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NUTRITIONAL GEOMETRY IN *ACHETA DOMESTICUS*

CAN NUTRITIONAL GEOMETRY MODULATE THE EFFECTS OF DIETARY RESTRICTION IN *ACHETA DOMESTICUS*?

By ZILLON LEBLANC, B.SC.

A Thesis Submitted to the School of Graduate Studies in Partial Fulfilment of the Requirements for the Degree of Masters of Science (Biology).

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Title: Can Nutritional Geometry Modulate the Effects of Dietary Restriction in *Acheta domesticus*? AUTHOR: Zillon LeBlanc, B.Sc. (McMaster University) SUPERVISOR: Professor C. David Rollo NUMBER OF PAGES: xi, 74

ABSTRACT

This study was performed to better understand the physiological responses of the cricket *Acheta domesticus* to dietary restriction and nutritional geometry (relative nutritional balance). Dietary restriction in crickets decreases the growth rate, survivorship, maturation mass and delays maturation, but it has the benefit of increasing their maximum longevity. Measurements of maturation mass, maturation age and longevity were obtained and used to calculate adult duration, growth rate and survivorship. This experiment combined both dietary restriction and nutritional geometry. Treatments were dietary restricted and provided with one of three macronutrients: lipid, carbohydrate or protein. The macronutrients were predicted to modulate the effects of dietary restriction while still producing an increase in maximum longevity. The lifetime restricted males and females obtained the highest maximum longevity of all treatments. The females of the carbohydrate treatment experienced significant increases in survivorship when compared to the lifetime restricted treatment. The males of the carbohydrate treatment achieved the second highest maximum longevity as well as a significant increase in longevity when compared to the lipid and protein males. A significantly earlier maturation age was obtained by the carbohydrate males when compared to the lifetime restricted treatment. The protein females had a significantly higher maximum longevity than the control treatment. The lipid treatment had an extremely low survivorship, a decreased adult duration as well as a low maturation mass. In summary, carbohydrates decreased the maturation age and

increased the survivorship of the female crickets and increased the longevity of the male crickets. The protein treatment did not obtain the expected increases in growth rates or maturation mass associated with high protein diets. Therefore, different high protein diets should be tested in conjunction with the carbohydrate diet, in order to offset the negative effects of dietary restriction.

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DECLARATION OF ACADEMIC ACHIEVEMENT

The following is a declaration that the content in this thesis has been completed by Zillon LeBlanc and recognises the contributions of Dr. C. David Rollo in both research process and the completion of the thesis.

CHAPTER 1: Introduction and Background

Nutritional geometry investigates how different ratios of macronutrients affect an organism's growth, reproduction, maturation and longevity (Simpson & Raubenheimer, 2010).This study was designed to answer the following question: can nutritional geometry alter the growth, maturation and longevity of dietary restricted crickets? The predictions of this experiment were:

1) Dietary restriction via every other day feeding should extend longevity
2) Carbohydrate with dietary restriction should result in the greatest increase in longevity while reducing the stress associated with dietary restriction
3) Protein with dietary restriction should result in a shorter longevity than the carbohydrate and lifetime restricted treatments
4) Lipid with dietary restriction should result in the shortest longevity

Dietary restriction is well known to increase the longevity of nearly all animals (Carey, et al., 2008; Inness & Metcalfe, 2008; Burger, Hwangbo, Corby-Harris, & Promislow, 2007). There is however, a cost associated to this benefit, and that is a decline in reproductive effort (Carey, et al., 2008; Inness & Metcalfe, 2008; Burger, Hwangbo, Corby-Harris, & Promislow, 2007). Free radicals generated by metabolic processes and associated oxidative stress are considered a major cause of aging, although some controversy has emerged recently (Heilbronn & Ravussin, 2005; Rollo, 2002; Joe, 2000). Consequently, dietary restriction results in less oxidative stress as well

as a decrease in the amount of offspring produced and a reduction in the overall body mass of an organism (Maklakov, et al., 2008; Le Galliard, Ferrière, & Clobert, 2005).

Dietary restriction has also been implicated in causing reductions in growth rate and delaying maturation (Lyn, Naikkhwah, Aksenov, & Rollo, 2011; Carey, et al., 2008; Segoli, Lubin, & Harari, 2007; Masoro, 2005). Dietary restriction as well as specific nutrients such as protein and carbohydrate has been shown to manipulate female egg laying and male reproductive effort in insects as well as their growth (Archer, Royle, South, Selman, & Hunt, 2009; Lee, et al., 2008; Maklakov, et al., 2008). Therefore, an increase in longevity without a decrease in reproductive effort would be considered highly advantageous to an organism. This is where dietary restriction coupled with nutritional geometry would be beneficial. Dietary restriction should cause an increase in longevity while specific nutrients should help to alleviate the costs associated with this process (Simpson & Raubenheimer, 2010; Archer, Royle, South, Selman, & Hunt, 2009; Lee, et al., 2008; Min & Tatar, 2006).

The initial dietary restriction experiment in this study was designed to set the frame work for the nutritional geometry experiment and also to examine the difference in the maximum longevity of crickets that were dietary restricted at different stages of their life cycle (juvenile versus fully matured adult). The lifetime restricted treatment was restricted for a much longer period of time than the adult restricted treatment. As such, the lifetime restricted treatment was expected to have a greater delay in

maturation, the lowest maturation mass and the largest increase in longevity when compared to the adult restricted and control treatments. This was because dietary restriction has a cumulative effect and the longer an organism is dietary restricted the greater the benefits and consequences (Goto, Takahashi, Radak, & Sharma, 2007; Stunkard, 1983). The adult restricted treatment was dietary restricted upon maturation and it should therefore experience an increase in longevity when compared to the control treatment. The adult restricted treatment should also achieve the same average maturation mass and age as the control treatment since it was not dietary restricted until maturation. Survival on the lifetime restricted treatment was expected to be lower than the other treatments since this treatment represented an environment where food was scarce (Masoro, 2005). As a result, the crickets that could not adapt to this new environmental stressor would perish.

The negative aspects of dietary restriction make it problematic to apply to humans but nutritional geometry should help to reduce or eliminate these problems (Simpson & Raubenheimer, 2010; Archer, Royle, South, Selman, & Hunt, 2009; Lee, et al., 2008). This research is applicable to humans as some people do practice dietary restriction and they exhibit several results that mice and rats possess that are thought to extend longevity (Holloszy & Fontana, 2007). However, this evidence is not conclusive (Holloszy & Fontana, 2007).

My nutritional geometry experiment observed differences in longevity, maturity and survivorship between treatments with diets consisting of increased amounts of carbohydrate, protein or lipid. When carbohydrate, protein or lipid are removed from an organism's diet there has been an associated decrease in reactive oxygen species (Sanz, Caro, & Barja, 2004; Mohanty, et al., 2002). The restriction of carbohydrate has had mixed results and it has not been shown to extend the longevity of crickets or *Drosophila* (Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Sanz, Gómez, Caro, & Barja, 2006; Heilbronn & Ravussin, 2005). Alternatively, the restriction of protein and lipid have resulted in much more pronounced increases in longevity in comparison to carbohydrate restriction in several different animals and insects (Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Lee, et al., 2008; Maklakov, et al., 2008; Sanz, Gómez, Caro, & Barja, 2006; Heilbronn & Ravussin, 2005).

Many scientists believe that protein restriction and not caloric restriction is the key to increasing longevity in dietary restriction experiments (Sanz, Gómez, Caro, & Barja, 2006; Heilbronn & Ravussin, 2005; Sanz, Caro, & Barja, 2004). Protein restriction extends longevity by reducing the generation of reactive oxygen species and free radicals which results in a decrease in DNA damage (Sanz, Caro, Sanchez, & Barja, 2006; Sanz, Gómez, Caro, & Barja, 2006; Sanz, Caro, & Barja, 2004). Specifically, the restriction of the amino acid methionine has been shown to decrease reactive oxygen species production without dietary or caloric restriction (Sanz, Gómez, Caro, & Barja, 2006; Piper, Mair, & Partridge, 2005). On the contrary, increased protein intake has been

linked to an increase in reactive oxygen species and as a result a reduction in longevity (Sanz, Caro, & Barja, 2004; Mohanty, et al., 2002; Nakagawa & Masana, 1971). As such protein has been shown to be a major contributor to oxidative stress when compared to carbohydrates (Sanz, Gómez, Caro, & Barja, 2006; Mark, et al., 1984).

In North America, fast food consists of high amounts of fat and this macronutrient has been seen as the cause for many diseases which ultimately results in a reduction in longevity (Ujvari, Wallman, Madsen, Whelan, & Hulbert, 2009; Mark, et al., 1984; Driver & Cosopodiotis, 1979). High lipid diets have been shown to accelerate aging and as a result reduce the longevity of rats and *Drosophila* (Archer V. E., 2003; Mark, et al., 1984; Driver & Cosopodiotis, 1979). The cause for this acceleration in aging is due to increased oxidative stress (Archer V. E., 2003; Mohanty, et al., 2002). Therefore, based on the current evidence and studies, caloric restriction is not as important as protein and lipid restriction when it comes to extending longevity.

The field of nutritional geometry has shown significant increases in longevity without the need for dietary restriction (Simpson & Raubenheimer, 2010; Archer, Royle, South, Selman, & Hunt, 2009; Lee, et al., 2008). Although the physiological relationship between dietary restriction and longevity extension has not been fully understood, many studies have shown that restricting specific nutrients can also extend longevity (Archer, Royle, South, Selman, & Hunt, 2009; Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Ujvari, Wallman, Madsen, Whelan, & Hulbert, 2009; Lee, et al., 2008;

Maklakov, et al., 2008). With nutritional geometry, longevity and reproduction can be manipulated for specific organisms by feeding them diets composed of certain ratios of protein (Archer, Royle, South, Selman, & Hunt, 2009; Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Maklakov, et al., 2008). For example, a diet with a low protein to carbohydrate ratio resulted in the maximum lifespan of crickets and *Drosophila* (Archer, Royle, South, Selman, & Hunt, 2009; Lee, et al., 2008). Females that consumed low amounts of protein incurred the cost of reduced egg production and less eggs laid overall (Archer, Royle, South, Selman, & Hunt, 2009; Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Carey, et al., 2008; Lee, et al., 2008; Maklakov, et al., 2008). Interestingly, an increase in the protein content of the diet increased the egg production but reduced the longevity of the females (Archer, Royle, South, Selman, & Hunt, 2009; Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Carey, et al., 2008; Lee, et al., 2008; Maklakov, et al., 2008). A high protein to carbohydrate ratio diet was shown to shorten the lifespan of insects due to an increase in reactive oxygen species production (Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Lee, et al., 2008; Maklakov, et al., 2008). Likewise, protein restriction was shown to reduce free radical generation in rats and increase their longevity (Sanz, Caro, Sanchez, & Barja, 2006; Sanz, Gómez, Caro, & Barja, 2006; Sanz, Caro, & Barja, 2004; Mark, et al., 1984). As such, the ratio of carbohydrate to protein intake must be taken into careful consideration when attempting to increase the lifespan or reproductive effort of organisms.

Several recent experiments have shown that the ratio of carbohydrates or lipids in an insect's diet can have a marked effect on their longevity (Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Ujvari, Wallman, Madsen, Whelan, & Hulbert, 2009; Lee, et al., 2008; Maklakov, et al., 2008). Longevity was maximized on a carbohydrate to protein ratio of 21:1 in the Queensland fruit fly and a ratio of 16:1 in *Drosophila* (Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Lee, et al., 2008). Reducing carbohydrates and putting the insects on a caloric restricted diet did not result in an increase in longevity (Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Lee, et al., 2008; Maklakov, et al., 2008). When blowflies were placed on a high fat diet they experienced a reduction in longevity and when on a low fat diet their longevity was extended when compared to the controls (Ujvari, Wallman, Madsen, Whelan, & Hulbert, 2009). Therefore, nutritional geometry seems to be quite promising in terms of life extension when compared to dietary restriction but the effects of each macronutrient must be examined.

Male and female crickets display a clear nutrient preference when selecting from a variety of diets (Archer, Royle, South, Selman, & Hunt, 2009; Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Lee, et al., 2008; Maklakov, et al., 2008). When given a choice, male and female crickets preferred diets with an intermediate carbohydrate to protein ratio which maximized their lifetime reproductive effort rather than longevity (Archer, Royle, South, Selman, & Hunt, 2009; Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Lee, et al., 2008; Maklakov, et al., 2008). Male and female crickets prefer

different ratios of protein to carbohydrate with females consuming more protein and males consuming more carbohydrate (Archer, Royle, South, Selman, & Hunt, 2009; Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Maklakov, et al., 2008). This difference has arisen because of the associated cost of producing eggs in females and the increased energy expenditure of males when performing mating calls (Archer, Royle, South, Selman, & Hunt, 2009; Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Maklakov, et al., 2008). The ratios of protein and carbohydrates in nutritional geometry experiments have different effects on male and female insects and as such gender differences must be carefully observed.

Dietary restriction is well known to increase longevity but it also delays maturation and results in slower growth rates (Lyn, Naikkhwah, Aksenov, & Rollo, 2011; Segoli, Lubin, & Harari, 2007; Masoro, 2005; Piper, Mair, & Partridge, 2005). As a result, the dietary restricted treatments were expected to have slower growth rates, delayed maturation ages, decreased maturation masses, and the greatest increase in longevity when compared to the control treatment. Likewise, by combining nutritional geometry and dietary restriction, we expect an even further increase in longevity without compromising maturation age, maturation mass and growth rates. This will be achieved by reducing the diet intake of the insects and increasing the quantity of specific macronutrients in their diets. An overall improvement of the quality of their diets, by switching from insect food to gerbil food, should reduce the mortality that was

associated with some of the harsher dietary restriction regimes (Lyn, Aksenov, LeBlanc, & Rollo, 2012).

High carbohydrate to protein ratio diets have been shown to increase longevity when compared to diets high in protein (Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Maklakov, et al., 2008). Since the carbohydrate treatment is dietary restricted and high in carbohydrates, it is expected to have one of the greatest increases in longevity. Diets high in carbohydrates also promote somatic maintenance which should therefore increase the treatment's survival to maturation and growth rates (Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; House, 1961; Phillips & Brockway, 1959). Due to the male cricket's mating rituals it was expected that the consumption of the carbohydrate diet would be higher since this diet increases male reproductive effort (Archer, Royle, South, Selman, & Hunt, 2009; Maklakov, et al., 2008).

The protein diet was expected to improve the growth of the male and female crickets as well as cause the crickets to mature earlier and at a greater mass. This was mainly because diets high in protein cause early maturation and increased growth rates (Segoli, Lubin, & Harari, 2007; Shahirose, Tanis, & Reg, 2006; Merkel, 1977). High protein intake has also been shown to reduce the longevity of many different organisms and it is for this reason that the protein treatment was also expected to have a decrease in longevity (Archer, Royle, South, Selman, & Hunt, 2009; Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Maklakov, et al., 2008; Sanz, Caro, Sanchez, & Barja, 2006). As

mentioned previously, this decrease in longevity is a result of an increase in reactive oxygen species associated with the protein intake and this accelerates aging because of increased DNA damage (Archer, Royle, South, Selman, & Hunt, 2009; Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Maklakov, et al., 2008; Sanz, Caro, Sanchez, & Barja, 2006; Sanz, Gómez, Caro, & Barja, 2006). Female crickets were expected to have a high diet consumption rate on the protein treatment since they require protein to produce eggs and this nutrient increases their reproductive effort (Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Maklakov, et al., 2008).

The lipid treatment is new to the dietary restriction paradigm as only carbohydrates and proteins have been focused on in the past (Archer, Royle, South, Selman, & Hunt, 2009; Lee, et al., 2008). Lipid has been implicated in an increase in free radicals, accelerated aging and decreases in longevity through disease (Mohanty, et al., 2002; Driver & Cosopodiotis, 1979). The lipid treatment was expected to have one of the shortest longevities as well as a slow growth rate and delayed maturation since this nutrient does not seem to be very beneficial in excessive quantities to insects (Ujvari, Wallman, Madsen, Whelan, & Hulbert, 2009; Mohanty, et al., 2002; Driver & Cosopodiotis, 1979). Therefore the lipid and protein treatments should have the shortest longevities of all treatments and this experiment should shed much needed light on the role of lipid in nutritional geometry.

The house cricket, *Acheta domesticus,* was chosen to be the experimental animal for these experiments since they have a short lifespan, approximately 120 days, and they can be housed easily and affordably. Various observations can also be made of the insects such as measurement of mass, consumption of food and age at maturation. In *Acheta domesticus,* gender can be determined before maturation and maturation is achieved when flightless wings are present which allows for the separation of sexes for experimental purposes (Lyn, Aksenov, LeBlanc, & Rollo, 2012). Males and females can be distinguished from one another by the presence of an ovipositor at the end of the abdomen (Lyn, Aksenov, LeBlanc, & Rollo, 2012). Lastly, Crickets are an excellent experimental organism to study longevity as their diet can be easily controlled and observations and measurements can be acquired easily throughout their lifespan..

CHAPTER 2: Methods

Colony

The breeding colony consisted of a large plastic enclosure that had a wire grid top to prevent crickets from escaping and to allow adequate air circulation (93 x 64 x 47 cm). The colony and egg-laden trays were placed inside of a 1.5 cm thick styrofoam enclosure with an ambient temperature of 30° C. The temperature was maintained with a space heater (*Sunbeam*) and verified daily with a thermometer. The dimensions of the cricket enclosures were 244 x 69 x 46 cm. The colony and experiments had a photoperiod of 12 hours light and 12 hours dark via 13 watt fluorescent light bulbs. Diet and distilled water were provided ad libitum. The diet used for the colony was chicken feed (*Quick Feeds*) placed in petri-dishes. Distilled water was given in petri-dishes containing a saturated cellulose rectangular sponge. The colony was cleaned every day. Several cardboard egg cartons were provided as shelter.

Cricket eggs were harvested by placing a plastic tray (41 x 29 x 7 cm) filled with 500mg of damp potting soil (premier pro-mix) into the colony. Egg saturated soil was removed after 24 hours of being inside the colony. The soil was then transferred into a plastic container (30 x 19 x 13 cm) with 1cm^2 openings covered with filter paper to allow adequate air circulation and to prevent the nymphs from escaping. The eggs were incubated at 30° C. The soil was sprayed with distilled water to prevent dehydration of the eggs. It took ten to fourteen days for the nymphs to hatch. At this point the nymphs

were given chicken feed and water *ad libitum* and kept as a group until they were old enough for the experiments.

General Experimental Protocol

The crickets were placed in plastic containers (10 x 10 x 10 cm). Approximately 30 small holes, 1mm in diameter, were made in the lids of the containers to ensure adequate airflow. Diet was placed in sterilized bottle caps and replaced daily. The diets were stored in a refrigerator. Distilled water was given to the crickets via microtubules with a 1cm^3 piece of sponge wedged in the mouth to prevent any spillage.

The cricket containers were placed in 1.5cm thick Styrofoam incubators with an ambient temperature of 30° C. The temperature was maintained with a heater (*Sunbeam*) and verified daily with a thermometer. There were ten crickets in each container. Experimental crickets hatched on the same day from the breeding colony. The crickets were observed every day to determine differences in mortality, maturation, sex differentiation and longevity between treatments. Females were distinguished from males by the presence of ovipositors. Mature males and females were distinguished from juveniles by the presence of flightless wings.

Dietary Restriction

One hundred crickets were used per treatment. The experiment began when the nymphs were 14 days old. First: This experiment was conducted to determine whether

crickets restricted in their juvenile stage of life would live longer than crickets that were restricted only after maturity. The dietary restriction was every-other-day feeding (24 hour feeding and 24 hour fasting). The diet used for this experiment was *Repashy Superfood's Insect Gutload* (purchased from www.repashy.com). There were three treatments in this experiment. The control group was provided with fresh diet every day. The lifetime restricted treatment was under dietary restriction and therefore had access to diet fresh every other day once the experiment began. The adult restricted treatment received fresh diet every day until maturity. After maturity the adult restricted treatment began it's every other day dietary restriction.

Nutritional Geometry

There were five treatments in this experiment and each treatment was composed of 110 crickets. The crickets used were 21 days old. Older crickets were used to reduce the impact of early mortality in the dietary restricted treatments. There were two control groups, one group was fed every day and the other was on every other day feeding dietary restriction. The other treatments in this experiment were the lipid, carbohydrate and protein treatments. The base diet used was the Extrusion Guinea Pig Feed purchased from Petsmart. The lipid was Gallo extra virgin olive oil (Fortinos Grocery), the carbohydrate was pure granulated white sugar (Redpath, purchased from Fortinos Grocery) and the protein was ISONatural 100% pure and unflavoured whey

protein (purchased from the Nutrition House). The lifetime restricted and macronutrient treatments were all on every other day feeding dietary restriction.

To create the control diet, 30 g guinea pig food was first blended into powder. To create the agar for this experiment, 300ml of water was boiled and then 2.07g of agar was mixed into the boiling water. Before the agar was solidified, the guinea pig food was added and the whole mixture was blended. The diet was then transferred into a 500ml Tupperware container and refrigerated.

The diets for the lipid, protein and carbohydrate treatments were all made similarly to the control diet. To make the carbohydrate diet, 22.5g of guinea pig food was mixed with 300ml of liquid agar. Exactly 7.5g of carbohydrate was blended into the guinea pig diet/agar mixture. This was repeated for the lipid and protein treatments. After the diets were then solidified via refrigeration and replaced on a weekly basis to ensure quality and freshness.

Diet Consumption

The average diet consumption was determined for each of the treatments in the nutritional geometry experiment. This was performed to determine if there was any compensatory feeding in the dietary restricted treatments or if there was any reduction in feeding that could be attributed to gender-specific macronutrient preference. Over the course of two weeks, mature male and female crickets of each treatment had their

food intake measured. The mean diet consumption rate was calculated from a minimum of ten samples for each gender within the respective treatment.

Statistics

Probabilities were calculated for increases in longevity, maturation age, maturation mass, diet consumption rate and growth rate using one-way ANOVA followed by Fisher's Least Significant Difference test. This test was used for the overall analysis of the males and females for all treatments at once. This test was conducted via Statistica software. Average maturation mass and average maturation age was used to determine the growth rate of the treatments. Maximum longevity and average maturation age were used to determine the adult duration of the treatments. The standard error of mean was calculated for all means. Survivorship probabilities were calculated with the Chi^2 test. The Chi^2 statistic for the survivorship of the lifetime restricted treatment was determined from a contingency table similar to the one below using a degree of freedom of one. This method was employed for all Chi^2 survivorship probability calculations.

	Survived	Deceased	Total
Control	44	56	100
Lifetime Restricted	21	79	100
Total	65	135	200

CHAPTER 3: Dietary Restriction Results

Survival to Maturation

The lifetime restricted treatment had the lowest number of crickets surviving to maturity (21%, $p < 0.0005$; Table 1). The adult restricted and control treatments had the highest percentage of crickets surviving to maturation, 50% and 44% respectively and there were no significant differences in survivorship between these two treatments (Table 1).

Longevity

The lifetime restricted crickets had the highest maximum longevity of any female treatment and this resulted in a significant increase in longevity (156 d on lifetime restricted, 124 d on adult restricted and 120 d on control, $p < 0.03$, Figure 1). There were no other significant increases in longevity between the treatments.

Maturation Age

The female lifetime restricted treatment matured in sixty seven days on average and this was significantly later than the adult restricted and control treatments (67.0 ± 2.2 d on lifetime restricted, 59.0 ± 0.7 d and 55.6 ± 1.2 d on control, $p < 0.0004$ on adult restricted and $p < 0.000003$ on control; Table 2). The female adult restricted treatment reached maturity significantly later than the control ($p < 0.03$; Table 2). The males of the lifetime restricted treatment matured later than the control and adult restricted

treatments and at sixty five days on average (64.9 ± 1.5 d on lifetime restricted, 59.5 ± 1.2 d on adult restricted and 60.0 ± 1.2 d on control, $p < 0.005$ on control and $p < 0.004$ on adult restricted; Table 3).

Adult Duration

The female lifetime restricted treatment had a significantly longer adult duration than the other treatments ($p < 0.00001$, control and adult restricted, lifetime restricted was 1.38-fold longer than control; Table 4). There were no significant differences between the adult durations of the control and adult restricted females (adult restricted was 1.01-fold longer than control; Table 4). The male lifetime restricted treatment had a significantly shorter adult duration than the control and adult restricted treatments ($p < 0.00001$, control and adult restricted, lifetime restricted was 0.74-fold shorter than control; Table 5). The male adult restricted treatment had a significantly longer adult duration than the control treatment ($p < 0.00001$, adult restricted was 1.18-fold longer than control; Table 5).

Growth Rate

The lifetime restricted females had a significantly slower growth rate than the other female treatments (2.9 ± 0.3 mg/d on lifetime restricted, $p < 0.01$ and 4.2 ± 0.3 mg/d on control, $p < 0.002$ and 4.6 ± 0.2 mg/d on adult restricted; Table 6). The lifetime restricted males had the slowest growth rate of the male treatments. (3.1 ± 0.2 mg/d on

lifetime restricted, $p < 0.005$ and 4.2 ± 0.2 mg/d on control, $p < 0.0006$ and 4.5 ± 0.3 mg/d on adult restricted; Table 6).

Maturation Mass

The lifetime restricted treatment group had the lowest mean maturation mass of the female crickets with one hundred and ninety four milligrams but it was not significantly less than the control groups maturation mass (228 ± 16.0 mg on control, $p < 0.02$ and 274 ± 13.1 mg on adult restricted; Table 7). The average maturation mass of male lifetime restricted treatment was one hundred and ninety nine milligrams and this was significantly smaller than all male treatments ($p < 0.02$ and 250 ± 9.8 mg on control, $p < 0.003$ and 270 ± 18.7 mg on adult restricted; Table 8).

CHAPTER 4: Dietary Restriction Discussion

Survival to maturation

Previous experiments with *Acheta domesticus* have shown an increase in juvenile mortality with the onset of dietary restriction (Lyn, Aksenov, LeBlanc, & Rollo, 2012). Dietary restriction decreased the number of crickets that survived to maturity in the lifetime restricted treatment compared to the control (Table 1). The adult restricted and control treatments had very similar numbers of crickets survive to maturation which was expected since neither treatment was dietary restricted before maturation (50% on adult restricted and 44% on control; Table 1). As such, less strenuous forms of dietary restriction as well as diets higher in carbohydrate should be investigated as they might be able to alleviate the poor survivorship found in the lifetime restricted treatment.

Longevity

Oxidative stress is well known to be one of the key factors linked to aging (Sanz, Caro, Sanchez, & Barja, 2006; Barja, 2004; Sanz, Caro, & Barja, 2004). The free radicals produced by cellular respiration cause DNA damage which ultimately results in aging (Sanz, Caro, Sanchez, & Barja, 2006; Barja, 2004; Sanz, Caro, & Barja, 2004). Based on previous studies, early dietary restriction is expected to result in a greater increase in longevity than dietary restriction at maturity (Goto, Takahashi, Radak, & Sharma, 2007; Stunkard, 1983). This is attributable to the fact that dietary restriction results in a cumulative effect of reducing free radicals (Sanz, Caro, Sanchez, & Barja, 2006; Barja,

2004; Sanz, Caro, & Barja, 2004). Therefore, the longer the duration of time the organism is protected from harmful free radicals, the less damaged its DNA is thought to become (Sanz, Caro, Sanchez, & Barja, 2006; Barja, 2004; Sanz, Caro, & Barja, 2004). This would result in a slower aging process and an increased life span (Sanz, Caro, Sanchez, & Barja, 2006; Barja, 2004; Sanz, Caro, & Barja, 2004).

The lifetime restricted and adult restricted treatments were the only dietary restricted treatments. The main difference between the two treatments was that the adult restricted treatment was only placed on dietary restriction after maturity. As such, the lifetime restricted treatment should have had a greater reduction in free radicals produced during the juvenile phase compared to the other treatments as it was the only treatment being dietary restricted at that stage of life. Therefore, the lifetime restricted treatment should have the greatest increase in maximum longevity.

The lifetime restricted females obtained the highest maximum longevity and lived significantly longer than the control treatment (Table 1). This result was expected given the duration of dietary restriction experienced by the lifetime restricted treatment. The males of the lifetime restricted treatment had a lower maximum longevity than the other treatments. The mortality of juvenile lifetime restricted crickets could have been associated with this result.

The lack of a significant increase in longevity for the adult restricted treatment could be attributed to the onset of dietary restriction and its duration. The adult

restricted treatment was restricted upon maturation and therefore its growth phase was completed. Growth is known to cause an increase in oxidative stress since the organism is producing new cells and tissues rapidly instead of just replacing and maintaining them (Goto, Takahashi, Radak, & Sharma, 2007; Mohanty, et al., 2002). The adult restricted treatment was dietary restricted after maturity and thus for a much shorter period of time than the lifetime restricted treatment. Therefore, it is possible that the benefits of dietary restriction were reduced in the adult restricted treatment.

Maturation Age

The maturation age of the cricket *Acheta domesticus* determines whether the cricket reproduces at an early age or later in its lifespan as only after this final moult are the cricket's reproductive organs fully developed. The maturation of crickets in this study was characterized by the presence of flightless wings as well as a fully developed ovipositor in females (Lyn, Naikkhwah, Aksenov, & Rollo, 2011).

Dietary restriction has been implicated in causing a delay in the maturation and reproduction of several insects including crickets (Lyn, Naikkhwah, Aksenov, & Rollo, 2011; Segoli, Lubin, & Harari, 2007; Mair, Sgrò, Johnson, Chapman, & Partridge, 2004). This correlation can be explained by Hormesis. Hormesis is the occurrence of advantageous adaptations due to environmental stress (Masoro, 2005). Based on hormesis, an extension in longevity is a developmental shift in the cricket that better suits it for an environment where food is scarce (Lyn, Naikkhwah, Aksenov, & Rollo,

2011; Segoli, Lubin, & Harari, 2007; Masoro, 2005; Mair, Sgrò, Johnson, Chapman, & Partridge, 2004). Without adequate nourishment, reproductive effort is expected to decrease (Maklakov, et al., 2008; Segoli, Lubin, & Harari, 2007). Therefore it is possible that an environment with poor nourishment induces a delay in sexual maturity in order to give the organism an opportunity to search for a better environment before reproducing (Segoli, Lubin, & Harari, 2007). This would increase fecundity if the environment was rich with nutrients and other resources. If the organism is not able to achieve their goal in finding a more reliable and plentiful food source then a delay in maturity and a reduction in offspring would occur (Maklakov, et al., 2008; Segoli, Lubin, & Harari, 2007; Mair, Sgrò, Johnson, Chapman, & Partridge, 2004). As such, the time it takes a cricket to achieve maturation is important with regards to their reproduction.

Dietary restriction was expected to prolong the maturation age of both males and females. The lifetime restricted treatment was expected to have a delay in maturation age when compared to the control and adult restricted treatments as it was the only treatment under dietary restriction before maturation. The females of the lifetime restricted treatment matured significantly earlier than the adult restricted and control treatments (Table 2). Likewise, the males of the lifetime restricted treatment matured significantly later than the control and adult restricted treatments (Table 3). This confirmed the hypothesis that dietary restriction would result in a delay in the maturation age of the male and female crickets.

Adult Duration

The duration of time a cricket spends after maturation till its death is known as its adult duration. Adult duration should therefore be extended with early maturation and increased lifespan. As such, treatments under dietary restriction which modify the maturation age or longevity of the crickets will have an impact on the adult duration.

The lifetime restricted treatment was hypothesized to have an increase in adult duration when compared to the control (Segoli, Lubin, & Harari, 2007; Mair, Sgrò, Johnson, Chapman, & Partridge, 2004). This was primarily due to dietary restriction causing a delay in the onset of maturation as well as an increase in longevity (Lyn, Naikkhwah, Aksenov, & Rollo, 2011; Segoli, Lubin, & Harari, 2007; Mair, Sgrò, Johnson, Chapman, & Partridge, 2004). The results supported this hypothesis as the females of the lifetime restricted treatment obtained a significantly longer adult duration than the other treatments (Table 4).

The adult restricted treatment was expected to have an extended adult duration as it was dietary restricted after maturation. This treatment's constant access to diet during its juvenile period should have decreased its maturation age and its maximum longevity should have been increased due to the dietary restriction protocol implemented after maturity (Segoli, Lubin, & Harari, 2007; Mair, Sgrò, Johnson, Chapman, & Partridge, 2004; Stunkard, 1983). The adult durations of the control and adult restricted treatments were not significantly different from one another (Table 4).

This could indicate an optimal adult duration based on certain environmental conditions in the juvenile phase. The adult restricted male treatment was found to have the longest male adult duration and the male lifetime restricted treatment had the shortest (Table 5). This resulted in a significant decrease in the adult duration of the lifetime restricted males ($p < 0.00001$; Table 5). This unexpected result could have been explained by the male lifetime restricted treatment experiencing a lower maximum longevity than anticipated which caused their adult duration to be shorter than expected.

Growth Rate

The growth rates of all organisms have been shown to decrease when they are dietary restricted (Masoro, 2005; Joe, 2000). The crickets of the lifetime restricted treatment group, being the only treatment on dietary restriction during the juvenile growth phase (pre-maturation), was expected to have the slowest growth rate. The results supported this hypothesis as the lifetime restricted males and females had significantly slower growth rates than the control and adult restricted treatments (Table 6).

The crickets of the adult restricted treatment group was expected to have an identical growth rate to the control group since it was only dietary restricted after it achieved maturity and was therefore finished growing. The results agreed with this hypothesis as there were no significant differences between the control and adult restricted male and female growth rates (Table 6). The dietary restricted crickets did in

fact have a slower growth rate than the treatments provided with constant access to diet, agreeing with the literature on dietary restriction.

Maturation Mass

Dietary restriction causes a decrease in growth rate and therefore crickets provided with diet ad libitum should achieve a larger maturation mass as the constant availability of nutrients would fuel their growth (Masoro, 2005; Joe, 2000). The lifetime restricted treatment was expected to have the lowest maturation mass of all treatments as it was dietary restricted during its growth phase. It essentially would not have enough nutrients to support a body size and growth rate as large as the other treatments (Masoro, 2005; Joe, 2000).

The female lifetime restricted crickets had a significantly lower maturation mass than the adult restricted treatment (Table 7). The males of the lifetime restricted treatment were significantly smaller than the other male treatments (Table 8). The difference in the maturation mass of the control and the adult restricted treatments were interesting as neither group was dietary restricted before maturation but the adult restricted crickets were significantly larger at maturation. The lifetime restricted crickets obtained the lowest mean maturation masses of all treatments although the females were not significantly smaller than the control treatment, partially confirming the hypothesis.

CHAPTER 5 – Nutritional Geometry Results

Survival to Maturation

The control crickets had the highest survival with eighty five percent reaching maturity. Crickets in the carbohydrate, lifetime restricted and protein treatment groups had the next highest survival (35% on carbohydrate, 23% on lifetime restricted and 18% on protein; Table 9). Ninety five percent of the crickets in the lipid treatment group died before reaching maturity and this resulted in the lowest survival of any treatment (Table 9).

The females of the lipid treatment had a significantly lower survival when compared to the lifetime restricted treatment ($p < 0.03$; Table 9). The carbohydrate females had a significantly higher survivorship than the lifetime restricted treatment ($p < 0.05$; Table 9). The protein females did not have a significantly different survivorship than the lifetime restricted treatment (Table 9).

The males of the lipid treatment were the only treatment to have a significantly lower survivorship than the lifetime restricted treatment ($p < 0.005$; Table 9). The protein and carbohydrate males did not have a significantly different survivorship compared to the lifetime restricted males (Table 9).

Longevity

Female crickets in the lifetime restricted treatment group had a maximum longevity of one hundred and thirty five days (Table 9). This resulted in the highest longevity of all crickets (Table 9). The females of the lifetime restricted treatment had a significant increase in longevity when compared to the control and carbohydrate females ($p < 0.004$ on control and $p < 0.04$ on carbohydrate, Figure 3). The female crickets of the protein treatment group had a maximum longevity of one hundred and nineteen days. This resulted in a significant increase in longevity when compared to the control females ($p < 0.03$, Figure 3). The lipid female crickets had a maximum longevity of ninety six days and this was the lowest maximum longevity of the female treatments (Table 9). There was a significant decrease in the longevity of the female lipid treatment when compared to the control, protein and lifetime restricted treatments ($p < 0.01$, Figure 3).

Male crickets in the lifetime restricted treatment group had the highest maximum longevity relative to the other male treatment groups with a one hundred and thirty two day lifespan (Table 9). The lifetime restricted males experienced a significant increase in longevity when compared to the protein and lipid treatments ($p < 0.000001$ on protein and $p < 0.00004$ on lipid Figure 4). The carbohydrate males also lived significantly longer than the protein and lipid treatments ($p < 0.000001$ on protein and $p < 0.00004$ on lipid Figure 4). The protein treatment had the lowest maximum longevity

for male crickets with a maximum longevity of ninety nine days (Table 9). This resulted in a significant decrease in longevity when compared to the control males ($p < 0.00004$, Figure 4).

Maturation Age

The control females had the earliest maturation age on average (68.1 ± 0.9 d; Table 10). The female protein and carbohydrate treatments matured significantly later than the control group ($p < 0.01$ on protein and $p < 0.03$ on carbohydrate; Table 10). There were no significant differences between the lipid and lifetime restricted female maturation ages (Table 10). The females of the protein treatment did however mature the latest on average (75.2 ± 3.8 d, N.S.; Table 10).

The control males, like the females, matured the earliest of all treatments on average (68.6 ± 0.8 d; Table 11). The lifetime restricted treatment reached maturity later than all of the other treatments on average (79.8 ± 2.2 d; Table 11). The male lifetime restricted and protein treatments achieved maturity significantly later than the control treatment ($p < 0.00007$ on control and $p < 0.01$ on protein; Table 11).The male crickets of the carbohydrate treatment matured significantly earlier than the lifetime restricted treatment ($p < 0.005$; Table 11).

Adult Duration

Crickets in the lifetime restricted treatment group had a significantly longer adult duration the other female treatments (lifetime restricted was 1.59-fold longer than control, lipid was 0.63-fold shorter than control, protein was 1.10-fold longer than control and carbohydrate was 1.06-fold longer than control; $p < 0.00001$; Table 12). The female lipid treatment had a significantly shorter adult duration than the other treatments (lipid was 0.63-fold shorter than control, $p < 0.0009$; Table 12). The male protein treatment had a significantly shorter adult duration than the other treatments (protein was 0.45-fold shorter than control, lifetime restricted was 1.04-fold longer than control, lipid was 0.97-fold shorter than control and carbohydrate was 1.10-fold longer than control, $p < 0.003$ on lipid, $p < 0.00001$ on carbohydrate, control and lifetime restricted; Table 13). The carbohydrate treatment had the highest adult duration of all the male treatments but it was only significantly longer than the control treatment (1.10-fold longer than control, $p < 0.04$; Table 13).

Diet Consumption

Crickets of the carbohydrate treatment group had the greatest rate of diet consumption of the females (63.01 ± 3.93 mg/d; Table 14). Crickets of the lipid treatment had the lowest rate of diet consumption for females (6.24 ± 6.10 mg/d; Table 14). The female crickets of the lifetime restricted treatment group consumed significantly more diet than the lipid and protein crickets ($p < 0.0002$, lipid and $p < 0.04$

on protein; Table 14). The crickets of the lipid and protein female treatment groups also consumed significantly less diet than the control treatment ($p < 0.00009$, lipid and 0.04 on protein; Table 14). The crickets of the carbohydrate female treatment group consumed significantly more diet than the lipid and protein females (0.000001, lipid and $p < 0.001$ protein; Table 14). The lipid and protein treatment's crickets had the lowest rates of diet consumption of the females.

For males, the crickets of the carbohydrate treatment group had the greatest rate of diet consumption (55.86 ± 3.06 mg/d; Table 11). The crickets of the lipid treatment group had the lowest rate of diet consumption for males (7.92 ± 6.41 mg/d; Table 11). The lifetime restricted males consumed significantly more diet than the lipid and protein treatment groups ($p < 0.002$, lipid and $p < 0.008$ on protein; Table 11). The lipid and protein males also consumed significantly less diet than the control treatment ($p < 0.001$, lipid and 0.008 on protein; Table 11). The carbohydrate males consumed significantly more diet than the lipid and protein males (0.00001, lipid and $p < 0.0009$ protein; Table 11). The lipid and protein treatments had the lowest rates of diet consumption of the males. Therefore the lipid and protein males and females had a significantly lower rate of diet consumption the males and females of the other treatments.

Growth Rates

The female control crickets had the fastest growth rate of any female treatment (3.3 ± 0.09 mg/day; Table 14). The females of the carbohydrate treatment had the slowest female growth rate (2.4 ± 0.1 mg/day Table 14). All female treatments grew significantly slower than the control females ($p < 0.00003$ for lifetime restricted, $p < 0.005$ for lipid, $p < 0.00001$ for protein and $p < 0.00001$ for carbohydrates; Table 14).

The male control crickets had the fastest male growth rate of any male treatment (3.0 ± 0.07 mg/day; Table 15). The protein treatment had the slowest male growth rate of any treatment (2.1 ± 0.2 mg/day; Table 15). Similar to the females, the lifetime restricted, protein and carbohydrate treatments possessed a significantly slower growth rate than the control males ($p < 0.00001$ for lifetime restricted, N.S. for lipid, $p < 0.00001$ for protein and $p < 0.0004$ for carbohydrate; Table 15). The lipid treatment was not significantly slower but it should also be noted that only one lipid male reached maturity.

Maturation Mass

The females of the control group had the highest average maturation mass for females with two hundred and twenty six milligrams (226 ± 6.5 mg; Table 16).The female control crickets had a significantly larger maturation mass than all female treatments ($p < 0.005$ for all treatments; Table 16). The carbohydrate females had the lowest average maturation mass (167 ± 5.9 mg). The male controls had the largest maturation mass

while the lipid treatment had the lowest (205 ± 4.6 mg on control, 144 mg on lipid). The male control treatment had a significantly larger maturation mass than the lifetime restricted, carbohydrate and protein treatments ($p < 0.009$ for all treatments except lipid; Table 17). There were no other significant differences between treatments.

CHAPTER 6: Nutritional Geometry Discussion

Survival to Maturation

Dietary restriction has been shown to decrease the survival of crickets (Lyn, Aksenov, LeBlanc, & Rollo, 2012). In this study, the control treatment was observed to have the greatest survival to maturity as it was the only treatment that was not dietary restricted. The lifetime restricted treatment represented an environment where food was scarce and not of increased nutritional value and as a result the crickets on dietary restriction showed increased mortality (Lyn, Aksenov, LeBlanc, & Rollo, 2012; Segoli, Lubin, & Harari, 2007). The lifetime restricted males and females were expected to have less crickets survive to maturation than the control, carbohydrate and protein treatments. The experiment partially confirmed this as the lifetime restricted treatment had a significantly lower survivorship than the carbohydrate females but not the protein treatment or the carbohydrate males ($p < 0.05$, carbohydrate females; Table 9). As a result, new methods must be investigated in order to reduce the mortality found in dietary restricted crickets.

Male and female insects require specific macronutrients such as carbohydrates and protein for reproduction and reproductive effort (Archer, Royle, South, Selman, & Hunt, 2009; Maklakov, et al., 2008). Male crickets, when given a choice, preferred higher carbohydrate diets and these diets have been shown to increase male reproductive effort (Simpson & Raubenheimer, 2010; Archer, Royle, South, Selman, & Hunt, 2009;

Maklakov, et al., 2008). As such, a diet with plentiful amounts of carbohydrate was anticipated to have a greater amount of male crickets survive to maturity as this is the macronutrient they require for reproduction (Archer, Royle, South, Selman, & Hunt, 2009; Maklakov, et al., 2008). The carbohydrate treatment was expected to have the highest survival to maturity for male dietary restricted crickets. However, this study did not produce significant results to confirm this hypothesis although the male crickets on the carbohydrate diet had the highest survival to maturity of the dietary restricted treatments (Table 9).

Female crickets were anticipated to have the highest survival to maturity on the protein diet since they produce an increased number of eggs on high protein diets (Simpson & Raubenheimer, 2010; Archer, Royle, South, Selman, & Hunt, 2009; Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Maklakov, et al., 2008). As such, a diet that consists of a protein, which required for their reproduction, is expected to result in a higher amount of females surviving to maturity. The experiment did not support this hypothesis as the protein treatment had one of the lowest survivorships of the treatments and only ten females achieved maturity. Another experiment is required to verify this with other sources of protein such as a base diet containing a higher amount of protein.

The lipid treatment was hypothesized to have a decreased male and female survivorship. This stems from previous experiments on lipid consumption which

indicated that excess dietary fat resulted in increased mortality and oxidative stress (Ujvari, Wallman, Madsen, Whelan, & Hulbert, 2009; Mohanty, et al., 2002; Driver & Cosopodiotis, 1979). The lipid treatment had the lowest survival to maturation for male and female crickets which agreed with the hypothesis (Table 9). In conclusion, dietary restriction coupled with carbohydrate and protein did not achieve the anticipated results, however, the lipid treatment did confirm its hypothesis.

Longevity

The lifetime restricted treatment was expected to have a significant increase in maximum longevity when compared to the control treatment as it was dietary restricted. This is because dietary restriction has been shown to extend the maximum longevity of many different organisms (Heilbronn & Ravussin, 2005; Sanz, Caro, & Barja, 2004; Mark, et al., 1984; Stunkard, 1983). The experiment maintained this hypothesis as the lifetime restricted male and female crickets had the highest maximum longevity of all treatments (Table 9). The lifetime restricted females had a significant increase in longevity compared to the controls (Figure 3). A less strenuous method of dietary restriction should be utilized in an attempt to increase the survivorship of the dietary restricted treatments and as such increase the statistical significance of the longevity results.

Male and female longevity were shown to become maximized on a high carbohydrate diet in several studies (Fanson, Weldon, Pérez-Staples, Simpson, & Taylor,

2009; Maklakov, et al., 2008). As such, the male and female carbohydrate treatments were hypothesized to have a maximum longevity that rivals the lifetime restricted treatment. Experiments in this study partially supported this hypothesis for the males but not the females (Table 9). Females of the carbohydrate treatment had a significantly lower maximum longevity than the lifetime restricted treatment (Figure 3). This decrease in longevity could have been caused by the proportion of carbohydrate to protein in the diet. Previous studies have used ratios of carbohydrate to protein as high as 16:1 in order to experience increased longevity (Archer, Royle, South, Selman, & Hunt, 2009; Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Maklakov, et al., 2008).The diet in this experiment may not have consisted of a high enough ratio of carbohydrate to protein for a significant increase in maximum longevity to be obtained.

Increased protein intake resulted in increased egg production in female crickets in several studies (Archer, Royle, South, Selman, & Hunt, 2009; Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Maklakov, et al., 2008). The increased egg production consequently reduced the maximum longevity of the female crickets (Archer, Royle, South, Selman, & Hunt, 2009; Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Maklakov, et al., 2008). It has been well documented that high levels of protein consumption causes increased free radicals which result in oxidative damage and decreased longevity of many different organisms (Sanz, Gómez, Caro, & Barja, 2006; Archer, Royle, South, Selman, & Hunt, 2009; Sanz, Caro, Sanchez, & Barja, 2006; Maklakov, et al., 2008). As such the males and females of the protein treatment were

expected to have one of the lowest maximum longevities of the dietary restricted treatments. The male crickets had a low maximum longevity which was expected but the females lived longer than hypothesized (Table 9). The protein females had the second highest maximum longevity of all female crickets (Table 9). This was contrary to what was expected. It is possible that a dietary restricted female with every-other-day access to a diet high in protein content could still receive the benefits of increased egg production while the dietary restriction increased their maximum longevity. Interestingly, the females could have been distributing resources towards somatic maintenance on restricted days and reallocating their resources towards reproduction on feeding days (Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Hatle, et al., 2008; Maklakov, et al., 2008). This would have allowed the females of the protein treatment to obtain a high maturation mass as well as a high maximum longevity.

Diets with increased lipid content have been shown to have a detrimental effect on an organism's lifespan (Ujvari, Wallman, Madsen, Whelan, & Hulbert, 2009; Heilbronn & Ravussin, 2005; Driver & Cosopodiotis, 1979). One mechanism behind the decreased longevity of crickets on high lipid diets could be increased free radical production which would result in DNA damage and accelerated aging (Mohanty, et al., 2002; Loft, Thorling, & Poulsen, 1998). Therefore the lipid treatment was not expected to live as long as the lifetime restricted treatment. The males and females of the lipid treatment supported this theory of aging as neither lived significantly longer than their conspecifics. This should be confirmed with varying amounts of lipid as excess lipid

increases the mortality of some insects and this would prevent the crickets from achieving a high maximum longevity (Ujvari, Wallman, Madsen, Whelan, & Hulbert, 2009).

Maturation Age

Dietary restriction has been shown to delay the onset of maturation in several different species (Pauwels, Stoks, & De Meester, 2010; Segoli, Lubin, & Harari, 2007; Heilbronn & Ravussin, 2005; Mair, Sgrò, Johnson, Chapman, & Partridge, 2004). As such, a delay in maturation was expected in the dietary restricted treatments and not the control treatment. The lifetime restricted treatment, which had no macronutrients added to enrich the diet, should have been the latest treatment to mature on average (Maklakov, et al., 2008; Segoli, Lubin, & Harari, 2007). This experiment partially supported this hypothesis as only the males of the lifetime restricted treatment achieved maturity later than the other treatments (Table 11). The mean maturation age of the control and lifetime restricted females were similar and this was unexpected as the latter treatment was dietary restricted (Table 10).

Experiments have shown that protein is preferred by female crickets and this allows them to improve their reproductive effort (Archer, Royle, South, Selman, & Hunt, 2009; Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Lee, et al., 2008; Maklakov, et al., 2008). Increased protein consumption in female crickets results in increased egg production (Archer, Royle, South, Selman, & Hunt, 2009; Maklakov, et al.,

2008). This nutritional preference could have hastened maturation in females that had access to protein. The experiment did not confirm the hypothesis that the protein treatment would mature faster than the other dietary restricted treatments. The protein females were the last treatment to achieve maturity on average but they were not significantly different from any of the other dietary restricted treatments (Table 10). The protein treatment did however mature significantly later than the control treatment on average. The palatability of whey protein should be analyzed with other sources of protein since this as well as other results stemming from the protein treatment were unexpected.

Studies have shown that male crickets prefer carbohydrates and this nutrient increases their reproductive effort in the form of mating calls (Archer, Royle, South, Selman, & Hunt, 2009; Maklakov, et al., 2008). Therefore, it is hypothesized that the males of dietary restricted treatments would achieve maturity fastest on the carbohydrate diet. The experiment confirmed the hypothesis as the carbohydrate males matured significantly earlier than the lifetime restricted treatment and had the earliest average maturation age of the dietary restricted treatments (Table 11). As a result, the carbohydrate males achieved its predicted early maturation.

The lipid treatment was expected to have delayed maturation for males and females since excessive amounts of this nutrient have not been shown to benefit longevity or reproduction in insects (Ujvari, Wallman, Madsen, Whelan, & Hulbert, 2009;

Driver & Cosopodiotis, 1979). Results of this experiment were not significant and therefore the lipid females matured around the same time as the other treatments (Table 10). Males of the lipid treatment were not taken into consideration as only one cricket survived to maturity (Table 11). With the exception of the protein treatment all female treatments took approximately seventy days to achieve maturation on average (Table 10). The lack of variability in average maturation age could have indicated that there was a goal age at which female crickets mature regardless of environmental conditions.

Adult Duration

Average maturation age and maximum longevity were the two components used to determine the adult duration of the crickets. An early maturation and a high maximum longevity it would result in an extended adult duration when compared to the control. As such, the different macronutrients should manipulate the adult duration as each treatment had different projected maturation ages and maximum longevities.

The females of the protein treatment were expected to experience early maturation and a decrease in longevity (Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Maklakov, et al., 2008). As such, the protein treatment should have had one of the shortest female adult durations. The results agreed with that hypothesis as the females of the protein treatment had a significantly shorter adult duration than the

lifetime restricted treatment (Table 12). The main reason the protein females achieved a short adult duration was because of its unexpected delay in maturation.

Male crickets being fed a high carbohydrate diet were expected to mature earlier than the lifetime restricted treatment and have a moderate extension in longevity since the carbohydrate diet possessed a higher amount of energy (Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Maklakov, et al., 2008). As a result, the carbohydrate treatment was expected to have one of the longest adult durations for male crickets and the results supported this hypothesis. The males of the carbohydrate treatment had a significantly longer adult duration than the control treatment (Table 13). In conclusion, both the carbohydrate and protein treatments have achieved their expected adult duration results.

The lifetime restricted treatment was hypothesised to have a delayed maturation age and greatest longevity. Therefore, this treatment should have had a shorter adult duration than the protein and carbohydrate treatments which were both expected to mature earlier. The experiment did not confirm this hypothesis for the lifetime restricted females or males. The females of the lifetime restricted treatment had a significantly longer adult duration than the other treatments (Table 12). The extended longevity of the lifetime restricted females resulted in a lengthened adult duration even though they experienced a delayed maturation. The lifetime restricted males did not have a significantly shorter adult duration than the other treatments (Table 13). The extended

adult durations of the lifetime restricted treatment can be explained due to its high maximum longevity.

The control and lipid treatments were expected to have the two shortest adult durations in this experiment. The control treatment was predicted to mature the earliest and also have the shortest lifespan. This combination should therefore have resulted in a decreased adult duration when compared to the other treatments and the results partially supported this for the female treatments. The females of the control treatment had a significantly shorter adult duration than the lifetime restricted treatment (Table 12). The lipid treatment should have produced short lived male and female crickets and also cause a delay in maturation (Ujvari, Wallman, Madsen, Whelan, & Hulbert, 2009). This was hypothesized to result in a reduced adult duration. The results indicated that the female lipid treatment had a significantly shorter adult duration than the other treatments (Table 12). The lack of variability in the male adult durations could have indicated optimal adult durations that male crickets achieved regardless of diet and food availability (Table 13). To conclude, the longest adult duration was possessed by the lifetime restricted female treatment and this 1.59-fold extension of the control's adult duration was not matched or exceeded by any male adult duration.

Diet Consumption

Compensatory feeding is not uncommon in organisms that are dietary restricted (Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Hatle, et al., 2008). In this

experiment, the dietary restricted treatments were expected to consume more food per day than the control treatment (Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Hatle, et al., 2008). There were no significant differences between the control, lifetime restricted and the carbohydrate treatments even though the latter two were dietary restricted (p = N.S.; Tables 14 and 15). This was not expected as the dietary restricted treatments should have had a much higher rate of diet consumption than the control which was not under dietary restriction (Tables 14 and 15). The unexpectedly high rate of diet consumption in the control treatment could have been due to increased energy expenditure which is a result of its faster growth rate and increased hypothesized reproductive effort (Le Galliard, Ferrière, & Clobert, 2005; Masoro, 2005; Joe, 2000). An experiment that measures sexual effort should be examined in terms of male mating calls and female egg production/laying in order to determine if this hypothesis was correct.

The female's preference for protein, which increases its reproductive effort, should result in the protein treatment having the highest female diet consumption (Ujvari, Wallman, Madsen, Whelan, & Hulbert, 2009; Maklakov, et al., 2008). The experiment did not support this as the females on the protein diet consumed significantly less diet per day than the lifetime restricted and control treatments (Table 15). This raises the recurring question of whether or not this specific protein diet was suitable for crickets as this result is quite unexpected.

Male crickets have a preference for diets that contain high amounts of carbohydrates which results in increased reproductive effort (Maklakov, et al., 2008; Segoli, Lubin, & Harari, 2007). Therefore, the males of the carbohydrate treatment should have consumed more diet per day than any other dietary restricted treatment. The carbohydrate males had the highest average rate of diet consumption of all male treatments and it was significantly higher than the lipid and protein treatments (Table 15). The results of this experiment confirmed this hypothesis for the carbohydrate males.

The lipid treatment was expected to have lower diet consumption than the other dietary restricted treatments. This was because increased lipid consumption does not produce any benefits in terms of reproductive effort or increased longevity (Mohanty, et al., 2002; Driver & Cosopodiotis, 1979). Males and the females of the lipid treatment supported this hypothesis as the lipid treatment had the lowest rate of diet consumption for males and females and it was significantly lower than the carbohydrate, lifetime restricted and control treatments (Tables 14 and 15).

Growth Rates

Dietary restricted animals have been shown to possess a reduced growth rate when compared to animals fed ad libitum (Inness & Metcalfe, 2008; Le Galliard, Ferrière, & Clobert, 2005). As such, the control treatment was expected to have the fastest growth rate since it had constant access to nourishment. Results in this study verified

this hypothesis as the control males and females grew significantly faster than all treatments (Tables 14 and 15). This result clearly set the dietary restricted treatments apart from the control males and females.

Protein is known to be essential for growth and development and many different organisms experience increased growth rates when on a high protein diet (Shahirose, Tanis, & Reg, 2006; Merkel, 1977; Nakagawa & Masana, 1971; Phillips & Brockway, 1959). The protein treatment was predicted to have the fastest male and female growth rates for the dietary restricted treatments. However, the results did not confirm this hypothesis as the males and females did not experience a significant increase in growth when compared to the other treatments (p = N.S. for both males and females). This is a profound result as protein has been well documented to increase growth rates and this treatment should have had a distinct increase in growth when compared to the other dietary restricted treatments.

Carbohydrates assist in somatic maintenance and also provide fuel for the energetically costly process of growth (Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Phillips & Brockway, 1959). Therefore, the carbohydrate treatment was expected to have an increased growth rate when compared to the lifetime restricted treatment. The males and females of the carbohydrate treatment did not confirm this. The males and females of the carbohydrate treatment did not have a significantly different growth rate than the lifetime restricted treatment (p= N.S.). This result could

have been due to the effects of dietary restriction on growth rate as no dietary restricted treatments growth rate was significantly different from one another (Le Galliard, Ferrière, & Clobert, 2005; Masoro, 2005).

Finally, the lipid treatment was expected to have a slower growth rate that the other treatments based on the detrimental effects of high lipid diets (Ujvari, Wallman, Madsen, Whelan, & Hulbert, 2009; Mohanty, et al., 2002; Driver & Cosopodiotis, 1979).The growth rate of the lipid treatment was not significantly different from any of the other dietary restricted treatments (Table 14 and 15). This indicates that there was possibly a minimum growth rate that both male and female crickets strive to achieve.

Maturation Mass

The control treatment was provided with constant access to diet and as a result had the resources available to achieve and support fast growth and a large body mass (Shahirose, Tanis, & Reg, 2006; Masoro, 2005; Merkel, 1977; Nakagawa & Masana, 1971). The control treatment was hypothesized to have the fastest growth rate and therefore the largest maturation mass of all treatments. The experiment supported this hypothesis for both males and females of the control treatment. The females achieved a significantly larger maturation mass than the females of the other treatments (Table 16). Likewise, the control males had a significantly larger maturation mass than the other treatments (Table 17). This confirmed the hypothesis that the control treatment would have a larger body mass than the other treatments.

In the dietary restricted treatments, the protein treatment was expected to produce the largest female crickets. This diet was hypothesized to result in an increased rate of growth and egg production (Archer, Royle, South, Selman, & Hunt, 2009; Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Maklakov, et al., 2008). The increased egg production and growth rate should have resulted in the greatest maturation mass for the dietary restricted females (Archer, Royle, South, Selman, & Hunt, 2009; Fanson, Weldon, Pérez-Staples, Simpson, & Taylor, 2009; Maklakov, et al., 2008). The egg production would have increased their mass because the females were not able to lay their eggs in this experiment. This would force them to retain their eggs and as a result increase their mass. The results of this experiment were not significant although the female protein treatment possessed the highest mean maturation mass of the dietary restricted treatments (Table 16).

The carbohydrate and protein treatments should have produced the males with the largest maturation masses because of the hypothesized increases in growth rates on either diet. Interestingly, the findings in this experiment did not support this prediction. The carbohydrate and protein treatments did not result in a significantly larger male maturation mass (Table 17). This could have been due to increased male reproductive effort which would have increased their energy expenditure (Maklakov, et al., 2008; Segoli, Lubin, & Harari, 2007). To validate this, further experiments are needed to assess the male reproductive effort in terms of chirps.

The lifetime restricted and lipid treatments were expected to produce the crickets with the lowest maturation mass since their expected growth rates were slower than the other dietary restricted treatments. The results of this experiment were not significant. Males of the lifetime restricted treatment did however have the largest mean maturation mass and the females had the second largest mean maturation mass of the dietary restricted males and females. The lifetime restricted males and females could have obtained a large maturation mass as a result of their delayed maturation (Segoli, Lubin, & Harari, 2007; Masoro, 2005; Joe, 2000). Their delayed maturation would have given them more time to grow albeit at a slower rate (Segoli, Lubin, & Harari, 2007; Masoro, 2005; Joe, 2000). The lack of significant differences between the dietary restricted treatments indicated that dietary restricted crickets in this experiment achieved the same mean maturation mass regardless of their diet.

CHAPTER 7: Conclusion

Both dietary restriction and nutritional geometry experiments have several obstacles to overcome in order to improve and verify their results. Both experiments were plagued with low survivorship of the dietary restricted treatments. Overall mortality was improved by switching from the insect diet to gerbil feed. However, a better method of dietary restriction must be obtained that is less stressful (in terms of mortality) for the crickets to further improve survival rates. One such method could be one-hour-interval feeding. The insects are fed for one hour every day in this method of dietary restriction. An alternative method could be dietary dilution. This would require the food to be diluted with the agar solution used in the nutritional geometry experiment. The cricket diet would therefore consist of a greater proportion of agar than is currently used but they would be fed every day. Both of these methods should be tested and the method that produces the greatest increase in maximum longevity and survival of the crickets should be used in future experiments.

Different sources of protein and lipid should be examined in order to improve the survivorship of crickets on the lipid treatment and the growth rate and diet consumption of crickets on the protein treatment. The lipid treatment had an alarmingly low survival rate compared to the other dietary restricted treatments in the nutritional geometry experiments. This treatment also had the lowest rate of diet consumption of all treatments and as such, other sources of lipid should be tested to see if this result

persists. The protein treatment did not display many of the key characteristics of a high protein diet. Traits such as an increased growth rate, maturation mass, decreased longevity and increased survivorship were not evident in this experiment given the nature of the diet. The low rate of diet consumption in the protein females was another indicator that the diet was not having the desired effect for which it was chosen. This leads one to believe that whey protein is not an adequate source of protein for crickets. As with the lipid diet, other sources of protein should be tested to determine the effects of high protein diets on dietary restricted crickets.

Based on the results from the dietary restriction and nutritional geometry experiments, the lifetime restricted crickets had a reduced survivorship and maturation mass as well as a delayed maturation age and a retarded growth rate. The lifetime restricted treatment did however have the benefit of a high maximum longevity when compared to the other treatments which resulted in an extended adult duration.

The different macronutrients used in this experiment have modified the longevity, maturation mass, maturation age and survivorship of males and females in different ways. The females of the carbohydrate treatment experienced increases in survivorship, but not longevity, maturation mass, maturation age or adult duration. The males of this treatment however had the second highest maximum longevity as well as a significant increase in longevity when compared to the lipid and protein males. Males of the carbohydrate treatment also experienced a significantly earlier maturation age when

compared to the lifetime restricted treatment. The protein treatment had a lower survivorship, growth rate and longevity than expected for both sexes but it did however have the second highest maximum longevity for females. The lipid treatment had an extremely low survivorship as well as a decrease in longevity and adult duration for both genders.

The reason why the field of nutritional geometry is so appealing is that by altering the ratio of nutrients in a diet, one can extend the longevity, enhance the reproduction and/or growth of an organism. The simplicity of this approach drives this emerging field and someday this can be applied to the pets, livestock or even humans. Feeding livestock various amounts of nutrients in different stages of their lifespan could maximize their size and reproduction and in pets their longevity. Imagine humans achieving increased growth and longevity just by altering the nutritional content of their diets. It is for these reasons that nutritional geometry should be pursued and studied so that its costs can be analyzed and its benefits can be maximized. The next step in the field of nutritional geometry experiments would be to test different sources of lipid, protein and carbohydrate to test for consistency in the results. Also the effects of each nutrient on reproductive effort should be determined to shed more light on the effects of nutrition on physiological processes. This would increase our understanding of dietary restriction on insects that consume different macronutrients and allow us to extrapolate this knowledge to humans in the future.

Works Cited

Barja, G. (2004). Aging in vertebrates, and the effect of caloric restriction: a mitochondrial free radical production–DNA damage mechanism? *Biological Reviews, 79*, 235-251.

Burger, J. M., Hwangbo, D. S., Corby-Harris, V., & Promislow, D. E. (2007). The functional costs and benefits of dietary restriction in *Drosophila*. *Aging Cell, 6*, 63-71.

Carey, J. R., Harshman, L. G., Liedo, P., Müller, H.-G., Wang, J.-L., & Zhang, Z. (2008). Longevity–fertility trade-offs in the *tephritid* fruit fly, Anastrepha ludens, across dietary-restriction gradients. *Aging Cell, 7*, 470-477.

Driver, C. J., & Cosopodiotis, G. (1979). The effect of dietary fat on longevity of *Drosophila melanogaster*. *Experimental Gerontology, 14*, 95–100.

Fanson, B. G., Weldon, C. W., Pérez-Staples, D., Simpson, S. J., & Taylor, P. W. (2009). Nutrients, not caloric restriction, extend lifespan in Queensland fruit flies (*Bactrocera tryoni*). *Aging Cell, 8*, 514-523.

Goto, S., Takahashi, R., Radak, Z., & Sharma, R. (2007). Beneficial biochemical outcomes of late-onset dietary restriction in rodents. *Annals of the New York Academy of Sciences, 1100*, 431-441.

Hatle, J., Wells, S., Paterson, C., Allen, I., Fronstin, R., Fuller, L., . . . Quattrochi, J. (2008). Reduced diet and reduced reproduction both increase lifespan in *lubber* grasshoppers, perhaps by independent mechanisms. In E. S. Morris, & A. Vosloo, *Molecules to migration: The pressures of life* (pp. 143-156). Bologna, Italy: Medimond Publishing Co.

Heilbronn, L. K., & Ravussin, E. (2005). Calorie Restriction Extends Life Span— But Which Calories? *PLoS Medicine, 2*, 721-723.

Holloszy, J. O., & Fontana, L. (2007). Caloric restriction in humans. *Experimental Gerontology, 42*, 709–712.

House, H. L. (1961). Insect Nutrition. *Annual Review of Entomology, 6*, 13-26.

Inness, C. L., & Metcalfe, N. B. (2008). The impact of dietary restriction, intermittent feeding and compensatory growth on reproductive investment and lifespan in a short-lived fish. *Proceedings of The Royal Society: Biological Sciences, 275* , 1703-1708.

Joe, M. M. (2000). The evolution of free radicals and oxidative stress. *The American Journal of Medicine, 108*, 652-659.

Le Galliard, J.-F., Ferrière, R., & Clobert, J. (2005). Juvenile growth and survival under dietary restriction: are males and females equal? *Oikos, 111*, 368-376.

Lee, K. P., Simpson, S. J., Clissold, F. J., Brooks, R., Ballard, J. W., Taylor, P. W., . . . Raubenheimer, D. (2008). Lifespan and reproduction in *Drosophila*: new insights from nutritional geometry. *PNAS, 105*, 2498-2503.

Loft, S., Thorling, E. B., & Poulsen, H. E. (1998). High fat diet induced oxidative DNA damage estimated by 8-oxo-7,8-dihydro-2-deoxyguanosine excretion in rats. *Free Radical Research, 29*, 595-600.

Lyn, J. C., Naikkhwah, W., Aksenov, V., & Rollo, C. D. (2011). Influence of two methods of dietary restriction on life history features and aging of the cricket *Acheta domesticus*. *AGE, 33*, 509–522.

Lyn, J., Aksenov, V., LeBlanc, Z., & Rollo, C. D. (2012). Life history features and aging rates: insights from intra-specific patterns in the cricket *Acheta domesticus*. *Evolutionary Biology, 1*, 1-17.

Mair, W., Sgrò, C. M., Johnson, A. P., Chapman, T., & Partridge, L. (2004). Lifespan extension by dietary restriction in female *Drosophila melanogaster* is not caused by a reduction in vitellogenesis or ovarian activity. *Experimental Gerontology, 39*, 1011–1019.

Maklakov, A. A., Simpson, S. J., Zajitschek, F., Hall, M. D., Dessmann, J., Clissold, F., . . . Brooks, R. C. (2008). Sex-specific fitness effects of nutrient intake on reproduction and lifespan. *Current Biology, 18*, 1062-1066.

Mark, D. A., Alonso, D. R., Quimby, F., Thaler, H. T., Kim, Y. T., Fernandes, G., . . . Weksler, M. E. (1984). Effects of nutrition on disease and life span. I. Immune responses, cardiovascular pathology, and life span in MRL mice. *The American Journal of Pathology, 117*, 110-124.

Masoro, E. J. (2005). Overview of caloric restriction and ageing. *Mechanisms of Ageing and Development, 126*, 913-922.

Merkel, G. (1977). The effects of temperature and food quality on the larval development of *Gryllus bimaculatus* (Orthoptera, Gryllidae). *Oecologia, 30*, 129-140.

Min, K.-J., & Tatar, M. (2006). Restriction of amino acids extends lifespan in *Drosophila* melanogaster. *Mechanisms of Ageing and Development, 127*, 643-646.

Mohanty, P., Ghanim, H., Hamouda, W., Aljada, A., Garg, R., & Dandona, P. (2002). Both lipid and protein intakes stimulate increased generation of reactive oxygen species by

polymorphonuclear leukocytes and mononuclear cells. *American Journal of Clinical Nutrition, 75*, 767-772.

Nakagawa, I., & Masana, Y. (1971). Effect of protein nutrition on growth and life span in the rat. *The Journal of Nutrition, 101*, 613-620.

Pauwels, K., Stoks, R., & De Meester, L. (2010). Enhanced anti-predator defence in the presence of food stress in the water flea Daphnia magna. *Functional Ecology, 24*, 322-329.

Phillips, A. M., & Brockway, D. R. (1959). Dietary calories and the production of trout in hatcheries. *The Progressive Fish-Culturist, 21*, 3-16.

Piper, M. D., Mair, W., & Partridge, L. (2005). Counting the calories: the role of specific nutrients in extension of life span by food restriction. *Journal of Gerontology: Biological Sciences, 60*, 549-555.

Rollo, C. D. (2002). Growth negatively impacts the life span of mammals. *Evolution & Development, 4*, 55-61.

Sanz, A., Caro, P., & Barja, G. (2004). Protein restriction without strong caloric restriction decreases mitochondrial oxygen radical production and oxidative DNA damage in rat liver. *Journal of Bioenergetics and Biomembranes, 36*, 545-552.

Sanz, A., Caro, P., Sanchez, J. G., & Barja, G. (2006). Effect of lipid restriction on mitochondrial free radical production and oxidative DNA damage. *Annals of the New York Academy of Sciences, 1067*, 200-209.

Sanz, A., Gómez, J., Caro, P., & Barja, G. (2006). Carbohydrate restriction does not change mitochondrial free radical generation and oxidative DNA damage. *Journal of Bioenergetics and Biomembranes, 38*, 237-333.

Segoli, M., Lubin, Y., & Harari, A. R. (2007). The effect of dietary restriction on the lifespan of males in a web-building spider. *Evolutionary Ecology Research, 9*, 697-704.

Shahirose, P., Tanis, F., & Reg, S. (2006). Higher versus lower protein intake in formula-fed low birth. *The Cochrane Library, 1*, 1-50.

Simpson, S. J., & Raubenheimer, D. (2010). The nutritional geometry of aging. In S. J. Simpson, & D. Raubenheimer, *Calorie Restriction, Aging and Longevity* (pp. 111-122). Netherlands: Springer.

Stunkard, A. (1983). Nutrition, aging and obesity: a critical review of a complex relationship. *International Journal of Obesity, 7*, 201-220.

Ujvari, B., Wallman, J. F., Madsen, T., Whelan, M., & Hulbert, A. (2009). Experimental studies of blowfly (Calliphora stygia) longevity: a little dietary fat is beneficial but too much is detrimental. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology, 154*, 383-388.

APPENDIX

DIETARY RESTRICTION

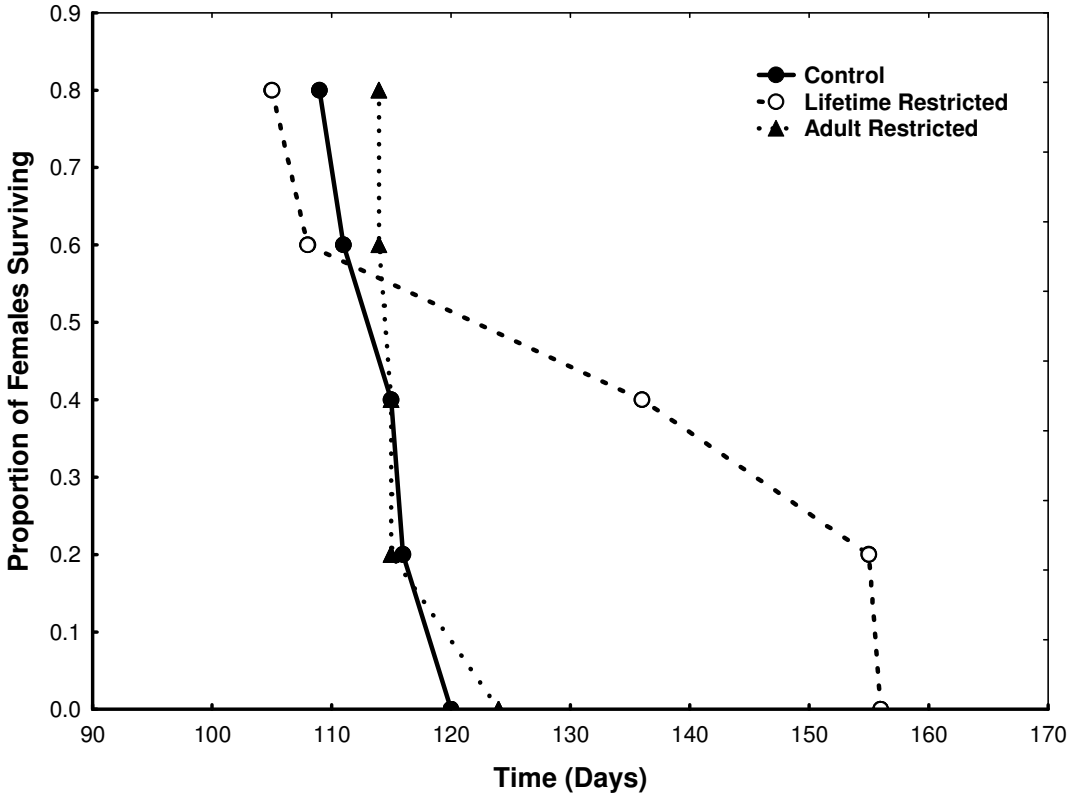

Figure 1: Five Longest Living Females. The lifetime restricted treatment had a significant increase in longevity when compared to the control treatment ($p < 0.03$). The Fisher's LSD one-way ANOVA test was used to calculate the probabilities.

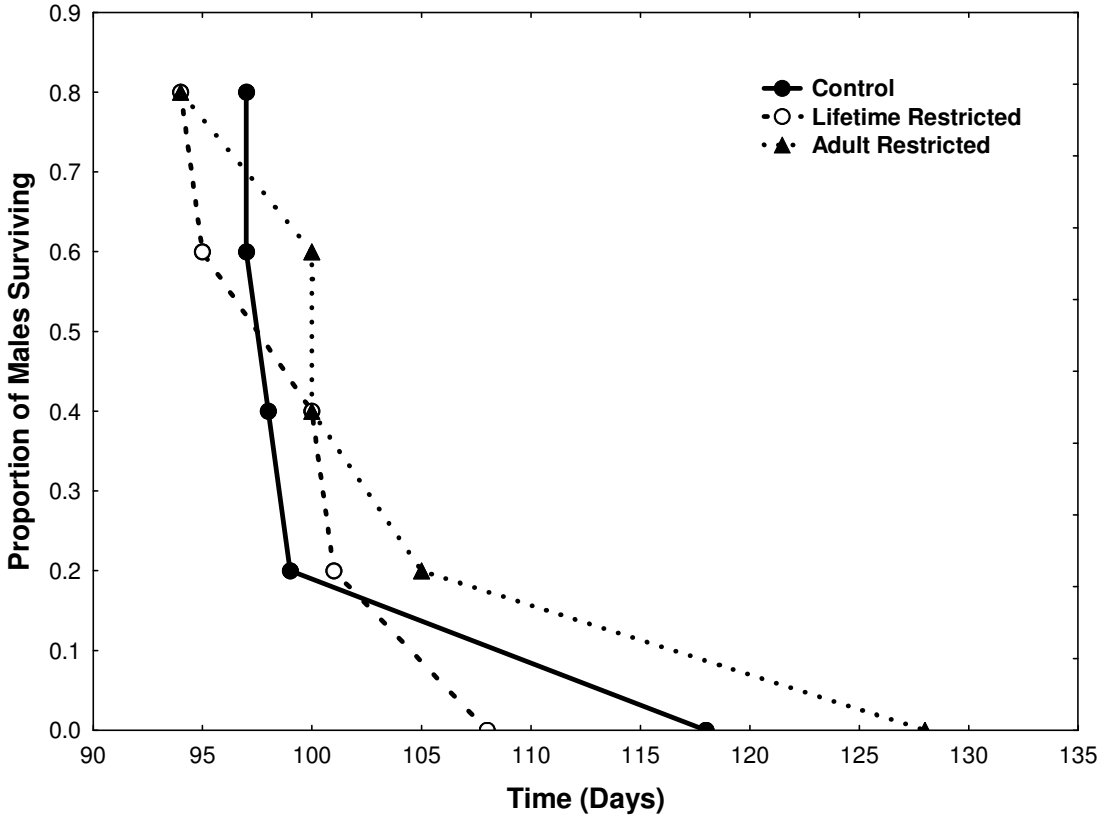

Figure 2: Five Longest Living Males. There were no significant differences between the treatments (p = N.S.). The Fisher's LSD one-way ANOVA test was used to calculate the probabilities.

NUTRITIONAL GEOMETRY

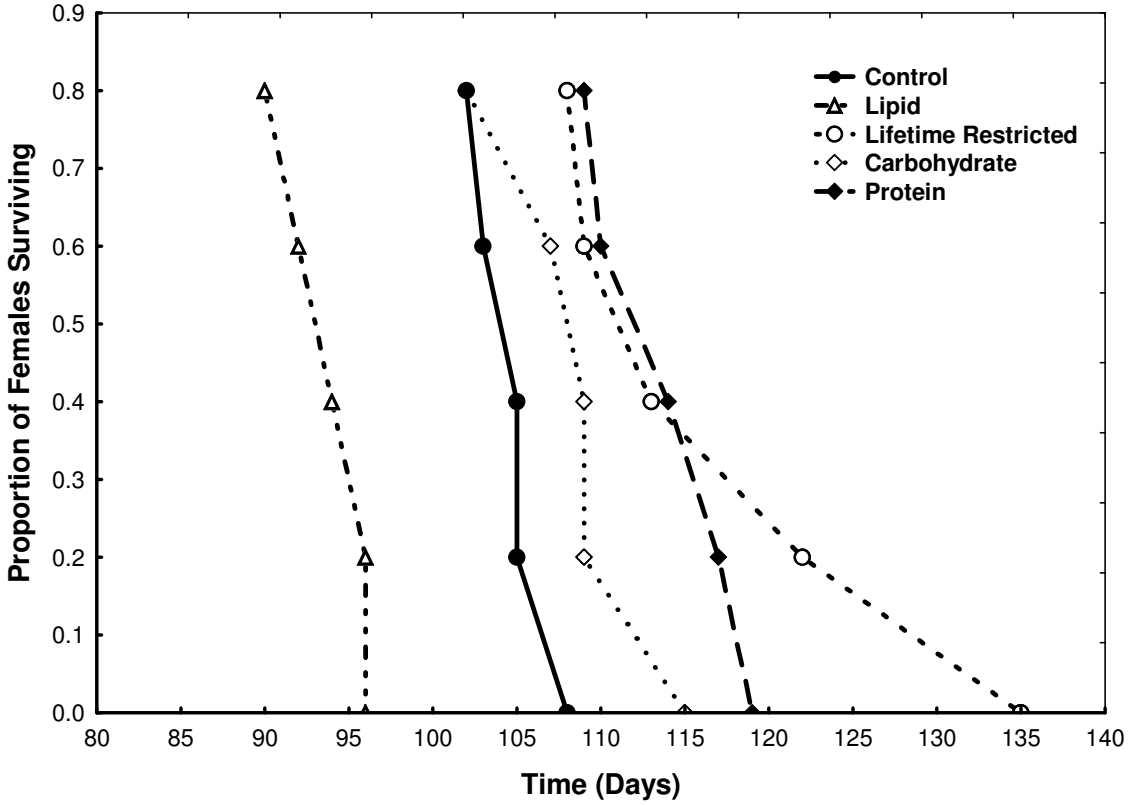

Figure 3: Five Longest Living Females. The female lifetime restricted and protein crickets had a significant increase in longevity when compared to the controls ($p < 0.004$ on lifetime restricted and $p < 0.03$ on protein). The lifetime restricted treatment lived significantly longer than the carbohydrate treatment ($p < 0.04$). The Fisher's LSD one-way ANOVA test was used to calculate the probabilities.

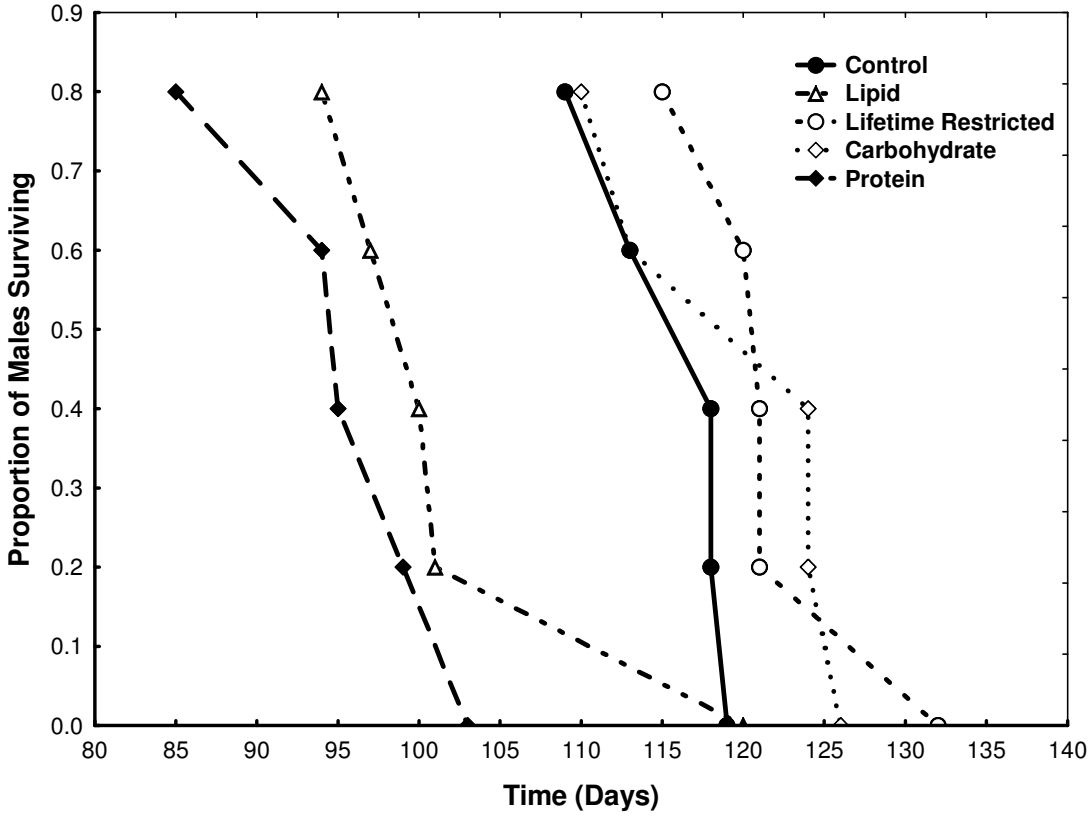

Figure 4: Five Longest Living Males. The lifetime restricted treatment had a significant increase in longevity when compared to the lipid and protein crickets ($p < 0.00004$ on lipid and $p < 0.000001$ on protein). The carbohydrate treatment also experienced an increase in longevity when compared to the lipid and protein treatments ($p < 0.00004$ on lipid and $p < 0.000001$ on protein). The male crickets on the protein treatment had a significantly lower longevity than the control treatment ($p < 0.00002$ on control). The Fisher's LSD one-way ANOVA test was used to calculate the probabilities.

DIETARY RESTRICTION

Treatment	Mean Longevity	Probability	Survivorship	Probability	Gender	Maximum Longevity
Control	54.2 ± 3.7 d		44%		Female	120 d
					Male	118 d
Adult Restricted	61.1 ± 3.9 d	$p < 0.001$, lifetime restricted	50%	N.S. control	Female	124 d
					Male	128 d
Lifetime Restricted	42.4 ± 3.5 d	$p < 0.03$, control	21%	$p < 0.0005$, control	Female	156 d
					Male	108 d

Table 1: Longevity and Survivorship. The lifetime restricted treatment had a significantly lower survivorship than the control treatment ($p < 0.001$). The dietary restricted probabilities were calculated via the Chi^2 test. The Fisher's LSD one-way ANOVA test was used to calculate the mean longevity probabilities.

Female	Minimum Age	Maximum Age	Mean Maturation Age	Probability
Control	47 d	63 d	55.6 ± 1.2 d	
Adult Restricted	53 d	69 d	59.0 ± 0.7 d	$p < 0.028$, control
Lifetime Restricted	62 d	77 d	67.0 ± 2.2 d	$p < 0.000003$, control and $p < 0.0004$, adult restricted

Table 2: Female Maturation. The Control treatment matured significantly earlier than the Adult restricted and Lifetime restricted treatments. The lifetime restricted treatment matured significantly later than the adult restricted treatment. The Fisher's LSD one-way ANOVA test was used to calculate the probabilities.

Male	Minimum Age	Maximum Age	Mean Age	Probability
Control	50 d	73 d	60.0 ± 1.2 d	
Adult Restricted	50 d	69 d	59.5 ± 1.2 d	$p < 0.0035$, lifetime restricted
Lifetime Restricted	52 d	73 d	64.9 ± 1.5 d	$p < 0.0054$, control

Table 3: Male Maturation. The Adult restricted treatment matured significantly earlier than the Lifetime restricted treatment. The lifetime restricted treatment matured significantly later than the control treatment. The Fisher's LSD one-way ANOVA test was used to calculate the probabilities.

Female	Adult Duration	Proportion of Control	Probability
Control	64.4 ± 1.2 d		
Adult Restricted	65.0 ± 0.7 d	1.01	N.S. control
Lifetime Restricted	89.0 ± 2.2 d	1.38	$p < 0.00001$, control and adult restricted

Table 4: Female Adult Duration. The lifetime restricted treatment had a significantly longer adult duration than the control and adult restricted treatments. The Fisher's LSD one-way ANOVA test was used to calculate the probabilities.

Male	Adult Duration	Proportion of Control	Probability
Control	58.0 ± 1.1 d		
Adult Restricted	68.6 ± 1.2 d	1.18	$p < 0.00001$, control
Lifetime Restricted	43.1 ± 1.5 d	0.74	$p < 0.00001$, control and adult restricted

Table 5: Male Adult Duration. The control treatment had a significantly shorter adult duration than the adult restricted treatment. The lifetime restricted treatment had a significantly shorter adult duration than the control and adult restricted treatments. The Fisher's LSD one-way ANOVA test was used to calculate the probabilities.

Treatment	Gender	Growth Rate (mg/d)	Probability
Control	Female	4.2 ± 0.3	
	Male	4.2 ± 0.2	
Adult Restricted	Female	4.6 ± 0.2	$p < 0.002$, lifetime restricted female
	Male	4.5 ± 0.3	$p < 0.0006$, lifetime restricted male
Lifetime Restricted	Female	2.9 ± 0.3	$p < 0.01$, control female
	Male	3.1 ± 0.2	$p < 0.005$, control male

Table 6: Male and Female Growth Rates. The lifetime restricted male and female crickets had a significantly slower growth rate than the adult restricted and control treatments.

Female	Minimum Mass	Maximum Mass	Mean Mass	Probability
Control	115 mg	397 mg	228 ± 16.0 mg	
Adult Restricted	139 mg	448 mg	274 ± 13.1 mg	$p < 0.021$, control
Lifetime Restricted	110 mg	235 mg	194 ± 16.7 mg	$p < 0.005$, Adult restricted

Table 7: Female Maturation Mass. The adult restricted treatment had a significantly greater mass at maturation than the control and lifetime restricted treatment. The Fisher's LSD one-way ANOVA test was used to calculate the probabilities.

Male	Minimum Mass	Maximum Mass	Mean Mass	Probability
Control	172 mg	342 mg	250 ± 9.8 mg	
Adult Restricted	141 mg	415 mg	270 ± 18.7 mg	$p < 0.0027$, lifetime restricted
Lifetime Restricted	111 mg	289 mg	199 ± 15.9 mg	$p < 0.023$, control

Table 8: Male Maturation Mass. The lifetime restricted treatment had a significantly lower mass at maturation than the control and adult restricted treatments.

NUTRITIONAL GEOMETRY

Treatment	Mean Longevity	Probability	Gender	Survivorship	Probability	Maximum Longevity
Control	85.2 ± 1.8 d	$p < 0.0001$, all treatments	Female	44.6 %		108 d
			Male	41.8%		119 d
Lifetime Restricted	57.2 ± 2.9 d	$p < 0.05$, carbohydrate	Female	11.8 %		135 d
			Male	10.9 %		132 d
Lipid	50.9 ± 2.3 d	$p < 0.0002$ carbohydrate	Female	3.6 %	$p < 0.03$	96 d
			Male	0.9 %	$p < 0.005$	120 d
Protein	56.3 ± 2.3 d	$p < 0.03$, carbohydrate	Female	10.0 %	N.S.	119 d
			Male	8.2 %	N.S.	99 d
Carbohydrate	64.0 ± 2.7 d	$p < 0.05$, all treatments	Female	21.8 %	$p < 0.05$	115 d
			Male	13.6 %	N.S.	126 d

Table 9: Longevity and Survivorship. The Lipid females had a significantly lower survivorship than the lifetime restricted treatment ($p < 0.03$). The carbohydrate females had a significantly higher survivorship than the lifetime restricted treatment ($p < 0.05$).The dietary restricted probabilities were calculated via the Chi^2 test. Lifetime

Restricted was the "expected" variable and the other treatments were the "observed" variable. The lipid males had a statistically lower survivorship than the lifetime restricted treatment ($p < 0.005$). Fisher's LSD test was used to calculate probabilities for mean longevity. All male and female dietary restricted treatments were had a significantly lower survivorship than the control males and females ($p < 0.0001$ for males and $p < 0.0003$ for females).

Female	Minimum Age	Maximum Age	Mean Maturation Age	Probability
Control	55 d	80 d	68.1 ± 0.9 d	
Lifetime Restricted	56 d	85 d	71.5 ± 2.4 d	N.S.
Lipid	60 d	79 d	71.0 ± 4.1 d	N.S.
Protein	58 d	103 d	75.2 ± 3.8 d	$p < 0.01$, control
Carbohydrate	56 d	100 d	72.6 ± 2.7 d	$p < 0.03$, control

Table 10: Female Maturation. The control female crickets matured significantly earlier than the protein ($p < 0.01$) and carbohydrate ($p < 0.03$) treatments. No other treatments were significant. The Fisher's LSD one-way ANOVA test was used to calculate the probabilities.

Male	Minimum Age	Maximum Age	Mean Maturation Age	Probability
Control	54 d	81 d	68.6 ± 0.8 d	
Lifetime Restricted	68 d	94 d	79.8 ± 2.2 d	$p < 0.00007$, control
Lipid	71 d	71 d	71 ± * d	N.S.
Protein	68 d	90 d	76.3 ± 2.4 d	$p < 0.01$, control
Carbohydrate	60 d	94 d	70.5 ± 2.4 d	$p < 0.005$, lifetime restricted

Table 11: Male Maturation. The male control crickets matured significantly earlier than the lifetime restricted ($p < 0.00007$) and the protein ($p < 0.01$) treatments. The lifetime restricted male crickets matured significantly later than the carbohydrate ($p < 0.005$) treatment. The Fisher's LSD one-way ANOVA test was used to calculate the probabilities.

Female	Adult Duration	Probability
Control	39.9 ± 0.9 d	
Lifetime Restricted	63.5 ± 2.4 d	$p < 0.00001$, control, lipid, protein and carbohydrate
Lipid	25.0 ± 4.1 d	$p < 0.0009$, control and carbohydrate
Protein	43.8 ± 2.8 d	$p < 0.0002$, lipid
Carbohydrate	42.4 ± 2.7 d	$p < 0.0002$, lifetime restricted and lipid

Table 12: Female Adult Duration. Adult durations for the female treatments as well as the associated probabilities. The Fisher's LSD one-way ANOVA test was used to calculate the probabilities.

Male	Adult Duration	Probability
Control	50.4 ± 0.8 d	
Lifetime Restricted	52.3 ± 2.2 d	$p < 0.00001$, protein
Lipid	49.0 ± * d	$p < 0.003$, protein
Protein	22.7 ± 2.4 d	$p < 0.00001$, control and carbohydrate
Carbohydrate	55.5 ± 2.3 d	$p < 0.04$, control

Table 13: Male Adult Duration. Adult durations for the male treatments as well as the associated probabilities. The Fisher's LSD one-way ANOVA test was used to calculate the probabilities.

Female	Diet Consumption (mg/d)	Probability	Growth Rate (mg/d)	Probability
Control	48.02 ± 10.32		3.3 ± 0.09	
Lifetime Restricted	50.10 ± 8.85	$p < 0.0002$, lipid and $p < 0.04$, protein	2.6 ± 0.1	$p < 0.00003$, control
Lipid	6.24 ± 6.10	$p < 0.00009$, control	2.5 ± 0.3	$p < 0.005$, control
Protein	26.20 ± 10.89	$p < 0.04$, control	2.5 ± 0.1	$p < 0.00001$, control
Carbohydrate	63.01 ± 3.93	$p < 0.000001$, lipid and $p < 0.001$ protein	2.4 ± 0.1	$p < 0.00001$, control

Table 14: **Female Diet Consumption and Growth Rate.** The lipid and protein treatments had the lowest rates of diet consumption of the females. The Fisher's LSD one-way ANOVA test was used to calculate the probabilities.

Male	Diet Consumption (mg/d)	Probability	Growth Rate (mg/d)	Probability
Control	39.54 ± 4.76		3.0 ± 0.07	
Lifetime Restricted	42.04 ± 6.89	$p < 0.002$, lipid and $p < 0.008$, protein	2.2 ± 0.1	$p < 0.00001$, control
Lipid	7.92 ± 6.41	$p < 0.001$, control	2.0 ± *	N.S. control
Protein	13.41 ± 8.54	$p < 0.008$, control	2.1 ± 0.2	$p < 0.00001$, control
Carbohydrate	55.86 ± 3.06	$p < 0.00001$, lipid and $p < 0.0009$, protein	2.4 ± 0.01	$p < 0.0004$, control

Table 15: Male Diet Consumption and Growth Rate. The lipid and protein treatments had the lowest rates of diet consumption of the males. The Fisher's LSD one-way ANOVA test was used to calculate the probabilities.

Female	Minimum Mass	Maximum Mass	Mean Mass	Probability
Control	124 mg	320 mg	226 ± 6.5 mg	
Lifetime Restricted	115 mg	234 mg	181 ± 9.2 mg	$p < 0.00009$, control
Lipid	140 mg	214 mg	173 ± 15.4 mg	$p < 0.005$, control
Protein	118 mg	243 mg	182 ± 11.7 mg	$p < 0.0004$, control
Carbohydrate	102 mg	232 mg	167 ± 5.9 mg	$p < 0.000001$, control

Table 16: Female Maturation Mass. All treatments were had a significantly lower mean maturation mass than the control treatment. The Fisher's LSD one-way ANOVA test was used to calculate the probabilities.

Male	Minimum Mass	Maximum Mass	Mean Mass	Probability
Control	132 mg	279 mg	205 ± 4.6 mg	
Lifetime Restricted	122 mg	253 mg	174 ± 12.0 mg	$p < 0.009$, control
Lipid	114 mg	114 mg	144 ± * mg	-
Protein	97 mg	204 mg	154 ± 10.8 mg	$p < 0.0002$, control
Carbohydrate	101 mg	215 mg	166 ± 7.4 mg	$p < 0.004$, control

Table 17: Male Maturation Mass. All treatments with the exception of lipid had a significantly lower maturation mass than the control treatment. The Fisher's LSD one-way ANOVA test was used to calculate the probabilities.