The effect of nutrition and diapause on longevity and aging in the pitcher-plant mosquito, *Wyeomyia smithii*

Undergraduate Honors Thesis

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Abstract:
Dietary restriction (DR) is a widely conserved intervention known to expand lifespan in worms, flies, mice and other organisms. Worms and flies undergo a hibernal diapause stage, and it remains poorly understood as to how DR interacts with diapause. We propose that the larval diapause of *W. smithii*, where individuals continue active metabolism with the capacity to feed and sequester lipids, represents a more relevant model for mammalian DR than the well-studied worms, flies and mice. Here we show that in the pitcher-plant mosquito, *Wyeomyia smithii*, DR experienced prior to diapause increases female adult longevity, diapause length increases male adult longevity and total survivorship, and DR during diapause increases survivorship. These findings have broad implications for understand whether diapause or a lowered metabolism increases in lifespan and reduces aging in model organisms.

1. Introduction

Dietary restriction (DR), the imposed reduction of available food when compared to the *ad lib* condition, is the only intervention which has been shown to increase lifespan, delay or prevent many age-related declines in function and diseases (Broughton, 2010; Yu, 1994). This has been observed in many species including mammals. Understanding this phenomenon in model organisms may one day lead to extending lifespan in humans (Helfand et. al., 2008; Yu, 1994; Katewa and Kapahi, 2010). Aging and longevity research has utilized three model organisms; the nematode worm (*Caenorhabditis elegans*), the fruit fly (*Drosophila meloganster)* and the mouse (*Musca domestica*). All of these organisms exhibit extended lifespan and slowed aging when exposed to DR. The extensive understanding of genomics of these organisms also makes them ideal candidates to research the underlying genetic mechanisms of this phenomenon (Yu, 1994).

Despite increased longevity, it is still unclear how DR extends lifespan and improves health during aging and there is a lack of consensus whether these mechanisms are universal or distinct among species (Broughton, 2010). Mice are different from worms in flies as they have an active metabolic rate for the duration of their life. Mice as model organisms also pose problems to extending research findings to humans because similarities in pathways related to aging in different organisms coul be due to domestication as well as a simpler mammal system (Reznick, 2005). Worms and flies both experience a dormant state of diapause, which results in a reduction in metabolic rate during diapause. This reduced metabolic rate may interact with DR in ways that are not currently understood. We propose that the pitcher-plant mosquito, *Wyeomyia smithii*, an organism which has an active metabolism during diapause, provides an opportunity to better understand whether it is diapause per se or metabolic repression that gives rise to increased longevity and reduced aging observed in worms and flies. These findings may have broad implications on the conservation of DR mechanisms that may or may not extend to humans.

1.1. Dietary Restriction and Diapause

Diapause is a state of dormancy that is widely observed in insects where metabolic processes are typically reduced. In extreme conditions, states of developmental repression (hibernation in certain mammals and diapause in insects) can restrict dietary intake that can result in delayed reproduction and extended organismal lifespan in diverse species (Fielenbach and Antebi, 2008). This adaptation permits individuals to persist in a dormant state for months or years until environmental conditions are more favorable for development (Fordyce et. al., 2006).

Two of the three commonly used organisms, worms and flies, experience forms of diapause. *C. elegans* demonstrates a type of diapause, called dauer, which causes the organism to undergo a stage of larval development that favors metabolic repression for survival in stressful environments. Dormant individuals are non-feeding and inactive while maintained at low temperature or low food levels (Fielenbach & Antebi 2008). In dauer, aerobic respiration is suppressed in favor of glycolysis and formative metabolism; fat in converted to glucose during dauer. In this state, *C. elegans* are highly resistant to forms of stress including starvation, heat and oxidative stress. They can survive for months under this metabolic arrest and, once diapause is terminated, can survive to reproduce once environmental conditions improve (Fielenbach and Antebi, 2008). Under DR, *C.elegans* experiences increased lifespan, reduced fertility, and slowed senescence (Walker et. al., 2005). Unlike flies and mice, *C.elegans* are hermaphrodites and exhibit no difference it longevity or fitness between sexes (Walker et. al., 2005).

The fruit fly, *Drosophila melanogaster*, experiences a reproductive diapause induced by exposure to low temperatures and shortened photoperiods. Exposure to diapause inducing conditions for both males and females results in life-span extension, reduced senescence during diapause, and increased resistance to environmental stress. This physiological state of dormancy arrests both reproductive development and aging in the fly (Tartar et. al., 2001). *Drosophila* experiences an increase

in both mean and maximum life-span under DR conditions (Patridge, 2007). Females exemplify significant differences in lifespan post-DR; males exhibit a less robust, but still significant, increased lifespan (Bross et. al., 2005). This is because low temperatures or short days specifically induce ovarian diapause in females and DR appears to influence female fertility patterns and reduce reproduction (Bross et. al., 2005). Under low temperatures, *Drosophila* experience low levels of mortality and increased survivorship (Pletcher et. al., 2002; Mair et. al., 2003; Bross et. al., 2005; Patridge, 2007). Daily and lifetime fecundities of females are reduced by food dilution throughout the DR intervention (Partridge, 2007). High temperature increases the rate of aging by inflicting permanent debilitation; which determines future mortality (Mair et. al., 2003).

Mice do not experience diapause; like most mammals, they are metabolically active for the duration of their entire lifespan. McCay(1935) and colleagues first demonstrated DR increased mean lifespan in both male and females rats, thus established the first observation of lifespan extension due to DR in mammals (Yu, 1993). Mice exhibit extended periods of growth and development, later sexual maturation, and increase in mean and max lifespan when carbohydrates and fats are restricted (Masoro, 1995). DR has been shown to slow deterioration of physiological systems, as well as the occurrence and progression of age-associated diseases in mice. This is not because metabolism becomes reduced, but instead due to a change in energy intake per unit of body mass. DR reduces the proportion of lean body mass to energy intake and causes a greatly disproportional decrease in body fat mass (Yu et al., 1982; Bertrand et al., 1980). It is not the intensity of fuel use but altered characteristics of fuel use that may explain the slowed aging in mice due to DR (Masoro, 1995). The nervous and/or endocrine system(s) act(s) may act as mediators that couple the reduced energy intake by the animal to aging processes in the tissues. Research suggests that reduced concentrations of plasma glucose and insulin reduce the damage caused by hormones (Masoro, 1995). These findings have lead researchers to manipulate insulin

signaling pathways in worms, flies and mice to better understand DR as an invention in genetic pathways.

1.2. Insulin Signaling Pathways

The slowed aging and long lifespan in response to changes in diets has shown to have a strong genetic component, influenced by insulin signaling pathway and transcriptional regulators (Clancy et al., 2002; Pletcher et al., 2002). Current genetic techniques have allowed interventions such as mutations in the insulin and insulin-like growth factor signaling pathway, both which have been shown to extend life-span in *C. elegans, Drosophila,* and mice (Kenyon et al., 1996; Partridge and Gems, 2002; Holzenberger et al., 2003; Bluher et al., 1993). These constancies imply that genes and pathways may have been evolutionarily conserved over time (Katewa and Kapahi, 2010).

Insulin pathways are important biochemical pathways at the cellular level that effect cell homeostasis. As an organism consumes, digests, and absorbs carbohydrates, the pancreas senses the subsequent rise in blood glucose concentration and releases insulin to promote an uptake of glucose from the blood stream (Rothwell, 1998). When insulin binds to the cellular insulin receptor, it causes a cascade of cellular processes that promote the usage or storage of glucose in the cell (Helfrand et. al., 2008). This pathway is also influenced by fed versus fasting states, stress levels, and hormones. Mutations of single genes within these pathways can extend lifespan dramatically, causing the animal to age normally but just more slowly (Kenyon et. al., 1993).

The IGF-1 is the best understood insulin signaling pathway that is known to influence lifespan in worms, flies and mice (Kenyon, 2005). This pathway was first linked to lifespan in *C. elegans*, where mutations in the *daf-2* regulatory gene encoding for the IGF-1 insulin receptor ortholog were shown to double lifespan. These findings demonstrated that aging in *C. elegans* is subject to regulation and is regulated hormonally. The insulin/ IGF-1 pathway not only regulates adult longevity but also regulates entry of juveniles into dauer (Gerisch et. al., 2001; Geanacopoulos, 2004). Harsh environmental

conditions trigger dauer formation by down regulating insulin/IGF-1 signaling. Reduced insulin/IGF-1 signaling allows dauer individuals to increase longevity and stress resistance. This is because many stress-response genes whose expression changes in long-lived adults also change when worms enter dauer (Gerisch et. al., 2001; In contrast, whereas some *daf-2* mutations produce dauer-like traits in adults, other mutations have normal metabolism, reproduction, behavior, and body morphology (Gems et al., 1998). Weak *daf-2* and age-1 mutants that do not arrest at the dauer stage also live much longer than the wild type (Kimura et. al., 1997). It is possible that DR causes a decline in insulin signaling to induce a partial diapause state, like that induced in weak *daf-2* and age-1 mutants. The induction of diapause-like states, or the changes in metabolism, may affect post-reproductive longevity in *C. elegans* (Kimura et. al., 1997).

This mutation of the insulin receptor has been shown extend the lifespan of flies; this suggested that the ability of lowered insulin signaling activity to extend lifespan was conserved over large evolutionary distances (Pletcher et. al., 2002, Kenyon et. al., 2005). Interventions to the insulin signaling pathways cause male and female lifespan extension, reduced female fecundity, altered fat storage and age-related heart function decline (Wessells et. al., 2004). *Daf-2* is the only member of the insulin receptor family in the *C. elegans* genome sequence and is equally distant from the human insulin, IGF-I, and insulin receptor-related receptors. This makes *daf-2* a likely homolog of the ancestor of these duplicated and diverged receptors, and thus may subserve any or all of their functions (Kimura et. al., 1997).

Rodents' exhibit extended longevity if either the insulin or IGF signaling pathways were disrupted, indicating that the evolutionary conservation may extend to mammals. Unlike worms and flies, which have a single insulin IGF-1-like receptor, mice have separate receptors for insulin and IGF-1. When the IGF-1 receptor is down regulated in mice, individuals have been shown to live 30% longer than wild-type mice (Holzenberger et al., 2003). Long-lived insulin/IGF-1 mutants have normal rates of

oxygen consumption and reproduce normally (Holzenberger et al., 2003). Studies in both flies and worms hope to extend life-span regulation by insulin-like metabolic control since it appears to be analogous to mammalian longevity enhancement induced by caloric restriction (Kimura, 1997). Despite what has been discovered, gene expression analyses of longevity-assurance mechanisms in flies, worms and mice reveal few overlapping pathways and even conserved human pathways will have unique features not present in lower life forms (Mc Elewee et. al, 2007).

1.3. Dietary Restriction and Wyeomyia Smithii

The effect of DR on aging have been extensively explored; however an aspect that has yet to be explored is if the state of diapause itself or metabolic repression during diapause, increases longevity and reduces aging in worms and flies. The pitcher-plant mosquito, *Wyeomyia smithii*, differs from worms and flies as they are able to diapause at high temperatures, are behaviorally active, feed, grow and sequester lipids and amino acids (Bradshaw and Lounibus, 1972). *W. smithii* overwinter in a larval diapause which is initiated, maintained and terminated by day length. In response to change in photoperiod, larvae terminate diapause and delay development until resources are available to secure a high reproductive success (Bradshaw and Holzapfel, 1983).

During all 4 instars, larval developmental stages are distinguished by a molt, and can ingest available nutrients and sequester lipids; however the pupal stage before eclosion to adulthood is a non-feeding stage. Thus, the lifespan of *W. smithii* is dependent on nutrients before and after the pupal stage. The ability for larvae to sequester lipids during the larval stage and diapause is advantageous after eclosion in the adult stage, as adults still depend on nutrition from fat stores obtained during the larval stage (Lounibus et. al., 1982). We propose that an active metabolism observed in *W. smithii* during diapause and non-diapause life stages simulates human behavior moreso than organisms such as flies and worms that experience metabolic arrest during diapause. Because they experience diapause, while mice do not, *W. Smithii* provides an physiological intermediate state between organisms that enter a

metabolically repressed diapause (worms, flies) to non-diapausing and metabolically active mammals. Hence, they provide the opportunity to determine whether it is metabolic rate or diapause, *per se*, that affects longevity.

2. Materials and Methods

Pre-diapause food, duration of diapause and food during diapause were used to determine the interaction of dietary restriction and diapause on survivorship and adult longevity in *W. smithii*. Prior to the experiment, diapausing larvae from the New Jersey Pine Barrens (PB) were pooled into a large pan, stirred, and then haphazardly allocated into 15 dishes of 35 individuals per dish. These individuals were raised to adulthood on long days (L:D = 18:6) at 80% relative humidity and a sine-wave thermoperiod from 13 °C: 35 °C that lagged the light cycle by three hours. Eggs were collected and maintained on short days (L:D = 8:16) at 21°C. Hatching first instars were pooled into a large pan and then haphazardly distributed into Petri dishes (150 x 25mm) with 35 individuals per dish. Dishes contained 50 mL distilled water with O.1 g/l mg*l^{-1} tetracycline (OVSAC) and 10 ppm Kodak Photo-Flo© (Amazon.com). Control individuals were exposed only to long days, did not experience diapause and developed directly to adulthood. Experimental treatments (Fig.1.) were assigned haphazardly to high or low food treatments (Table 1) on the day of hatch. High food consisted of 13.5 mg*$dish^{-1}$on the day of hatch, 17.5 mg*$dish^{-1}$ on the day half the larvae molted to the second instar and 52.5 mg mg*$dish^{-1}$on the day half the larvae molted to the third instar. Low food consisted of 3.5 mg*$dish^{-1}$on the day of hatch, 5.25 mg*$dish^{-1}$ on the day half the larvae molted to the second instar and 17.5mg*$dish^{-1}$on the day half the larvae molted to the third instar (Table 1).

Table 1. Food concentrations before diapause and after diapausing larvae were placed on long days (post-diapause)

Instar	Low (mg*$dish^{-1}$)	High (mg*$dish^{-1}$)
I	3.50	17.5
II	5.25	17.5
III	17.5 mg*$week^{-1}$	52.5 mg*$week^{-1}$
Sum	36.00	87.5
Post-diapause	105.00	105.0

When half of the individuals of each dish reached the third instar, larvae were transferred to petri dishes with fresh water. The 75 total dishes were randomized using a table of random numbers and separated into 24 treatments with three replicates and three controls. Larvae were maintained in diapause on short days for the duration of diapause, liquid was replaced and high or low food (Table 1) was added to each dish. The three new food treatments during third-instar diapause were high food, low food or no food for four different durations of diapause; 1 week, 12 weeks, 24 weeks and 36 weeks. High food dishes received 52.5 mg*week^{-1}, low food dishes received 17.5 mg*week^{-1} once a week during diapause (Fig.1). No food dishes received no food once diapause was initiated. At the end of the assigned duration of diapause, dishes were transferred to long days (L:D = 18:6) at 21° C to terminate diapause and induce development to adulthood. Post-diapause, all dishes received 105 mg*dish^{-1}.

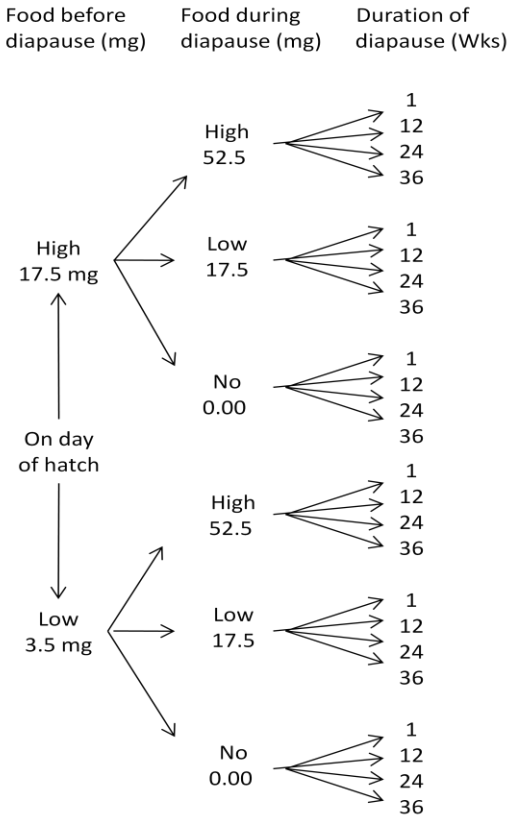

Fig. 2. Pre-adult food and duration of diapause. There were three replicates of 105 individuals for each of the 24 total treatments plus three replicates of the long-day (L:D = 18:6).

Dishes with developing larvae on longs days were checked three times per week and pupae were removed, blotted to remove water, weighed on analytical balance (Mettler Toledo AT261 Delta Range, Mettler inc. Toledo Off) to the nearest 0.01 mg, and then transferred to 0.95 L adult-cohort cages. Cohort cages were housed on long days (L:D = 18:6), 80% relative humidity, and a 13 °C: 35 ° C sin-wave thermoperiod that lagged the light cycle by three hours. Each cage was provided with organic raisins for adult nutrition, an open 50 mL jar of distilled water for pupae and adult eclosion, and a single cut leaf of *Sarracenia purpurea* for oviposition. Raisins, water and leaves were changed on a weekly basis to ensure optimal conditions for oviposition. The bottom of the cage was covered with Whatman 3MM, Fischer Scientific chromatography paper and was soaked with distilled water three times per week. Cohort cages were checked three times per week for pupal exuviae, dead adults, and eggs. Pupal exuviae and dead adults were sexed and used to calculate survivorship to adulthood (fraction of original cohort emerging as adults) and sex-specific adult lifespan (time from mean adult eclosion to mean adult death).

2.1 Data Analysis

We calculated mean number of longs days between adult eclosion and adult death for males and females (adult longevity), survivorship to adulthood (% eclosed adults), sex-ratios (% females/total surviving to long days), the mean number of long days to eclosion for males and females (pre-adulthood survivorship), and pupal mass for both sexes. Fractional data were arcsin transformed to approximate normality and, if necessary, further $\log_{10}$ transformed to reduce heteroscedasticity. All statistics and graphics were performed in Microsoft Excel.

3. Results

Neither male or female development time from the onset of long days until adult eclosion (Fig. 3) nor pupal mass (Fig. 2) varied with food or the duration of diapause. Pre-adult survivorship was negatively correlated with the duration of diapause (Fig. 4C) but not food, either before or during

diapause (Fig. 4A, B). Sex ratio (% females) was not correlated with food, either before or during diapause (Fig. 4D, F), or with the duration of diapause (Fig. 4E) and was biased towards males. Female adult longevity was negatively correlated with pre-diapause food (Fig. 5E) but not food during diapause or the duration of diapause (Figs. 5F, G). Male adult longevity was positively correlated with the duration of diapause (Fig. 5B) but not food either before or during diapause (Fig. 5A,C).

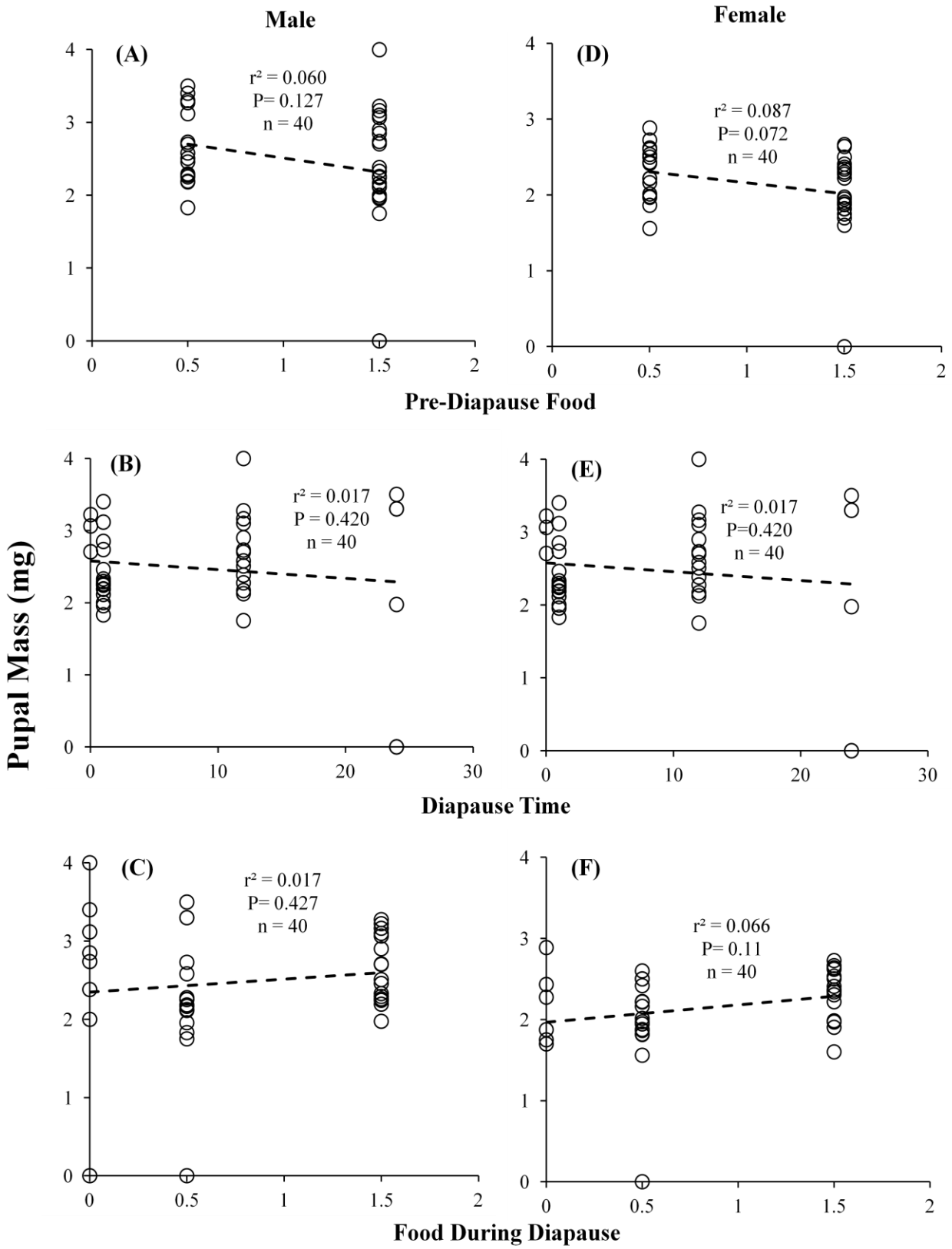

Fig. 2. Pupal Mass. Food in mg/larva. Duration of diapause in weeks. Dashed lines; non-significant correlation.

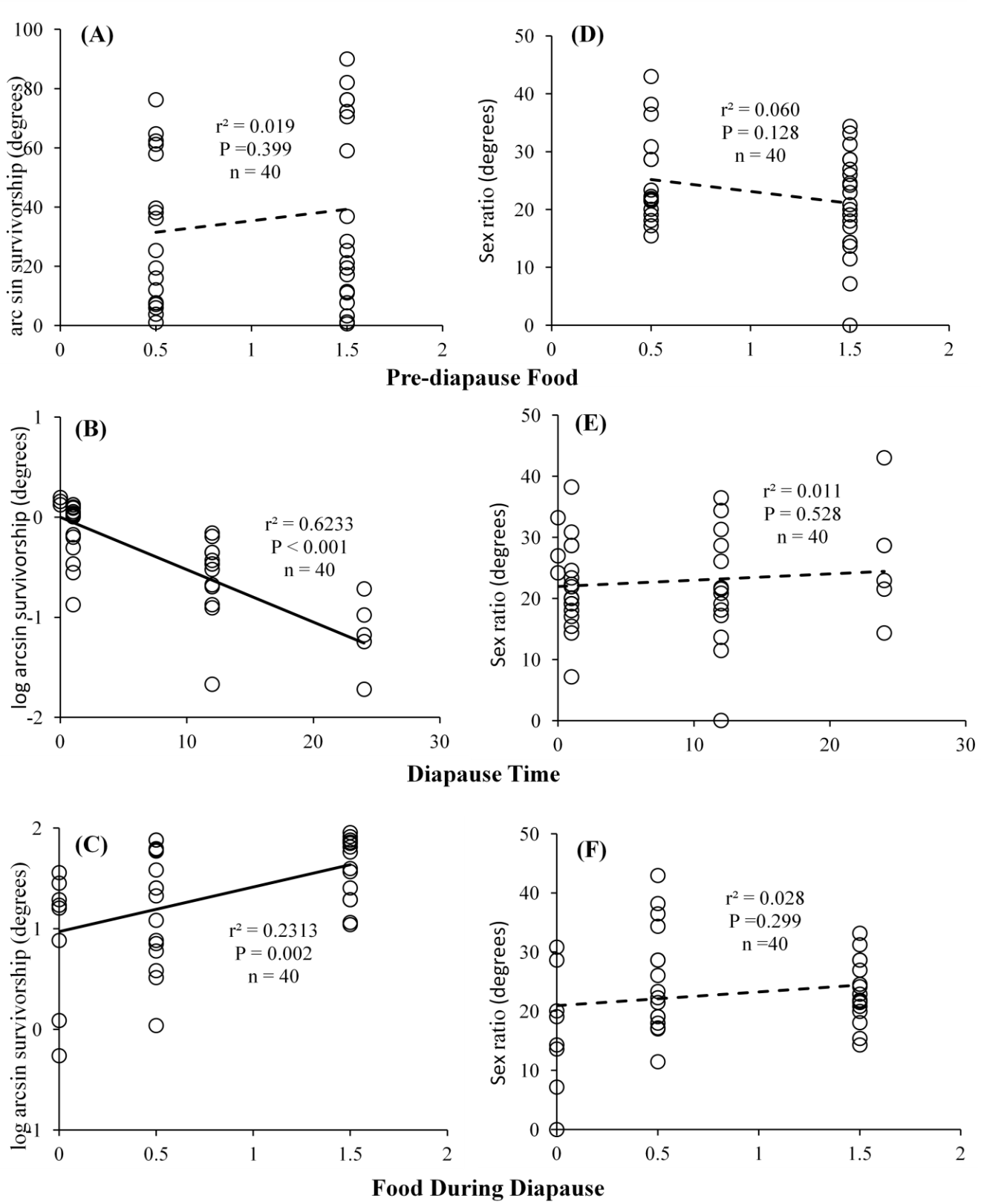

Fig. 4. Survivorship to adulthood. A. Log-arcsin transformed survivorship to adulthood. B. Log-arcsin transformed sex ratio of females among eclosed adults. Horizontal axes and regression lines the same as Figure 2.

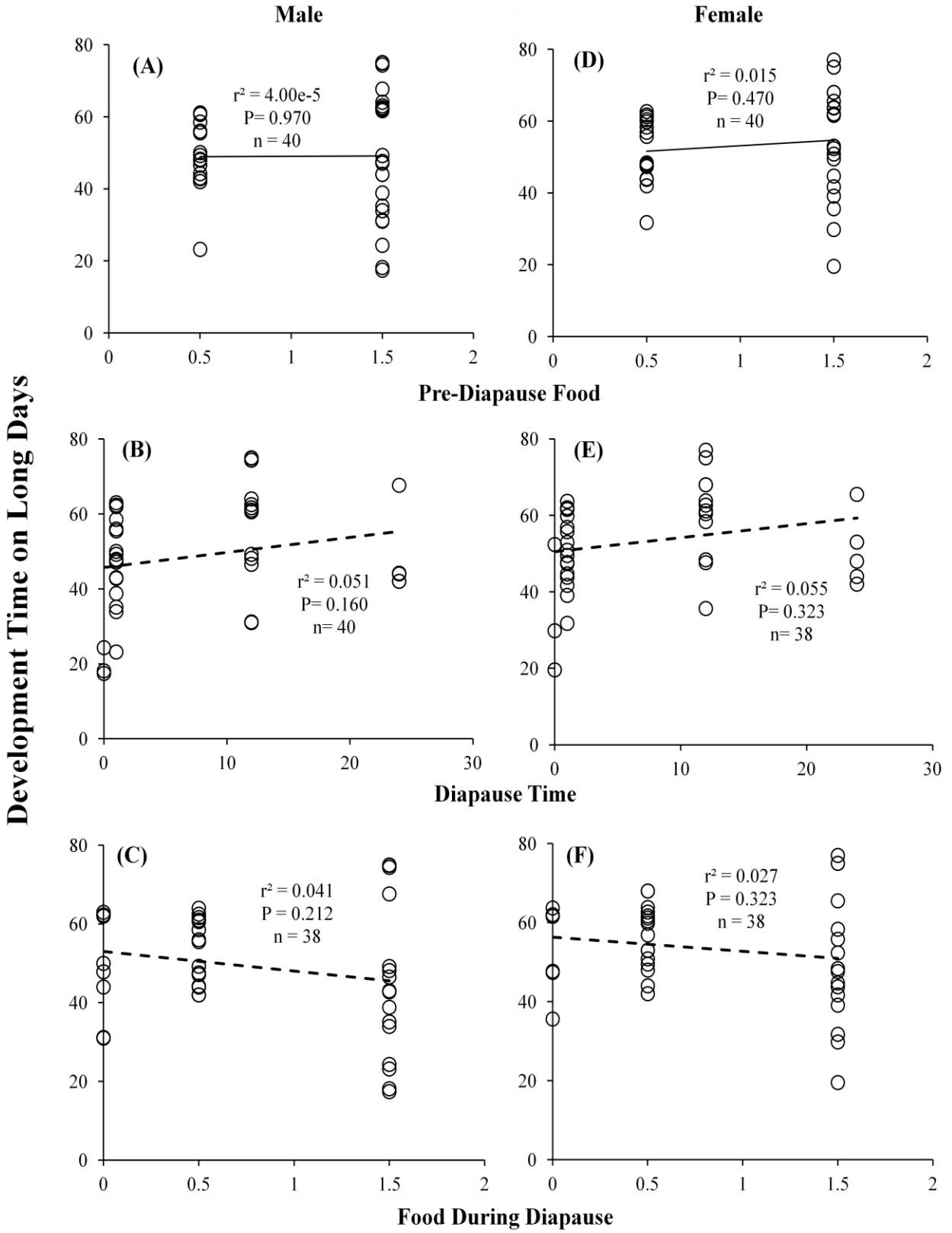

Fig. 3. Development time (days from the onset of long days until adult eclosion). Horizontal axes and regression lines the same as Figure 2.

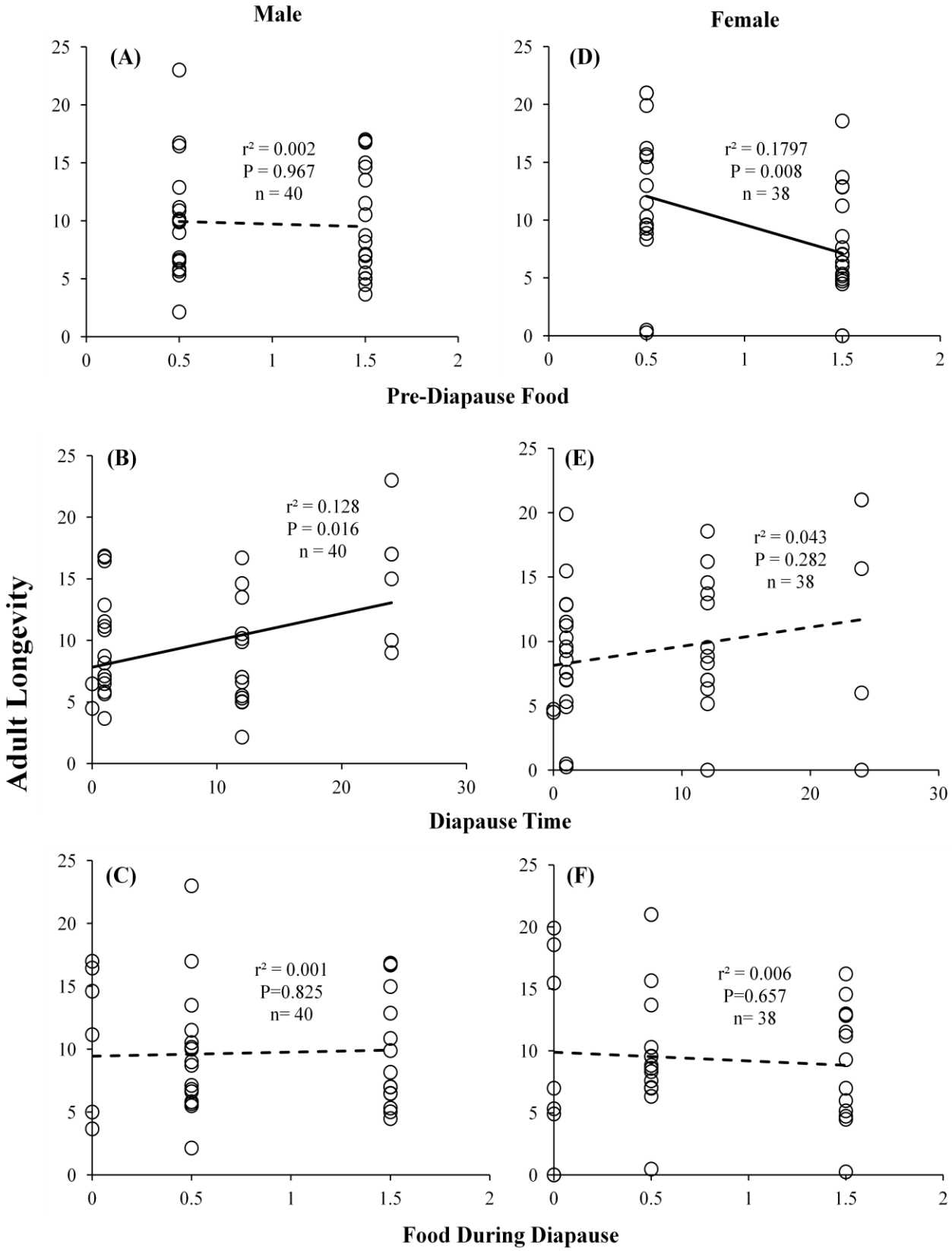

Fig. 5. Adult Longevity. Horizontal axes and regression lines the same as Figure 2.

4. Discussion

4.1. Pupal mass analysis

Pupal mass in both males and females was not influenced by nutrition or diapause time. This was included to verify whether both treatments of initial food (either 0.5 mg or 1.5 mg) were enough to ensure larval survivorship to adulthood. Neither amounts of food before or during diapause affected male or female pupal mass.

4.2. Survivorship to adulthood and female survival analysis

There is no correlation between survivorship to adulthood and pre-diapause food; therefore an individual's survival is not influenced by food availability before diapause. Survivorship to adulthood is negatively correlated with increased diapause time; this indicates that longer individuals spend in diapause, the lower their probability of surviving to adulthood. Individuals surviving through diapause may experience increased adult longevity depending on available nutrition. While diapause can increase fitness, diapause can also constitute a severe stress and can incur the cost lower survivorship to adulthood, lower female fecundity and lower egg fertility under stressful conditions (Bradshaw et. al., 1998). The positive correlation between survivorship and food during diapause demonstrates that survivorship increases significantly with the availability of nutrition during diapause. The more nutrition an individual has during diapause the higher the chance of survival to adulthood. Sex ratio was not correlated with pre-diapause food, diapause time, or food during diapause. Increased nutrition or diapause time does not favor the development of females over males.

4.3. Development time on long days analysis

The duration of pre-adult development was not affected by pre-diapause food, diapause time or food during diapause in either males or females. Individuals do not experience an extended pre-adult development regardless of the larval nutritional conditions or the duration of diapause. None of the variables appear to effect aging (development time) in either sex of *W. smithii.*

4.4. Adult Longevity

These analyses demonstrate there is no effect of food before or during diapause on adult longevity in males; however, females with lower nutrition available during their pre-diapause larval development experienced prolonged adult longevity. The latter has also been observed in female flies during DR (Bross et. al., 2005). This observation may be explained by the thrifty phenotype hypothesis (Hales and Barker, 2001), which posits that the biochemical parameters of malnourished individuals are set during their developmentally plastic stages, resulting in the ability to conserve energy and store fat in energy-deficient environments. Since females invest energy in the form of lipids into egg development, this may explain why females experiencing nutritional restriction before diapause would experience extended longevity. Resources allocated to reproduction are used to produce progeny, whereas resources allocated to maintenance are used to conserve state (Harrison and Archer, 1988). Low nutrition may give rise to lower fecundity; therefore females can invest nutritional resources in extending life instead of reproduction costs.

Male adult longevity increases as diapause time increases, while female adult longevity is not affected by diapause time. Male fitness is less dependent on size than females, and males expend less fat stores on reproduction (Fairbairn, 1997). With a large investment in maintenance during diapause with no expectation of using these stores in the adult stage, their state remains relatively unchanged, which may correspond longer adult lifespan in males. The availability of food during diapause does not influence male or female adult longevity; DR prior to diapause only increases adult longevity in females. An active metabolism during diapause (not prior to diapause) thus represents a way to accumulate available resources for maintenance during and after diapause that does not increase adult lifespan.

5. Conclusion

Dietary restriction before the onset of diapause does not influence aging or survivorship during diapause, and extends only female adult lifespan in *W. smithii*. Diapause does not influence aging, but

decreased survivorship and increased male adult longevity. DR during diapause does not influence aging or adult longevity, but decreased survivorship. This implies that *W. smithii* uses all available nutrition to maintain an active metabolism during diapause. It is not diapause that gives rise to increased longevity, but instead the active metabolic functioning during diapause. DR conditions prior to diapause may influence female plasticity to pre-program their physiology for a nutrient poor adulthood as suggested by the thrifty phenotype hypothesis.

This information contrasts with decreased survivorship observed in flies and worms experiencing DR. In both flies and worms, temperature and duration of diapause has been documented to extend lifespan; however both of these organisms experience a reduction in metabolic processes during diapause. Clearly, there is a physiological tradeoff between survivorship to the reproductive stage and increasing longevity; this tradeoff may be explained by the difference between an active metabolism and metabolic arrest. *C.elegans* are hermaphrodites and experience increased lifespan, reduced fertility, increased survivorship and slowed senescence under DR (Walker et. al., 2005). Female flies experience longer lifespan, delayed aging, decreased reproductive efficiency at young ages due to under developed female ovaries during diapause under DR. In flies the intervention of DR initiates a trade-off that extended lifespan while lowering female fecundity. Though female *W. smithii* may live longer, their chance of surviving and reproducing to the adult stage is substantially reduced compared to Drosophila females experiencing extending mean lifespan, reduced aging and increased survivorship (Crews, 2003).

These findings imply that DR and metabolism rate likely interact during diapause in flies and worms. Diapause does not represent an escape from an unfavorable season; it represents an investment in an alternative lifestyle with its own benefits, costs, and tradeoffs (Bradshaw and Holzapfel 1992). Diapause involves allocation of resources to cold- hardiness or somatic maintenance, which may incur costs to survivorship and/or reproductive performance (Bradshaw et. al., 1998). The reduced metabolic rate experienced by worms and flies in diapause and dauer, respectively, may play an undetermined role

in increasing longevity and reducing aging. While DR effects on lifespan and aging are widespread amongst animal species, there may be different biological mechanisms involved in invertebrates, small mammal and human systems. These observations can inform current genetic research and future research should focus on understanding if longevity due to DR is conserved (or not) in genetic pathways before discoveries in longevity and aging can be applied to larger mammals such as humans.

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Glossary

Aging: Deteriorative changes with time during post-maturational life that underlie an increasing vulnerability to challenges, thereby decreasing the ability of the organism to survive (Masoro, 1995).

Age-1: A mutant allele in two different genetic pathways that are identified as longevity assurance genes that determine lifespan in *C.elegans* (Crews, 2003).

Daf-2: Another mutant allele that increases longevity in *C.elegans.* This is considered to be a homolog of the human insulin/insulin-like growth factor receptor in humans. (Crews, 2003)

Diapause: a hormonally controlled cessation of development that is associated with stress tolerance and increased lipid sequestration. Diapause is life history tradeoff that enables arthropods to mitigate the negative effects of harsh seasons (Fordyce et. al., 2006).

Dietary Restriction: the imposed reduction of available food when compared to the *ad lib* condition (Masoro, 1995).

Downregulating: is the when a cell decreases the quantity of a cellular component, such as RNA or protein, in response external stimuli (Gordh and Headrick, 2001)..

Dauer: stage of larval development that favors metabolic repression for survival in stressful environments (Fielenbach and Antebi, 2008).

Eclosion: the emergence of an insect larva from the egg or an adult from the pupal case (Bradshaw 1998).

Fecundity: The number of eggs a female produces in her lifetime (Gordh and Headrick, 2001).

Fertility: The ability to reproduce or produce viable offspring (Gordh and Headrick, 2001).

Fitness: the ability for an organism to both survive and reproduce, and is equal to the average contribution to the gene pool of the next generation that is made by an average individual of the specified genotype or phenotype (Bradshaw et. al., 1998).

Gene: a unit of inheritance occupying a specific site on a chromosome that has one or more specific effects on an organism and can both recombine with other units and mutate independently to other allelic forms (Rothwell, 1993).

Homologous (homolog): corresponding genetic loci indicating presence of structure due to common ancestor (Rothwell, 1993).

Hormones: a chemical produced by the body organs, specialized cells, tissues or Endocrine glands, released into the body which mediate behavioral or physiological actions (Gordh and Headrick, 2001).

Instar: The postembryonic, immature insect between moults (Gordh and Headrick, 2001).

Insulin: synthesized in the fetal pancreas and regulates nutrient transport across cell membranes while controlling the synthesis of peptide growth factors and their binding proteins (Crews, 2007).

Insulin signaling pathway: a series of enzymatically facilitated reactions that bind insulin at the cell surface and transfer that hormonal signal to the nucleus or connect insulin with other cell-signaling pathways (King et. al, 2006).

Longevity: adult lifespan (Masoro, 1995).

Metabolism: Chemical reactions that sustain body maintenance and function in an organism (Rothwell, 1993).

Mutation: A sudden inheritable change that includes gene mutations and chromosome aberrations in the broadest sense (Rothwell, 1993).

Mutant: an individual, organism, new character, or new DNA sequence arising or resulting from genetic mutation (Crews, 2007).

Ortholog: Members of a multi-gene family in two or more species (King et. al., 2006).

Oviposition: The reproductive act of oviposition, laying the eggs. The eggs are fertilized inside the body, embryogenesis occurs after oviposition (Gordh and Headrick, 2001).

Regulatory gene: a DNA sequence that functions primarily to control the expression of other genes by modulating the synthesis of their products (Rothwell, 1995).

Senescence: a biological change by which organisms become less capable of maintaining physiological function and homeostasis with increasing survival (Crews, 2007).

Transcription factor: a varied group of regulatory molecules that are not parts of the enzymes of transcription but that recognize specific sequences in promoters and are necessary to initiate transcription in eukaryotes (Rothwell, 1993).

Wild type: the form of a gene or an individual that is considered the standard type typically found in nature (Rothwell, 1993).