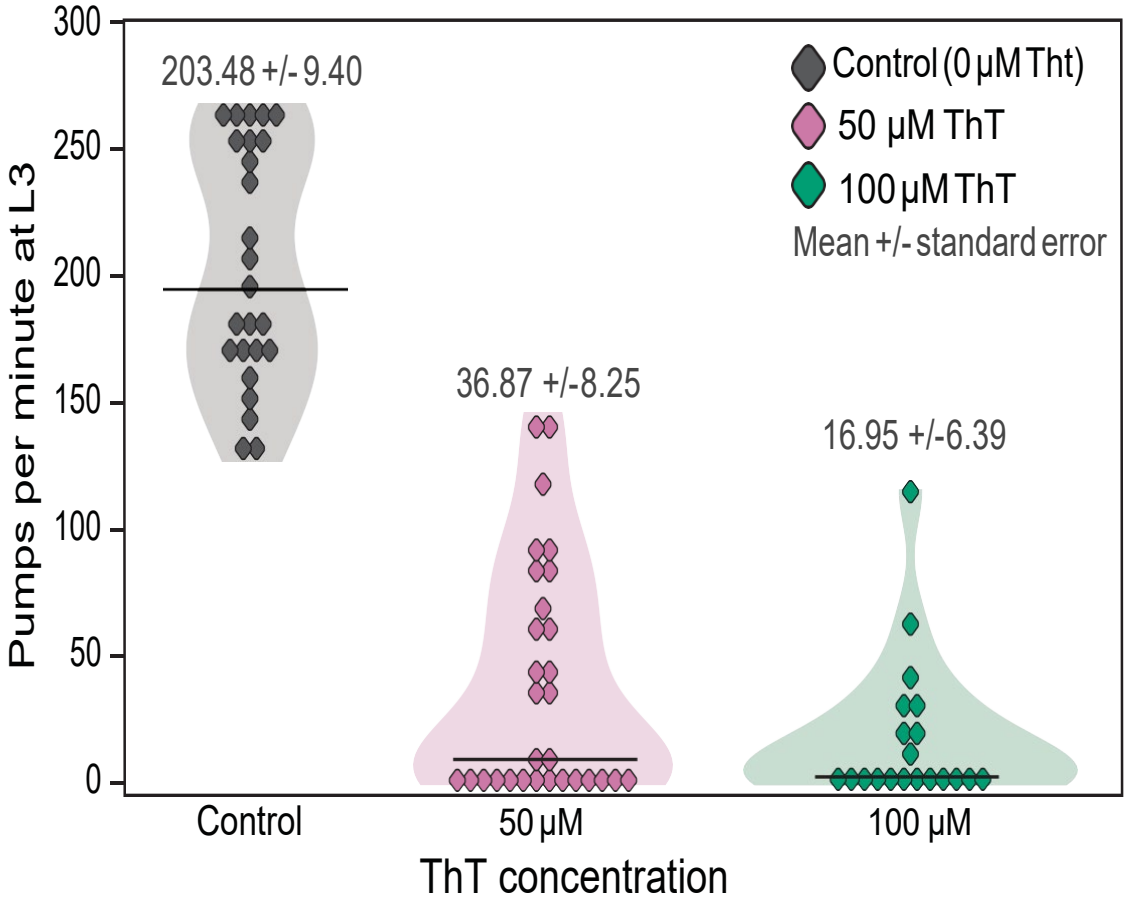

Figure 2.2 ThT Exposure Reduces Larval Pharyngeal Pump Rates L3 pharyngeal pumping declines in response to [ThT] exposure. Each diamond represents 1 individual per treatment. n=15 individuals per biological replicate, total of 2 experimental replicates. Bar represents the mean and +/- is the standard error of the mean. Violin plots represent the distribution of the points with respect to the mean.

ThT Exposed Larvae are Sensitive to 1% SDS Exposure

Following our observation that larval ThT exposure leads to a significant reduction in pharyngeal pump rates, we sought to determine if ThT might be inducing dauer formation. While *C. elegans* progresses through the four larval stages with reproducible timing under favorable experimental conditions, when conditions are stressful it can enter an alternative stage, the dauer, following the L2 stage (Kenyon et al.

1993). The dauer is long-lived, exhibits no pharyngeal pumping, and is SDS resistant (Reape & Burnell 1991). To characterize the development of WT N2 in the presence of ThT as described above, we used size measurements over time to distinguish among the larval stages. While size measurements are a reliable proxy for classic *C. elegans* developmental milestones, it is not a reliable proxy to distinguish dauer (Androwski et al. 2017). We therefore tested *C. elegans* larvae on ThT for sensitivity to SDS, as survival following 1% SDS exposure is indicative of a buccal plug, expected in *C. elegans* dauers(Mörck & Pilon 2006). To test for the presence of a functioning buccal plug in ThT treated larvae, we maintained synchronized populations of WT N2 on 100 μM ThT for three days and then exposed them to 1% SDS solution for 30 minutes. In order to generate positive dauer controls, one group of populations were subject to 10-days of starvation 20°C, while a second group was generated using a strain possessing the temperature sensitive *daf-2(e1370)* allele. In contrast to both of these dauer controls, the L3-like animals generated by growth on 100 μM ThT, remain sensitive to 1% SDS (Figure 2.3), indicating that they are indeed not dauers.

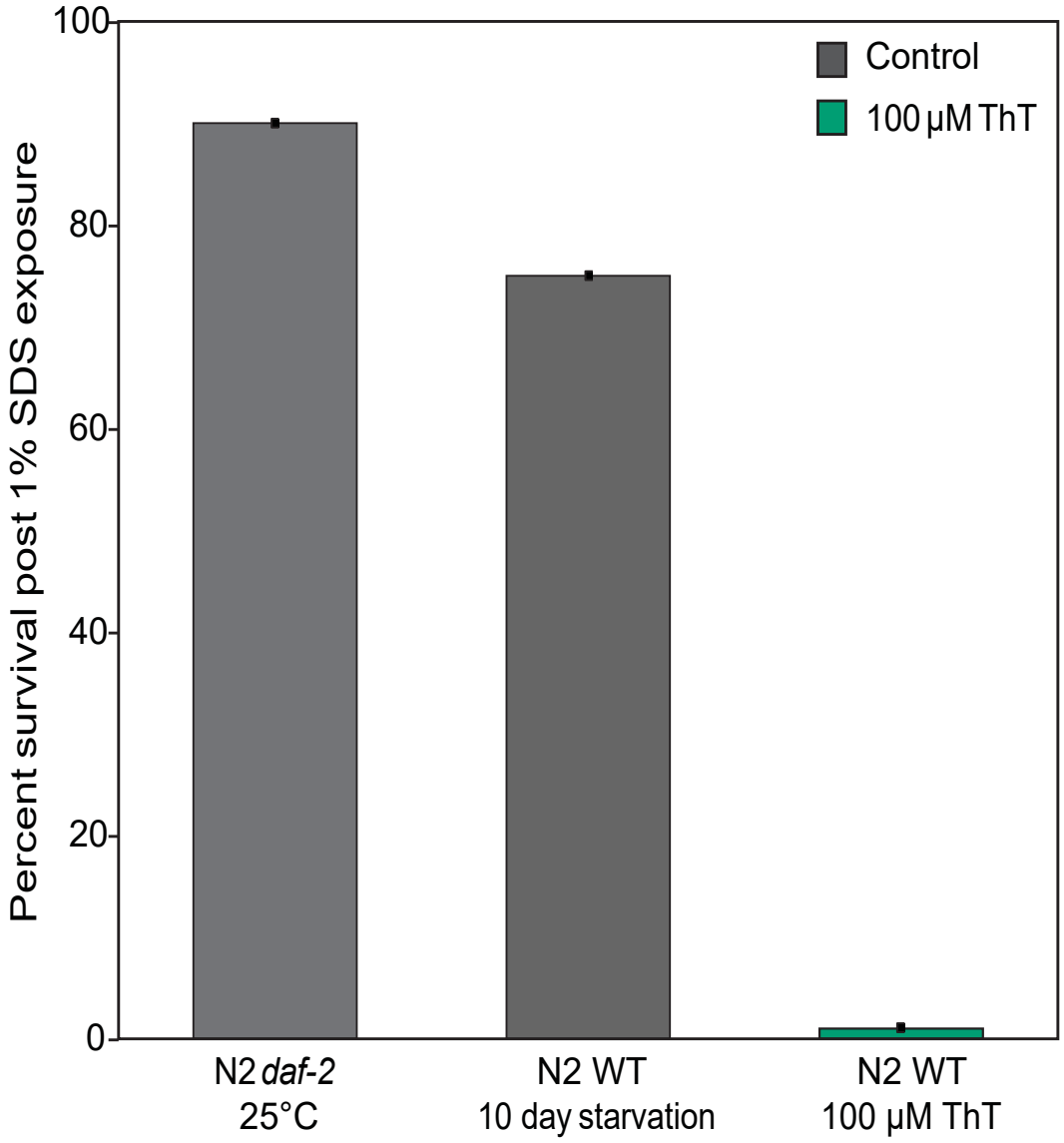

Figure 2.3 ThT Exposed Larvae are Sensitive to 1% SDS Exposure ThT induced L3s are sensitive to SDS. Following ThT exposure N2 WT is sensitive to 1% SDS and does not produce viable dauers. n=1,500 individuals per biological replicate, 2 experimental replicates total per treatment.

Larval Recovery Rate Post ThT Exposure

After we determined that larval exposure to ThT results in a major developmental delay, we attempted to reverse the effects by removing larvae from ThT. Upon removal from ThT on day 3, we found that N2 WT and *daf-16* animals took 3-4 days to reach day 1 of adulthood, while *hsf-1* mutants took approximately 6-7 days to reach day 1 of adulthood. Removing larvae from ThT on days 4-7 post treatment resulted in higher population larval mortality coupled with longer than 10 days to recover to day 1 of adulthood. We optimized our ThT treatment to take place at L1 for 72 hours.

Larval ThT Exposure Alters Adult Lifespan

Because larval ThT exposure is reversible, this shift provides the opportunity to determine the potential influence of early ThT exposure on later adult lifespan. Here, we used the automated Lifespan Machine (LM) to measure adult lifespan following 3 days of larval ThT treatment. When compared to animals who underwent normal development in the absence of ThT, we find a significant increase in lifespan in response to larval exposure to 100 μM ThT (Figure 2.4 Log-Rank $p = 0.00012$). This lifespan extension we see under larval treatment recapitulates the previously published adult 100 μM ThT exposure paradigm (to a lesser degree) with early life mortality coupled with a late life extension. This response is not simply caused by slow larval development though, as 50 μM larval ThT exposure results in population wide developmental delays but no observable lifespan extension. Indicating the dose dependent treatment effect. For adult ThT exposure, the Lithgow lab reported a 60% increase in median lifespan and a 43-73% increase in maximal lifespan for N2 WT adult *C. elegans* on 50 or 100 μM of ThT during

adulthood. Under our 100 μM N2 WT ThT larval treatment and recovery assay, we observe a 7.8 % increase in median adult lifespan, and a 22.2% increase in maximal adult lifespan.

The insulin signaling pathway is involved in larval arrest in response to adverse conditions and can regulate adult lifespan (GIANNAKOU & PARTRIDGE 2007). The FOXO transcription factor, DAF-16, which is the downstream target of the insulin signaling pathway in *C. elegans* is critical for insulin-dependent regulation of lifespan (Murphy et al. 2003). We therefore attempted to determine the increase adult lifespan post larval ThT exposure is *daf-16* dependent. Unlike the wildtype response, we see that larval exposure to 100 μM of ThT exposure in the absence of *daf-16* leads to a significant decrease in longevity (Figure 2.4 Panel C Log-Rank $P < 0.0001$). *Daf-16* mutants have a shorter lifespan than N2 WT individuals. Under our *daf-16* 100 μM larval ThT exposure and recovery assay we observe a 17.26 % decrease in adult median lifespan and no change in maximal adult lifespan at 20°C. The additional decrease *daf-16* lifespan following ThT intervention, indicates that *daf-16* is involved in the ThT lifespan extension response in WT N2 with respect to larval ThT exposure. This is notably different than the lifespan increase upon adult treatment with ThT which is independent of *daf-16* (Alavez et al. 2011).

Because the previous characterization of ThT lifespan extension under chronic adult ThT exposure implicated *hsf-1* as a necessary mediator of the lifespan extension (Alavez et al. 2011), we measured *hsf-1* mutant adult lifespans post 0 and 100 μM larval ThT exposure (Figure 2.4 Panel D). Our results indicate that developmental exposure to 100μM of ThT in *hsf-1* mutant individuals leads to a significant decrease in longevity

(Log-Rank $p < 0.0001$). Under our *hsf-1* 100 μM larval ThT exposure and recovery assay we observe a 13.01 % decrease in adult median lifespan and no change in maximal adult lifespan at 20°C. The decrease in lifespan among *hsf-1* individuals in the presence of ThT indicates that *hsf-1* is also involved in the ThT lifespan extension response.

In addition to *hsf-1*, the *skn-1* pathway is has also been implicated in the lifespan extension observed in adults treated with ThT (Alavez et al. 2011). To determine if *skn-1* is also necessary for the changes in adult lifespan seen in N2 adults post ThT larval treatment, we induced *skn-1* RNAi for N2 adults after recovery from larval ThT exposure using the same RNAi method outlined in Alavez et al. 2011. Similar to N2 WT grown on the standard OP50-1 diet, N2 WT animals grown on the RNAi competent *E. coli* strain HT115 in adulthood shows some early life mortality and late life extension, although this result is not significant, it trends in a similar manner to N2 WT animals. N2 WT animals grown on the RNAi competent *E. coli* strain HT115 have a longer early life mortality period, and a shorter late life extension period under larval ThT exposure. (Log-Rank P = 0.1815) (Figure 2.4 Panel B). For our adult *skn-1* RNAi post 100 μM larval ThT exposure, we observe a 14.91% decrease in median adult lifespan, and no change in maximal adult lifespan.

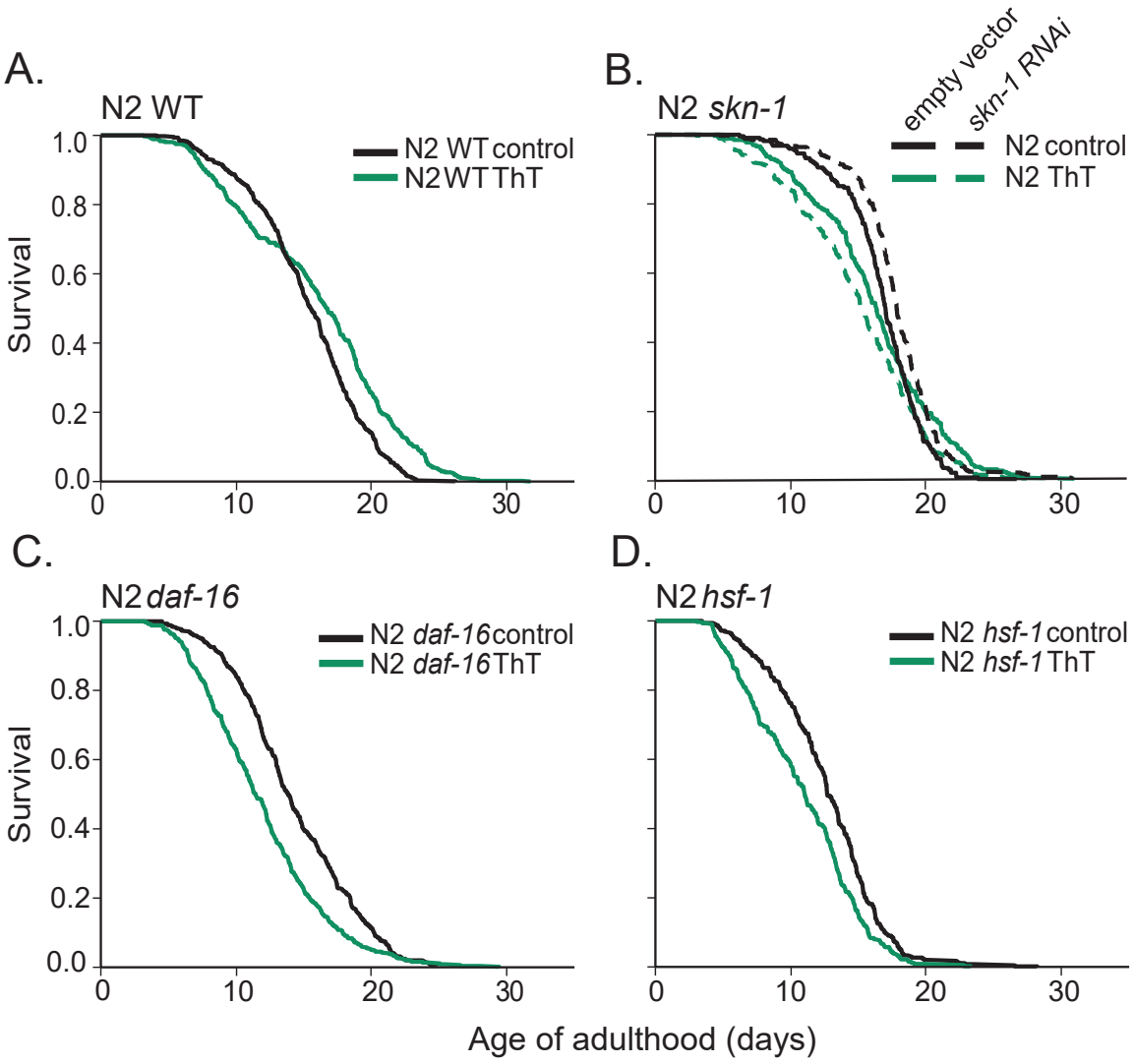

Figure 2.4 Larval ThT Exposure Alters Adult Lifespan Shows the adulthood lifespan trajectories per strain in response to larval ThT exposure. **Panel A** shows that in the N2 WT background there is a significant an early life mortality and a late life extension in response to larval ThT Exposure (P = 0.0072). **Panel B** shows that N2 WT on HT115 (RNAi Empty Vector Control, solid line) shows the same trend as N2 WT (early life mortality and late life extension P=0.005). However, *SKN-1* RNAi (dashed line) results in an overall decrease in lifespan (P = 0.016). **Panel C** shows that with a *daf-16* mutation, N2 larval ThT treatment results in a significant decrease to adult lifespan (P =0.011). **Panel D** shows that with a *hsf-1* mutation N2 larval ThT treatment results in a decrease to adult lifespan (P = 0.33).

Larval ThT Exposure Reduces Adult Fecundity

While treatment with ThT early in life can have a positive impact on longevity, it clearly can disrupt development and likely causes other challenges within developing worms. To examine this more closely, we measured the fecundity of N2, *daf-16*, *hsf-1*, *skn-1(RNAi)*, and HT115 (empty vector) adults post recovery from 100µM of larval ThT exposure (Figure 2.5). In each case, larval exposure to ThT leads to a substantial decrease in total fecundity. ANOVA reports $p = 0.0001$ for N2, *daf-16*, *hsf-1*, *skn-1*, and HT115 (empty vector).

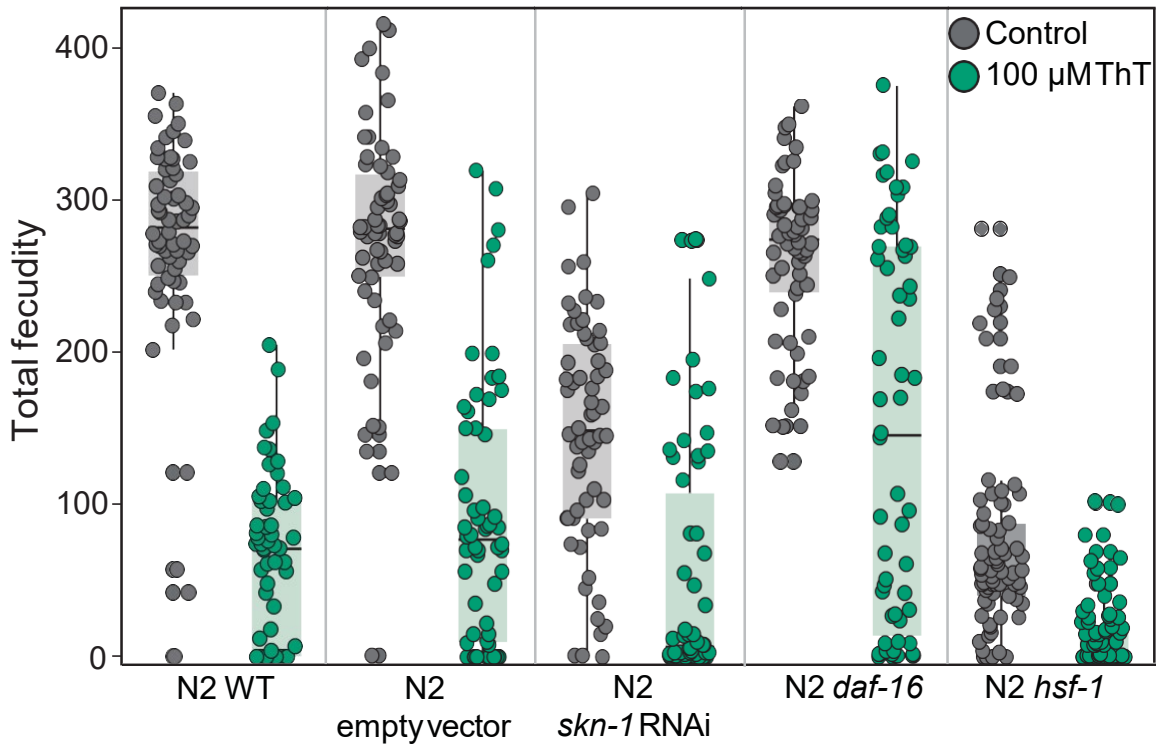

Figure 2.5 Larval ThT Exposure Reduces Adult Fecundity Larval ThT exposure results in significantly decreased fecundity across all strains tested (P > 0.001) n=30 individuals per treatment, each dot represents an individual > L3 stage, individual hermaphrodites are isolated at 4th larval stage to capture total reproduction

Discussion

Here, we sought to examine if the mechanism for ThT lifespan extension found in adults could be activated by exposure earlier in life. In particular, we were interested in if short term early-life treatment would be sufficient for inducing late-life longevity benefits. We made the surprising discovery that early-life exposure leads to a population-wide developmental delay. This is especially surprising since the proposed the mechanism for ThT induced lifespan extension in adults is enhanced protein homeostasis (Alavez et al. 2011), which would seem to be potentially favorable regardless of age. Apart from the adult early life mortality at high concentrations, there was little evidence suggesting that ThT had any toxicity effects in adults. The diverse and complex effects that we observe following larval treatment of ThT (developmental delay, decreased pharyngeal pump rate, bi-phasic N2 adult lifespan and decreased fecundity) suggest the potential for substantially different mechanisms of action for larval and adult response to treatment. Overall, our findings suggest that both ThT induction of larval developmental delay, and recovery post exposure is complex and likely a hormetic stress response, given the observation that *daf-16*, *hsf-1* and *skn-1* all prevent the late life extension observed in N2 WT (Jones & Candido 1999). In addition, all strains show decreased fecundity post treatment, suggesting that developmental ThT exposure is acting through multiple stress response pathways and that removing them results in reduced lifespans (Kumsta & Hansen 2017). We were also able to show that fecundity is significantly reduced following larval ThT exposure, highlighting the impact of this stressor to differentially allocate resources between somatic maintenance and reproduction early in development.

Larval ThT Exposure Causes Novel Developmental Delay

Identification of a compound resulting in a sustained severe developmental delay was a surprise finding. Especially, considering that *C. elegans* has two programmed developmental arrest states to survive harsh conditions (Baugh & Sternberg 2006)(Angelo & Gilst 2009). The ability of larval ThT exposure to bypass predetermined developmental arrest pathways, both L1 arrest and dauer, highlights the complexity of ThT's mechanism of action during development. Preliminary data on alternative adult lifespan extension compounds, such as green tea extract, do not result in developmental delay. This is suggestive that ThT is unique in its temporally restrictive response. ThT treatment during embryogenesis, L1, L2, and L3 development lead to a prolonged L3 developmental delay, eventually resulting in death after 3 weeks of exposure (Figure 2.1). It is also associated with a significant reduction in pharyngeal pump rates. ThT treatment during L4 results in facultative vivipary as (opposed to L4 reproductive diapause). Lastly, chronic ThT treatment during adulthood results in a significant lifespan extension (Alavez et al. 2011; Lucanic et al. 2017; Banse, Lucanic, Sedore, Coleman-Hulbert, Todd Plummer, et al. 2019). Remarkably, we show that early developmental delay effects of ThT can be reversed upon removal from the compound with sustained effects to overall adult fitness.

ThT Induced Developmental Delay Results in Adult Fitness Tradeoffs

In 2011, the Lithgow lab examined that adult exposure to ThT leads to a major adult lifespan extension. Their studies concluded that the lifespan extension of ThT in adults relies on HSF-1 and SKN-1 and that ThT is potentially acting as a stress response mimic to promote protein homeostasis. In 2017, a *Caenorhabditis* Intervention Testing Program

longevity assay including 22 *Caenorhabditis* strains across three independent laboratories, confirmed the response to adult ThT exposure was both potent and robust across all strains tested (Lucanic et al. 2017). Our results on the examination of ThT adult lifespan post larval developmental delay recapitulate the N2 adult lifespan extension to a lesser degree. Furthermore, our larval treatment paradigm allows us to examine the effects of ThT on reproduction. The fitness tradeoff we observe among increased lifespan and decreased reproduction suggests changes in nutrient allocation resulting in a fitness-tradeoff. We interpret these findings to mean that the enhanced lifespan following larval ThT treatment is achieved by a reallocation of nutrient resources to somatic maintenance at the expense of germline resources. Taken together, our findings support that developmental ThT exposure results in a hormetic stress response in adulthood, conversely to sustained adult ThT exposure resulting in a lifespan extension independently of *daf-16* but dependent on *hsf-1* and *skn-1*.

Our observations show that the effects of larval exposure to ThT are different than adult ThT exposure in a variety of ways, most distinctly, that it requires multiple stress response pathways (*daf-16*, *skn-1*, and *hsf-1*) to attain late adult lifespan extension. We characterize larval ThT exposure as hormesis due to the biphasic response of larval toxicity coupled with late lifespan extension and reduced fecundity in adults. Additionally, we observe a dose dependent toxicity effect of larval ThT exposure. Such that, under a moderate exposure concentration (100~150μM) for a short (~3 days) exposure period the toxicity effects of ThT are buffered by activation of multiple stress response pathways (hormesis), which remains activated throughout adulthood resulting in the observed adult lifespan extension. Taken together, these data suggest that the

consequences of larval ThT exposure on adults is complex, possibly polygenic, and temporally sensitive.

Conclusion

The developmental delay induced by larval ThT exposure examines an alternative paradigm for how *C. elegans* can potentially respond to environmental stress. Unlike the traditional L1 arrest or dauer larvae, *C. elegans* response to larval ThT exposure is bypassing the predetermined developmental stages for surviving adverse environmental conditions. These experiments highlight the need to examine the potential toxicity effects, and tradeoffs that can be associated with exposure to various compounds. It also highlights the various pathways that can be used to metabolize compounds, and that the temporal onset of stressors can result in varied pharmacodynamics and have the potential to lead to adverse consequences.

CHAPTER IV

CONCLUSION

In an effort to advance the field of senescence and address gaps in our knowledge concerning the biological fitness tradeoffs within the aging process, I address two fundamental questions using *C. elegans* as a model. First, I examine how nutrient deprivation can impact fitness trajectories within and across generations. Then I examine how compound intervention via Thioflavin T (ThT) can also impact fitness characteristics within a generation and affect various life history traits.

In Chapter II I address the role of transgenerational changes under an acute maternal starvation effects paradigm. Through the development and implementation of a novel microfluidic device fabricated to impose maternal starvation and collect laid progeny simultaneously, while mitigating the adverse biological and physiological constraints associated with adult starvation in nematodes. My findings demonstrate that under acute starvation *C. elegans* decreases overall fecundity while maintaining equal investment per embryo. Progeny laid during maternal starvation display no significant differences in lifespan nor fecundity trajectories when raised under normal environmental conditions. In this study we did not evaluate the potential for increased stress resistance of progeny when exposed to adverse environmental conditions, but rather chose to examine how incongruent environments for adults and progeny shape fitness trajectories under ideal circumstances. We sought to examine if increasing environmental resources could mitigate the consequences of prior adverse conditions. Under a specific set of conditions, we show that *C. elegans* progeny laid as a result of maternal starvation does not exhibit the same transgenerational consequences as extended L1 arrest nor extended

dauer. This highlights that, in *C. elegans*, transgenerational epigenetic effects are tightly regulated dependent upon the temporal resolution stress exposure. In this system, we see that developmental stravation has a stronger impact on progeny fitness than adult starvation. However, this phenomenon is likely to be inconsistent across biological systems given variation in the dynamics of reproduction and diverse biological tradeoffs between reproduction and lifespan.

This finding can be explained by various evolutionary life history strategies that organisms employ to maximize survival and reproductive success in adverse conditions. Senescence is accompanied by a decrease in protein and cellular homoeostasis irrespective of external nutrient availability. Following sexual and reproductive maturity there is a shift in the allocation of resources towards somatic maintenance instead of germline maintenance and gamete formation. This shift in resource allocation that happens naturally during senescence and can be induced via external manipulations that alter nutrient availability prior or during sexual and reproductive maturity. Reproduction is an energetically costly process across all systems; however, it is necessary for species survival. Thus, even when faced with less than ideal conditions, organisms continue to invest in progeny.

In Chapter III I address the role of epigenetic changes under compound intervention with the use of the amyloid-binding compound ThT. To examine how a compound that promotes protein homeostasis can impact life history trajectories I developed an early exposure paradigm to treat developing animals. I found that early exposure to ThT results in a population wide developmental arrest that is only recoverable within 6 days of exposure. I also found that the long-term consequences of

early life exposure to ThT to be an increased early life mortality, coupled with a late life extension for individuals who are able to overcome the early life mortality. I found a significant reduction to overall fecundity post sexual maturation in hermaphrodites as a result of early life exposure, suggesting a shift in the allocation of resources from reproduction to somatic maintenance. Through the use of insulin signaling mutants and RNAi knockdown I was able to determine that the mechanism for ThT exposure impacting lifespan varies from the previously reported adult exposure treatment such that it causes a widespread hormetic response, as opposed to acting through specific stress response pathways independently of insulin signaling in adults.

Using our developmental exposure treatment paradigm, we first show that the previously reported assertion of the impacts of ThT on adult lifespan is not applicable to a developmental exposure treatment paradigm. This further highlights that temporal variation in the treatment exposure is critical to downstream fitness consequences. The incongruent effects of ThT exposure leading to developmental delay in larvae and ThT exposure leading to a two-fold increase in overall lifespan in adults can be explained by a hormesis stress model. That is, exposure to something that is toxic can have beneficial effects later in life. Again, there is a shift in the allocation of resources from germline development to somatic maintenance as a function of age. Similar to the adult starvation model explored in Chapter II, we show that the total progeny output is affected due to adverse environmental conditions.

Taken together, both chapters of my dissertation increase our understanding of senescence and highlight the necessity that temporal onset of interventions, both through use of compounds and caloric restriction, is essential in mitigating inheritance within and

across generations. We show that the impacts of such perturbations can be sustained within a generation, but also have the potential to impact succeeding generations through high mortality rates. Although, we did not examine the transgenerational responses to species fitness in these studies, that is a reasonable future direction for this research given the information. In addition to protein homeostasis regressing as a function of age, so does the ability of natural selection to purge deleterious mutations from the population. Due to population drift (induced via high mortality rates), pleiotropy, linkage disequilibrium, and genetic hitchhiking there are a multitude of ways for selection to favor alleles that are early life beneficial and late life deleterious. In the case of ThT exposure, we see a late life extension and an early life mortality. While this may not be the case for all lifespan extension compounds, our study highlights that the ability to use a model system to understand the pharmacodynamics of compound interventions both during and after development yields to better insights on senescence as a function of time.

Overall, this thesis shows that the when evaluating the fitness consequences of environmental perturbations, it is imperative to consider temporal onset of the stressor. While this may not be applicable or translational to all stressors or model systems, altering temporal onset of environmental perturbations can yield to a better understanding of how biological fitness tradeoffs impact population fitness within and across generations.

REFERENCES CITED

Akazawa H (2016) Historical view of aging research. Nihon Rinsho Jpn J Clin Medicine 74, 1422–1426.

Alavez S, Vantipalli MC, Zucker DJS, Klang IM & Lithgow GJ (2011) Amyloid-binding compounds maintain protein homeostasis during ageing and extend lifespan. Nature 472, 226–229.

Albrecht DR, Bargmann CI. High-content behavioral analysis of Caenorhabditis elegans in precise spatiotemporal chemical environments. Nat Methods. 2011;8: 599–605. doi:10.1038/nmeth.1630

Androwski RJ, Flatt KM & Schroeder NE (2017) Phenotypic plasticity and remodeling in the stress-induced Caenorhabditis elegans dauer. Wiley Interdiscip Rev Dev Biology 6, e278.

Angelo G & Gilst MRV (2009) Starvation Protects Germline Stem Cells and Extends Reproductive Longevity in C. elegans. Science 326, 954–958.

Banse SA, Lucanic M, Sedore CA, Coleman-Hulbert AL, Plummer WT, Chen E, et al. Automated Lifespan Determination Across Caenorhabditis Strains and Species Reveals Assay-Specific Effects of Chemical Interventions. Biorxiv. 2019; 757302. doi:10.1101/757302

Baptista MS, Duarte CB & Maciel P (2012) Role of the ubiquitin–proteasome system in nervous system function and disease: using C. elegans as a dissecting tool. Cell Mol Life Sci 69, 2691–2715.

Baugh LR (2013) To Grow or Not to Grow: Nutritional Control of Development During Caenorhabditis elegans L1 Arrest. Genetics 194, 539–555.

Baugh LR & Sternberg PW (2006) DAF-16/FOXO Regulates Transcription of cki-1/Cip/Kip and Repression of lin-4 during C. elegans L1 Arrest. Curr Biol 16, 780–785.

Baugh LR. To Grow or Not to Grow: Nutritional Control of Development During Caenorhabditis elegans L1 Arrest. Genetics. 2013;194: 539–555. doi:10.1534/genetics.113.150847

Bishop NA, Guarente L. Genetic links between diet and lifespan: shared mechanisms from yeast to humans. Nat Rev Genet. 2007;8: 835–844. doi:10.1038/nrg2188

Bulmer M (2005) The theory of natural selection of Alfred Russel Wallace FRS. Notes Rec Roy Soc 59, 125–36.

Chen J, Caswell-Chen EP. Facultative Vivipary is a Life-History Trait in Caenorhabditis elegans. J Nematol. 2004;36: 107–13.

Ching T-T & Hsu A-L (2011) Solid plate-based dietary restriction in Caenorhabditis elegans. J Vis Exp Jove.

Doudna J (2017) CRISPR System: From Adaptive Immunity to Genome Editing. Mol Frontiers J 01, 1–16.

Fetoui H, Mahjoubi-Samet A, Jammousi K, Ellouze F, Guermazi F, Zeghal N. Energy restriction in pregnant and lactating rats lowers bone mass of their progeny. Nutr Res. 2006;26: 421–426. doi:10.1016/j.nutres.2006.06.016

Flatt T & Partridge L (2018) Horizons in the evolution of aging. Bmc Biol 16, 93.

Frézal L, Félix M-A. C. elegans outside the Petri dish. Elife. 2015;4: e05849. doi:10.7554/elife.05849

GIANNAKOUM & PARTRIDGE L (2007) Role of insulin-like signalling in Drosophila lifespan. Trends Biochem Sci 32, 180–188.

Gladyshev VN (2016) Aging: progressive decline in fitness due to the rising deleteriome adjusted by genetic, environmental, and stochastic processes. Aging Cell 15, 594–602.

Gladyshev VN (2016) Aging: progressive decline in fitness due to the rising deleteriome adjusted by genetic, environmental, and stochastic processes. Aging Cell 15, 594–602.

Greer EL, Dowlatshahi D, Banko MR, Villen J, Hoang K, Blanchard D, et al. An AMPK-FOXO Pathway Mediates Longevity Induced by a Novel Method of Dietary Restriction in C. elegans. Curr Biol. 2007;17: 1646–1656. doi:10.1016/j.cub.2007.08.047

Hales CN, Barker DJP. The thrifty phenotype hypothesis. Brit Med Bull. 2001;60: 5–20. doi:10.1093/bmb/60.1.5

Hibshman JD, Hung A, Baugh LR. Maternal Diet and Insulin-Like Signaling Control Intergenerational Plasticity of Progeny Size and Starvation Resistance. Plos Genet. 2016;12: e1006396. doi:10.1371/journal.pgen.1006396

Jagota S & Rajadas J (2012) Effect of Phenolic Compounds Against Aβ Aggregation and Aβ-Induced Toxicity in Transgenic C. elegans. Neurochem Res 37, 40–48.

Jobson MA, Jordan JM, Sandrof MA, Hibshman JD, Lennox AL, Baugh LR. Transgenerational Effects of Early Life Starvation on Growth, Reproduction, and Stress Resistance in Caenorhabditis elegans. Genetics. 2015;201: 201–212. doi:10.1534/genetics.115.178699

Jobson MA, Jordan JM, Sandrof MA, Hibshman JD, Lennox AL, Baugh LR. Transgenerational Effects of Early Life Starvation on Growth, Reproduction, and Stress Resistance in Caenorhabditis elegans. Genetics. 2015;201: 201–212. doi:10.1534/genetics.115.178699

Kamath RS & Ahringer J (2003) Genome-wide RNAi screening in Caenorhabditis elegans. Methods 30, 313–321.

Kang C, You Y -j, Avery L. Dual roles of autophagy in the survival of Caenorhabditis elegans during starvation. Genes Amp Dev. 2007;21: 2161–2171. doi:10.1101/gad.1573107

Kenyon C, Chang J, Gensch E, Rudner A & Tabtiang R (1993) A C. elegans mutant that lives twice as long as wild type. Nature 366, 461–464.

Kezik JM, Hill ME. Data Management Essentials Using SAS and JMP. 2016; 122–132. doi:10.1017/cbo9781316335307.011

Kim J, Ishihara N & Lee TR (2014) A DAF-16/FoxO3a-dependent longevity signal is initiated by antioxidants. Biofactors 40, 247–257.

Kirkwood TBL & Austad SN (2000) Why do we age? Nature 408, 233–238.

Kirkwood TBL. Evolution of ageing. Mech Ageing Dev. 2002;123: 737–745. doi:10.1016/s0047-6374(01)00419-5

Kogenaru M, Vos MGJ de & Tans SJ (2009) Revealing evolutionary pathways by fitness landscape reconstruction. Crit Rev Biochem Mol 44, 169–174.

Kujjo LL, Chang EA, Pereira RJG, Dhar S, Marrero-Rosado B, Sengupta S, et al. Chemotherapy-induced late transgenerational effects in mice. Plos One. 2011;6: e17877. doi:10.1371/journal.pone.0017877

Lee I, Hendrix A, Kim J, Yoshimoto J & You Y-J (2013) Correction: Metabolic Rate Regulates L1 Longevity in C. elegans. Plos One 8.

Li C & Casanueva O (2016) Epigenetic inheritance of proteostasis and ageing. Essays Biochem 60, 191–202.

Lockery SR, Lawton KJ, Doll JC, Faumont S, Coulthard SM, Thiele TR, et al. Artificial dirt: microfluidic substrates for nematode neurobiology and behavior. J Neurophysiol. 2008;99: 3136–43. doi:10.1152/jn.91327.2007

Ludewig AH, Izrayelit Y, Park D, Malik RU, Zimmermann A, Mahanti P, Fox BW, Bethke A, Doering F, Riddle DL & Schroeder FC (2013) Pheromone sensing regulates Caenorhabditis elegans lifespan and stress resistance via the deacetylase SIR-2.1. Proc National Acad Sci 110, 5522–5527.

Lumey LH, Martini LH, Myerson M, Stein AD & Prineas RJ (2012) No relation between coronary artery disease or electrocardiographic markers of disease in middle age and prenatal exposure to the Dutch famine of 1944–5. Heart 98, 1653.

Maxwell CS, Antoshechkin I, Kurhanewicz N, Belsky JA & Baugh LR (2012) Nutritional control of mRNA isoform expression during developmental arrest and recovery in C. elegans. Genome Res 22, 1920–1929.

Maxwell CS, Antoshechkin I, Kurhanewicz N, Belsky JA, Baugh LR. Nutritional control of mRNA isoform expression during developmental arrest and recovery in C. elegans. Genome Res. 2012;22: 1920–1929. doi:10.1101/gr.133587.111

McGinley MA, Temme DH, Geber MA. Parental Investment in Offspring in Variable Environments: Theoretical and Empirical Considerations. Am Nat. 1987;130: 370–398. doi:10.1086/284716

Miersch C, Döring F. Paternal dietary restriction affects progeny fat content in Caenorhabditis elegans. Iubmb Life. 2012;64: 644–648. doi:10.1002/iub.1042

Moorad JA (2016) Encyclopedia of Evolutionary Biology. Life Hist Evol Article Titles, 49–55.

Mörck C & Pilon M (2006) C. elegansfeeding defective mutants have shorter body lengths and increased autophagy. Bmc Dev Biol 6, 39.

Murphy CT, McCarroll SA, Bargmann CI, Fraser A, Kamath RS, Ahringer J, Li H & Kenyon C (2003) Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. Nature 424, 277–283.

Neelsen S, Stratmann T. Effects of prenatal and early life malnutrition: Evidence from the Greek famine. J Health Econ. 2011;30: 479–488. doi:10.1016/j.jhealeco.2011.03.001

Ni JZ, Kalinava N, Chen E, Huang A, Trinh T, Gu SG. A transgenerational role of the germline nuclear RNAi pathway in repressing heat stress-induced transcriptional activation in C. elegans. Epigenet Chromatin. 2016;9: 3. doi:10.1186/s13072-016-0052-x

Orr HA (2009) Fitness and its role in evolutionary genetics. Nat Rev Genet 10, 531–539.

Perez MF, Francesconi M, Hidalgo-Carcedo C, Lehner B. Maternal age generates phenotypic variation in Caenorhabditis elegans. Nature. 2017;552: 106–109. doi:10.1038/nature25012

Perez MF, Lehner B. Vitellogenins - Yolk Gene Function and Regulation in Caenorhabditis elegans. Front Physiol. 2019;10: 1067. doi:10.3389/fphys.2019.01067

Phillips P, Lithgow GJ & Driscoll M (2018) Reproducibility & Robustness in Pursuing Life-Extending Compounds: Caenorhabditis Intervention Testing Program. Innovation Aging 2, 808–808.

Reape TJ & Burnell AM (1991) Dauer larva recovery in the nematode Caenorhabditis elegans—II. The effect of inhibitors of protein synthesis on recovery, growth and pharyngeal pumping. Comp Biochem Physiology Part B Comp Biochem 98, 245–252.

Rechavi O, Houri-Ze'evi L, Anava S, Goh WSS, Kerk SY, Hannon GJ, et al. Starvation-Induced Transgenerational Inheritance of Small RNAs in C. elegans. Cell. 2014;158: 277–287. doi:10.1016/j.cell.2014.06.020

Rincon M, Muzumdar R, Atzmon G, Barzilai N. The paradox of the insulin/IGF-1 signaling pathway in longevity. Mech Ageing Dev. 2004;125: 397–403. doi:10.1016/j.mad.2004.03.006

Rollinson N, Edge CB, Brooks RJ. Recurrent violations of invariant rules for offspring size: evidence from turtles and the implications for small clutch size models. Oecologia. 2013;172: 973–982. doi:10.1007/s00442-012-2565-8

Rollinson N, Hutchings JA. Environmental Quality Predicts Optimal Egg Size in the Wild. Am Nat. 2013;182: 76–90. doi:10.1086/670648

Rollinson N, Hutchings JA. The relationship between offspring size and fitness: integrating theory and empiricism. Ecology. 2013;94: 315–324. doi:10.1890/2-0552.1

Roseboom TJ, Meulen JHP van der, Ravelli ACJ, Osmond C, Barker DJP, Bleker OP. Effects of Prenatal Exposure to the Dutch Famine on Adult Disease in Later Life: An Overview. Twin Res. 2001;4: 293–298. doi:10.1375/twin.4.5.293

Roux AE, Langhans K, Huynh W & Kenyon C (2016) Reversible Age-Related Phenotypes Induced during Larval Quiescence in C. elegans. Cell Metab 23, 1113–1126.

Saastamoinen M, Hirai N, Nouhuys S van. Direct and trans-generational responses to food deprivation during development in the Glanville fritillary butterfly. Oecologia. 2012;171: 93–104. doi:10.1007/s00442-012-2412-y

Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 2012;9: 671–675. doi:10.1038/nmeth.2089

Sciacca MFM, Romanucci V, Zarrelli A, Monaco I, Lolicato F, Spinella N, Galati C, Grasso G, D'Urso L, Romeo M, Diomede L, Salmona M, Bongiorno C, Fabio GD, Rosa CL & Milardi D (2017) Inhibition of Aβ Amyloid Growth and Toxicity by Silybins: The Crucial Role of Stereochemistry. Acs Chem Neurosci 8, 1767–1778.

Smith CC, Fretwell SD. The Optimal Balance between Size and Number of Offspring. Am Nat. 1974;108: 499–506. doi:10.1086/282929

Stahlschmidt ZR, Adamo SA. Food-limited mothers favour offspring quality over offspring number: a principal components approach. Funct Ecol. 2014;29: 88–95. doi:10.1111/1365-2435.12287

Stein AD, Pierik FH, Verrips GHW, Susser ES & Lumey LH (2009) Maternal Exposure to the Dutch Famine Before Conception and During Pregnancy. Epidemiology 20, 909–915.

Stiernagle T. Maintenance of C. elegans. Wormbook. 2006; 1–11. doi:10.1895/wormbook.1.101.1

Stroustrup N, Ulmschneider BE, Nash ZM, López-Moyado IF, Apfeld J, Fontana W. The Caenorhabditis elegans Lifespan Machine. Nat Methods. 2013;10: 665–670. doi:10.1038/nmeth.2475

Timmons L, Court DL & Fire A (2001) Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in Caenorhabditis elegans. Gene 263, 103–112.

Tissenbaum HA (2014) Using C. elegans for aging research. Invertebr Reprod Dev 59, 59–63.

Vaag AA, Grunnet LG, Arora GP, Brøns C. The thrifty phenotype hypothesis revisited. Diabetologia. 2012;55: 2085–8. doi:10.1007/s00125-012-2589-y

Weismann A, Thomson JA (Trans) & Thomson MR (Trans) (1904) The evolution theory, Vol 1. , 210–239.

WELLS JCK (2003) The Thrifty Phenotype Hypothesis: Thrifty Offspring or Thrifty Mother? J Theor Biol 221, 143–161.

WELLS JCK. The Thrifty Phenotype Hypothesis: Thrifty Offspring or Thrifty Mother? J Theor Biol. 2003;221: 143–161. doi:10.1006/jtbi.2003.3183

Whitesides GM, Ostuni E, Takayama S, Jiang X, Ingber DE. Soft Lithography in Biology and Biochemistry. Annu Rev Biomed Eng. 2001;3: 335–373. doi:10.1146/annurev.bioeng.3.1.335

Xia Y, Kim E, Zhao X-M, Rogers JA, Prentiss M, Whitesides GM. Complex Optical Surfaces Formed by Replica Molding Against Elastomeric Masters. Science. 1996;273: 347–349. doi:10.1126/science.273.5273.347

Xue C, Lin TY, Chang D & Guo Z (2017) Thioflavin T as an amyloid dye: fibril quantification, optimal concentration and effect on aggregation. Roy Soc Open Sci 4, 160696.

Yoshimura J, Ichikawa K, Shoura MJ, Artiles KL, Gabdank I, Wahba L, et al. Recompleting the Caenorhabditis elegans genome. Genome Res. 2019;29: 1009–1022. doi:10.1101/gr.244830.118

Zambrano E, Martínez-Samayoa PM, Bautista CJ, Deás M, Guillén L, Rodríguez-González GL, et al. Sex differences in transgenerational alterations of growth and metabolism in progeny (F 2) of female offspring (F 1) of rats fed a low protein diet during pregnancy and lactation. J Physiology. 2005;566: 225–236. doi:10.1113/jphysiol.2005.086462