The relationship between diet, aging and longevity in an orb-web spider.

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Declaration

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All other research described in this report is my original work.

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Abstract

The macronutrient composition of natural diets varies and affects aging and longevity of a variety of taxa. The relationship between diet and longevity is more prominent in model species than wild populations suggesting this may be a laboratory artefact. I applied dietary and protein restriction to understand how diet influences aging and longevity in a non-model species, the orb web spider *Argiope keyserlingi*. To assess whether longevity and performance vary with the amount and type of food, I set up treatments that manipulated how much food and protein the spiders received in a random assignment. My initial experiment showed that the amount of food (standard vs half the amount of food), did not affect longevity. In a subsequent experiment, I compared the spiders' performance, such as running speed, weight and web area on a weekly basis between a high and low protein feeding treatment. My results showed that there were no significant interactions between the amount of protein in the spiders' diet and its effects on a spiders' lifespan. However, spiders on the low protein diet built significantly smaller webs than their high protein counterparts, but as spiders aged, overall, they built smaller webs. Overall patterns of aging were affected by the duration of the treatment, but diet only affected the web area. In conclusion, my experiments only weakly support the notion that diet affects aging, which is prominently found in model species and less common in wild populations.

Keywords: Protein, *Argiope keyserlingi*, aging, longevity, diet, model species, non-model species.

Introduction

Aging and longevity have received significant research attention, particularly as they relates to humans (McDonald & Ruhe 2011). Beyond our own biology, aging has been documented in many long-lived species, such as mice, rats, fish and monkeys (Nakagawa et al. 2012). Patterns of aging and longevity vary widely across many species. For instance, human beings live for approximately eighty years, baboons for thirty-three years, dogs for fourteen, guppies for five years, while many invertebrates (including butterflies, bees, fruit flies) only live for a few months (Arking 2006).

While aging and longevity are related phenomena, they capture different aspects of life history. Longevity refers to the lifespan of an individual from birth to death, which is influenced by extrinsic factors such as predation and intrinsic factors such as senescence and aging (Bonsall & 2006). Aging is a decline in performance and function, which increases age specific mortality and thus influences longevity (Bonsall & 2006).

There are several mechanistic and evolutionary explanations for aging and its effect on longevity. Mechanistic explanations focus on the molecular, cellular or physiological mechanisms that result in aging, including telomere functioning, oxidative stress, nutrient signal transduction pathways, and hydrogen sulphide exposure (Monaghan et al. 2008).

Telomeres are DNA-protein structures that form protective caps at the end of chromosomes. They constitute safeguards of chromosome degradation and are responsible for maintaining genomic integrity, but shorten during cell division cycles (Tzanoakou et al 2014). After a certain number of cell divisions, the telomeres shorten significantly, which can stop the cell from dividing any further, hence contributing to old age or senescence (Tzanoakou et al 2014).

There are several signal transduction pathways that have been linked to the rate of aging and longevity. Signal transduction pathways are involved in the transmission of a molecular signal that ultimately triggers a biochemical event in the cell. Masoro (1988) suggested that the glucose signal pathway is a moderator of aging, because

it combines proteins, lipids and nucleic acids into a positive reaction that can lead to a reduction in aging (Masoro 1988).

Another mechanistic explanation for aging and its effects on longevity, is hydrogen sulphide, which has many beneficial biological effects including, increased stress resistance, and lifespan extension. But sulphide damages cells, tissues and organs, which reduces the lifespan of the animal (Hine & Mitchell 2015). However at low dosage, hydrogen sulphide will delay aging and extend longevity (Hine & Mitchell 2015). The effect of this transsulfuration pathway on ageing has been successfully demonstrated in mice and rats, vinegar flies and worms (Hine et al. 2015).

Another example of a nutrient signalling pathway is the downregulation of the target rapamycin (TOR) pathway in yeast (*Caenorhabditis elegans)* (Nakagawa et al. 2012). This TOR pathway is activated by nutrients and once activated promotes aging and growth (Simpson & Raubenheimer 2009; McCormick et al. 2011). The TOR pathway is related to both the circulation and balance of nutrients (Simpson & Raubenheimer 2009). The TOR pathway is activated when animals are fed high protein diets, with the effect of reducing an animals life span and accelerate their aging processes (Raubenheimer et al. 2009; Raubenheimer & Simpson 1999).

The molecular, cellular and physiological aspects of aging can be seen in many forms of damage to DNA, cells, tissues and organs. This is known as the Free-Radical Theory of Aging. Endogenous oxygen radicals produced during aerobic respiration can cause cumulative oxidative stress resulting in the aging and eventual death of cells and organs (Harman 1968). For example, the rate of energy expenditure varies inversely in the longevity of *Drosophila melanogaster* (Bonsall & 2006). Similarly, rockfish that live at a deeper depth with lower oxygen exposure experience lower metabolic activity that ultimately leads to an increase in longevity (Bonsall & 2006).

Aging can induce profound effects but not many researchers have looked at the behavioural changes due to aging. Anoteux et al (2012) examined the orb web spider, Zygiella x-notata, and measured alterations in web geometry (Anoteux et al 2012). What they found was two distinct groups of spiders – short and long lived –

150 and 236 days respectively. Changes with age were found in both groups and these related mostly to silk investment, cognitive and locomotor functions. The authors interpretation was that age-related changes in body mass was the main reason for variations in web investment (Anoteux et al 2012).

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Evolutionary mechanisms focus on the fitness consequences of aging and relate patterns of aging to life history traits, such as fecundity (Monaghan et al. 2008). Evolutionary explanations of aging evoke diet through a life-history trade-off between allocating resources to body repair or reproduction, but not both at once (Nakagawa et al. 2012). This is also known as the “resource re-allocation hypothesis” (Adler & Bonduriansky 2014). This hypothesis proposes that animals with ample food invest resources into reproduction and away from somatic maintenance. This means that these animals maximise fecundity at the cost of survival. However, under dietary restriction, animals re-allocate resources from reproduction to somatic maintenance, which enhances their survival (Adler & Bonduriansky 2014) (Fig 1).

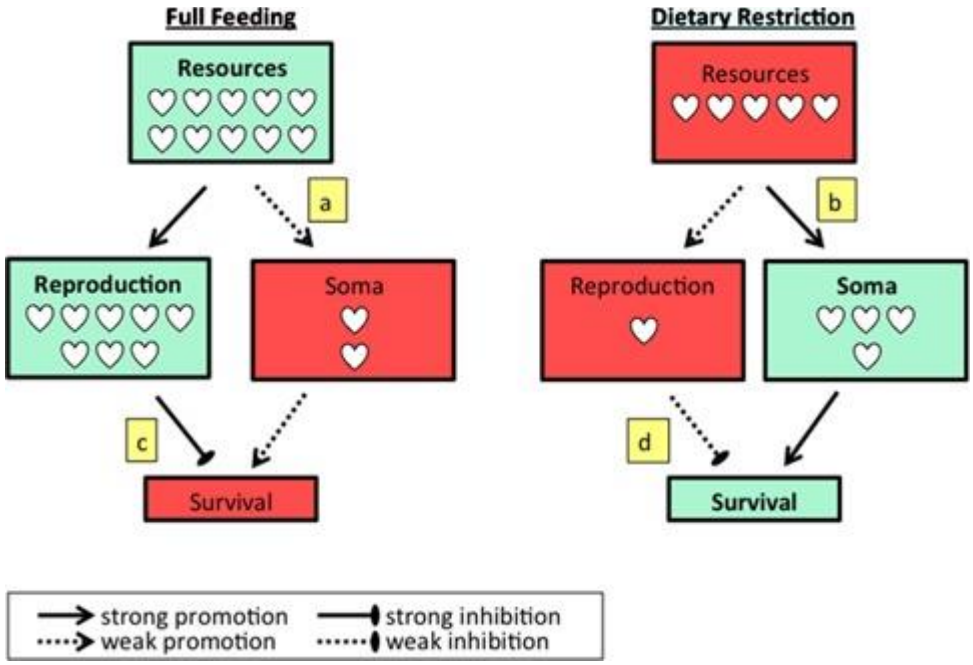

Fig 1: This is a flow diagram of the Resource re-allocation hypothesis that details how an animal reacts during both dietary restriction and full feeding diets. The boxes in red refer to the process that has been largely ignored, while the green boxes show the mostly used pathway on either treatment. a) refers to a weak promotion of the resources; b) refers to the

strong re-allocation of resources; while c) is the weak inhibition of resources for survival and d) is a strong inhibition of resources to survival (Adler & Bonduriansky 2014).

There is evidence that diet, especially dietary restriction affects aging and longevity in animals. Dietary restriction refers to a significant reduction in the calorie intake of an animal, without starvation (McCay et al. 1935; Piper et al. 2011; Simpson & Raubenheimer 2011; Le Couteur et al. 2013). Dietary restriction was first studied in the 1930s during the Great Depression, as the American Government was concerned with the effects of reduced calorie intake on human health (Piper et al. 2011). In an early study, McCay et al. (1935) restricted the calorie intake of two groups of white rats: one group of rats was fed only enough to maintain a low body weight, while the control rats received unlimited access to food (McCay et al. 1935). Although the latter reproduced more, they also died sooner (McCay et al. 1935). The authors concluded that reducing the calorie intake in rats appeared to extend their lifespans (Piper et al. 2011; Le Couteur et al. 2013).

Caloric restriction has since been demonstrated in a wide variety of taxa, and immensely impacts our understanding of the interactions between diet, aging and longevity. Most researchers conducted dietary restriction experiments on model species (Zuk et al. 2014).

There are five principal model species in aging research: yeast (*Saccharomyces cerevisiae*), the fruit fly (*Drosophila melanogaster),* the nematode worm (*Caenorhabditis elegans*), the rat (*Rattus norvegicus*) and the mouse (*Mus musculus*) (Willcox et al. 2006; Zuk et al. 2014). These model species have been raised in the laboratory, under lab conditions and this presents an environment of relaxed natural selection that is free from parasites, predators and diseases unlike the environment of wild species. Rearing animals in the lab also imposes new selection, resulting in adaptions to lab conditions (Zuk et al. 2014). Model species have several advantages such as that they are easy to rear, observe and experimentally manipulate (Zuk et al. 2014). However, the vast number of species throughout the world is not raised in a laboratory, and these are known as non-model species. Non-model species include species such as albatrosses, pigs, chickens, birds, spiders and humans. These species have not been selected for intense study

by the research community, either because of historic reasons or because they lack the features that make model species so easy to investigate. Non-model species usually have longer life spans than model species and as a result different aging patterns, as well as a low fecundity rates or poorly known genetics (Piper et al. 2011; Simpson & Raubenheimer 2011; Zuk et al. 2014).

Over the last couple of decades, researchers have branched out to investigate how dietary restriction affects aging and longevity in these non-model species. Not all studies on non-model species have confirmed that dietary restriction positively affects longevity, inferring that dietary restriction is not universal (Nakagawa et al. 2012). For example, studies of house flies (*Musca domestica)* failed to show any life span extension effects when exposed to dietary restriction (Cooper et al. 2004). To test this relationship, houseflies were given six different diets that differed in the amount of sucrose used (Cooper et al. 2004). The group fed *ad libitum* lived the longest, while restricting the calories in male house flies shortened their lifespans (Cooper et al. 2004). Additionally, recent research from Nakagawa et al. (2012) shows that the life extending effects of dietary restriction has less influence on males than females (Nakagawa et al. 2012).

Deficits and excesses of macronutrients can have negative effects on the growth, reproduction and longevity of animals. Consequently, there is strong selection for animals to regulate their intake of nutrients to an intake target. This is known as the geometric framework, developed by Simpson and Raubenheimer. The development of the geometric framework for nutrition (Simpson and Raubenheimer 1999; Simpson & Raubenheimer 2011), has shifted the focus of dietary studies from the amount of food to the composition of the macronutrients in the food. Specifically, the framework measures the responses of animals to variation in the nutritional composition of foods and to determine what food choices the animals make (Kohl et al. 2015). This framework argues for a protein intake target (Fig 2), which represents trade-offs that are reached by animals when regulating their nutritional balance. Many animals have the capacity to independently regulate their intake of protein and non-protein food - this is known as nutritional homeostasis (Simpson & Raubenheimer 2007; Hawley et al. 2014). Mayntz et al. (2005) demonstrated that invertebrate predators, such as wolf spiders and ground beetles can regulate their

protein and lipid intake in their diet and can use these nutrients to ”empty and fill” any imbalances that they have in their system as a result of the previous diet (Mayntz et al. 2005). However, many predators are passive hunters, meaning that they “sit and wait” for their prey to come into proximity (Mayntz et al. 2005). Therefore, their ability to balance nutrients in their prey many be less pronounced in sit and wait predators than those hunters that actively seek out their prey (Mayntz et al. 2005; Jenson et al. 2011). For example, the wolf spider *Pardosa prativaga* is normally a wanderer, but some individuals build traps or burrows, that they leave open at night, to trap prey. They have been shown to regulate their nutrient intake by extracting more dry mass from a prey item that contained a higher proportion of the nutrient that they were lacking from the spider’s previous meal (Mayntz et al. 2005; Jenson et al. 2011; Hawley et al. 2014).

Following the publication of the geometric framework of nutrition, studies have started to look at the effect of protein on aging and longevity. Simpson and Raubenheimer (2007) have argued that lifespan and reproduction each have different optimal diets. Lifespan is then, maximised on the higher carbohydrate diet while reproduction is maximised on the higher protein diet, because protein is used by my species as energy (Kirkwood et al 2007).

This idea (the Disposable Soma theory by Simpson and Raubenheimer) was tested by Lee et al (2007). This experiment looked at the relationship between diet, nutrient intake, lifespan and reproduction in the fruit fly, *Drosophila melanogaster* (Lee et al 2007). These fruit flies were fed several diets, differing in the carbohydrate: protein ratio. Calorie restriction had no effect on extending the lifespan in the experimental flies, but egg-laying production and reproduction were maximised at a P:C ratio of 1:4, while longevity was maximised at a P:C ratio of 1:16 (Lee et al 2007). When flies were offered a choice of complementary foods they regulated intake to maximise egg production (Lee et al 2007). However, these results do not indicate a resource re-allocation mechanism, as lifetime egg production remains near constant as P:C rises (Lee et al 2007). These results support the Disposable Soma hypothesis as fruit flies intake those nutrients that will ultimately maximise lifetime egg production, over longevity (Lee et al 2007).

Nakagawa et al (2012) undertook the first comprehensive meta-analysis of studies that investigated the relationship between diet and longevity. They analysed approximately 2000 experimental studies from which the effect sizes on longevity were extracted (Nakagawa et al. 2012). Overall, Nakagawa et al. (2012) found that dietary restriction significantly increased an animals' lifespan, however, they also detected some interesting finer scale patterns: 1) that males do not show this life extending effect, unlike females of the same species; 2) that dietary restriction is not universal for all species, hence the distinction between model and non-model species; 3) and that the proportion of protein intake in the diet is more important for the life extending effect than dietary restriction (Nakagawa et al. 2012).

The difference between model and non-model species, per Nakagawa et al. (2012) is particularly intriguing and the authors propose that being raised for several generations in the laboratory is responsible for this life extending effect. Model species are subject to a relaxed natural selection due to the lack of predators, parasites and diseases that are prevalent in the wild, suggesting that the life-extending effect of dietary restriction could be "a laboratory artefact" (Nakagawa et al. 2012).

In this forest plot (Fig 3) there are 10 species that exhibit confidence intervals in the negative range, which infers that a lower caloric diet had a positive effect on their individual lifespans. These include monkeys, *(Macaca mulatta),* dogs *(Canis lupus),* rodents (*R. norvegicus and M. musculus),* insects (*D. melanogaster),* and certain bacteria and nematode worm (*C. elegans).* However, most of the species that have been examined in this analysis are wild species, and exhibit confidence intervals that cross the zero, inferring a negative relationship dietary restriction and survival (Nakagawa et al. 2012).

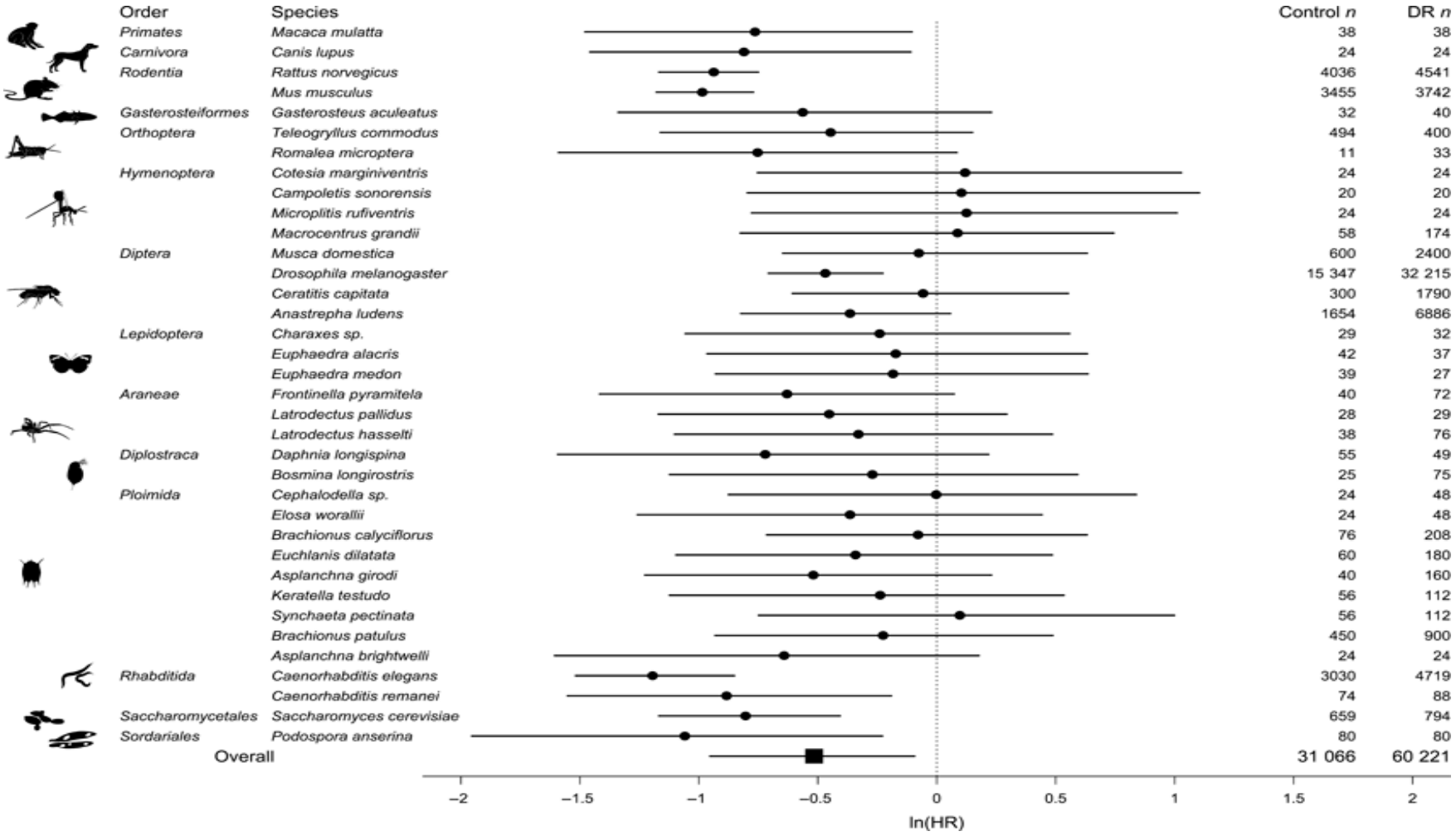

Fig 3: Forest plot of the effect size, logarithm of the hazard ratio and estimates of the relationship between dietary restriction and survival. The species that have negative values have a positive effect of dietary restriction and survival, whereas the species whose hazard ratios cross the zero, into positive values, have a negative effect on survival. The hazard ratio is plotted here, and refers to the ratio of the survival analysis (Nakagawa et al 2012).

Since Nakagawa et al (2012) research has focused more on the non-model species or wild species that have either been brought into the laboratory and reared for one generation or examined in the field. Most papers, post 2009 focused on the protein: carbohydrate ratio and the effect the macronutrient content has on the model and non-model species.

Using only articles that described experimental studies that were conducted after December 2009 I created a table that looked at the type of restriction, the effect of the restriction on the longevity, and the effect size (Table 1). I found a total of eleven experimental studies conducted after December 2009.

Most of the studies listed in Table 1 manipulated the amount of protein in the diet. Overall however, there was no clear pattern on how diet affects longevity. Four of the studies found a life extending effect while six of the studies did not, but instead found a positive effect on the growth of the animal, in terms of aging. Of the studies that detected a life extending effect in the animal, half of the studies were model species and the other half were non-model species. But three of these studies were using protein restriction.

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292 Table 1: A review of experimental studies after December 2009, that examine whether the species in question was model or non-model, the
293 type of restriction that was undertaken (i.e. protein of caloric restriction), the response of the species of the species to the type of restriction (i.e.
294 extended longevity and reduced aging), and the effect size of the study.

Paper	**Year**	**Common name**	**Scientific name**	**Broader taxonomic group**	**model species y/n**	**Type of diet restriction**	**Effect of restriction on longevity - increased, decreased, no effect**	**Effect size**
Wilder, Hallway et al.	2011	Ant	*Solenopsis invicta*	Insect	N	restriction of carbohydrates and/or amino acids	Positive effect on growth (ageing)	1.75
Jenson, Mayntz, Toft, Raubenheimer & Simpson	2011	Wolf spider	*Pardosa prativaga*	spider	N	Protein: lipid composition	Positive effect on growth (lean body mass and carapace length) when spiders fed more protein rich flies.	1.56
Chen, Wei, Wei, Yuan & Wang	2013	Oriental fruit fly	*Bactrocera dorsalis*	insect	N	Protein: carb ratio (yeast: Sugar)	lifespan increased, as ratio of P:C declines	2.54
Wang	2013	Fruit Fly	*Drosophila melanogaster*	Insect	Y	Protein: carb ratio	extended lifespan in high sugar-low protein	2.45
Niitepold,	2014	Butterfly	*Colias*	Insect	N	Calorie	No effect on lifespan -	1.34

Perez & Boggs			*eurytheme & Speyeria mormonia*			restriction		
Hawley, Simpson & Wilder	2016	Flesh flies	*Sarcophaga crassipalpis*	Insect	N	Nutrient restriction	No effect on lifespan, but shown a balance of nutrients.	
Mathison, Roth et al	2012	Rhesus monkeys	*Macaca mulatta*	Mammal	N	Calorie restriction	Increased lifespan (30%)	1.78
Sentinella, Crean & Bonduriansky	2013	neriid fly	*Telostylinus angusticollis*	Insect	N	Protein restriction	shortened survival of juveniles to adulthood	2.54
Solon-Biet, McMahon et al	2014	mice	(*Mus musculus*)	Mammal	Y	Protein restriction	increased longevity	1.67
Zajitschek et al.	2009	Black field crickets	*Teleogryllus commodus*	Insect	N	Diet quality – nutrient restriction	No effect on life span;	2.10

Orb web spiders possess all the advantages of invertebrates for the study of aging. The following is a list of reasons as to why I chose the orb web spider, *Argiope keyserlingi* as my study species (Fig. 4). 1) it is easy to establish age related changes and behavioural modifications due to their ability to construct a geometrical web (Anoteux et al 2012); 2) the orb web is a complex structure with a visible regularity (Anoteux et al 2012); 3) these species of spider are easy to catch and raise in the laboratory and 4) orb webs can indicate aging, as aging affects locomotor activity during web construction.

This study had two aims: first to understand whether the quantity of food affects longevity and second to understand whether the quality, specifically the amount of dietary protein affects patterns of aging and longevity. To overcome the potential limitations of model species (Nakagawa et al 2012), I selected an orb-web spider, *A. keyserlingi* to address these aims (Fig 4).

For experiment 1, the effect of the feeding frequency on spider longevity, I predicted that the spiders on the lower caloric diet will live longer. For experiment 2, the food quality experiment, I predicted that the spiders on the low protein treatment will live longer, while spiders on the higher protein treatment will have faster patterns of aging and a reduced lifespan (Herberstein et al. 2000a; Herberstein et al. 2000b).

Fig 4: A male and female *Argiope keyserlingi* inhabitating the female spiders' web. This photograph was taken at West Pymble park, West Pymble New South Wales, Australia. (Photograph by Nicole O'Donnell).

Materials and Methods

Study species

Argiope keyserlingi is a species of orb-web spider (Araneidae), mainly found on the East coast of Australia, from central NSW to southern Queensland (Australian Museum 2014). Juvenile *A. keyserlingi* were collected at both Bicentennial Park in West Pymble, and Macquarie University campus, North Ryde Sydney, NSW. In total, I collected 122 juvenile spiders from 2014 to 2016, during the months of September through to February. These orb web spiders are active from August through to February, with their breeding season occurring September to November. The spiders were collected from *Lomandra longifolia* bushes (Rao et al. 2007), using small specimen jars. The spiders were transported in these specimen bottles to the laboratory.

The juvenile spiders were brought into the laboratory, where they were placed in small plastic cups measuring 9.5 x 6.4 x 12.1cm with a mesh covering to allow for airflow. The spiders were fed approximately 30 *D. melanogaster* reared on a standard medium (see description below) twice a week. A regular misting with a water sprayer was applied three times a week. They were kept in these containers until they matured and were allocated to experimental treatments (see below). To check that the female spiders were mature, I examined the epigynum (the external genitalia of female spiders) on the dorsal side of the abdomen. The epigynum is only visible in adult females (Zschokke & Herberstein 2005). The diets for adult spiders differed depending on the treatment that the spiders were assigned to.

Once the female spiders matured they were placed in Perspex frames where they could build complete orb webs and forage. Adult *A. keyserlingi* build large orb webs and require frames that are at least 40 x 40 x 15cm in size (Zschokke & Herberstein 2005). The frames were open on the sides to allow measurements of the webs. When they were not being measured, thin transparent Perspex sheets were placed between the frames (Zschokke & Herberstein 2005). In addition to their diet a regular misting with a water sprayer was applied three times a week.

Manipulation of Diet in Prey

Adult spiders received the same type of prey, which differed either in the amount of prey or the macro nutrient composition of the prey (Wilder 2011). *Drosophila melanogaster* was cultured on a standard medium of 77% Edgell mashed potato mix (Edgell, Melbourne, Victoria, Australia), 1.3% Nature's Way protein supplement, 21% Homebrand caster sugar, 1% Lowan whole foods yeast and 30ml water. The mixture was placed in 300 millilitre bottles with 8-10 *D. melanogaster* to start the culture and the bottle was stoppered with a foam stopper. This was a standard mixture for growing *D. melanogaster* under Macquarie University laboratory conditions.

Experiment 1: The effect of feeding frequency on spider longevity

During 2015, I conducted a preliminary study that examined whether restricting the caloric intake would extend the longevity of *A. keyserlingi* spiders. This assumption was based on McCay et al. (1935) where they significantly reduced the caloric intake in white rats and successfully extended their longevity (McCay et al 1935). Here I examined the longevity of 44 adult female *A. keyserlingi*. 18 spiders were

placed on a low-calorie treatment, and 30 were placed on a high calorie treatment. I also measured 30 adult male spiders, 15 individuals on the low calorie and 15 on the high calorie treatment. The spiders on the low-calorie treatment were fed a restricted diet of flies, only once a fortnight, while the spiders on the high calorie treatment were fed flies once a week. The number of flies that were fed to both groups of spiders did not change - both groups of spiders were fed approximately 30 flies per feeding session. Spiders were maintained on these feeding treatments until they died.

As the spiders matured, they were weighed using a Mettler Toledo electronic balance, to three decimal places. This measurement was used as a baseline to determine whether the feeding treatment resulted in weight gain or loss. The weight of the adult male and female spiders was measured once a fortnight until they died.

The spiders were monitored every second day and the date of death was recorded. Longevity was measured as the number of days that the spider was alive, from the age of maturity when the treatment started until death. After every measurement session, the frame threads of the webs were cut and the web destroyed to encourage the spider to build a new web (Zschokke & Herberstein 2005).

Experiment 2: The effect of protein restriction on longevity and performance

During 2016, I manipulated the amount of protein in the spiders' diet but keeping the feeding frequency the same. I measured 52 female adult spiders, 25 of which were placed on the high protein treatment, while 27 were placed on the low protein treatment. Mature female spiders were randomly assigned to either a high or a low protein treatment. Protein intake was manipulated by rearing *D. melanogaster* on substrates that contain different amounts of protein. The standard fly mixture (1.3% protein) was used in this experiment, only for the juvenile spiders reared in the laboratory. The low protein mixture only contained 0.56% protein, with no additional protein supplement added to the standard potato mash mixture. The high protein mixture contained 2.67% protein from the addition of protein supplement.

Table 2: Table of the feeding treatments for the protein restriction experiment. The standard mix was only used for juvenile spiders in the protein restriction experiment and for the spiders on the calorie restriction in experiment 1.

	High Protein mix	**Low Protein mix**	**Standard mix**
Potato mix	2750g = 76.6%	2750g = 78.6%	2750g = 77.6%
Caster sugar	700g = 19.4%	750g = 21.4%	750g = 21.2%
Protein supplement	90g = 2.5%	0g = 0%	45g = 1.3%

To ensure that the live prey (*D. melanogaster*) contained the desired amount of protein, I fed the flies within 2 to 3 weeks after establishing the culture. This is sufficient to allow the protein to assimilate into the flies, but not too much time for the flies adjust to the protein diet thus not passing on the nutrient change to the spiders (Wilder 2011).

Every week I measured spider weight, web size and spider running speed. Body condition was measured using the weekly weight of the spider against the tibia-patella leg length, which is commonly used as a proxy for body size. The spiders were sitting on the central hub of their web as I begun the performance experiments. The horizontal and vertical web diameters were measured with a ruler from the outermost sticky spiral to the opposite outermost sticky spiral. Web area was calculated using the following formula:

$$\text{Web area} = \text{horizontal diameter}/2 * \text{vertical diameter}/2 * \pi$$

Running speed was estimated from high-speed video footage of the spiders responding to a vibrational stimulus. In preparation, a sticker was placed at the junction of a horizontal radial thread and the outmost sticky spiral. A Colgate 360 degree Micro Sonic Power toothbrush was placed against the sticker to stimulate the vibrations of prey. The response of the spider to the stimulus was filmed at 300 frames per second using a Casio EX-F1 digital camera (Japan, 12x optical zoom lens, 6.0 megapixels) on a tripod approximately 30 cm from the web. A black velvet background and a small light source (Kyowa Fibre optic FLG, Japan, 50-60Hz, 10/9W) were placed behind the frame to increase the visibility of the web for filming.

The spiders appeared to have a stereotyped response to the artificial stimulus, which could be broken up into three distinct actions: 1) the initial response of the spiders to the stimulus (Response Time), which was time between when the stimulus was applied and when the spider first moved; 2) the reorientation time when the spider readjusts its position to face the stimulus) and 3) the duration of the run from when it first moves in the direction of the stimulus to the time it reaches the stimulus (straight-line speed). For analyses, only actions 1 and 3 were used as they were expected to most likely deteriorate over time. If a spider did not react within 5 minutes, a 'no run' was recorded and the trial was terminated.

In order to obtain progressive measurements of the spiders' web building throughout their lifespan it was necessary to damage their webs on a regular basis (Zschokke & Herberstein 2005). Once a week, following the web measurements and running speed observations, I cut the frame threads similar to Experiment 1.

Statistical Data Analyses

I tested whether longevity was affected by calorie and/or protein restriction. The age of mortality was compared between treatments using a Mann-Whitney U test. To determine if the rate of mortality differed between treatments a Kaplan-Meier survival analyses was conducted, using the R package survival (Therneau et al 2000) in which the number of individuals that died per week was estimated and compared between treatments. I further investigated if the treatments had any effect on spiders aging. The performance variables (web area, running speed and body condition) were used as surrogates of aging and were expected to deteriorate over time. Weight and leg length was used to create an index of body condition (weight over leg length) generating a slope-adjusted ratio index (Jakob et al. 1996). The straight-line speed was divided by the horizontal radius of the web generating a relative running speed, to correct for variation in the size of the web. The influence of the treatments on spider aging was examined using linear mixed models (LMM) using the R-package lme4 (Bates et al. 2015) model with the following structure:

Performance ~ Treatment + Weeks + Treatment: Weeks + (1/ID)

Each model tested the influence of treatment on 'performance over time' (=aging).

Individual ID was incorporated as a random factor to account for the confounding influence of repeated weekly measurements of individuals. A LMM assumes residuals are normally distributed and lack heteroscedasticity. To reduce the likelihood of violating these assumptions the distributions of the performance variables were assessed using a repeated measures analysis. If they varied significantly from a normal distribution an appropriate transformation was used. Running speed and body condition were log transformed but web area was already normally distributed. A log-likelihood ratio test was used to extract P-values for each of the variables in the models. This involved comparing the fully specified model with a model without one of the variables. The interactions were tested first. If the interaction was not significant it was removed from the model. Data for the spiders' weight at maturity is given as an arithmetic mean and standard deviation unless stated otherwise. All plots were created in either ggplot (Wickham 2009) or the base (RStudio 2015) R packages. Statistical tests were conducted using both R Studio and Minitab statistical software (R Studio 2016; Minitab 17.0).

Results

Experiment 1: The effect of feeding frequency on a spiders' longevity

Both female and male *Argiope keyserlingi* spiders were randomly allocated to either a restricted caloric diet (once a fortnight feeding frequency) or an unrestricted diet (once a week feeding frequency).

Female *A. keyserlingi* spiders were allocated to two diet treatments, either a restricted diet (n = 30, weight at maturity = 0.1144 ± 0.0262g) or an unrestricted diet (n = 18, average weight at maturity = 0.1253 ± 0.0452g). There was a size bias between spiders on the two treatments (Mann-Whitney U test: W = 2538.0, n_1 = 22, n_2 = 16, p=0.0187). Overall, longevity was quite variable, ranging from 20 days to 181 days (Fig. 5). However, there was no overall effect of diet on longevity (Mann-Whitney U test: W = 3626, n_1 = 22, n_2 = 16 p=0.1). Likewise, the rate of mortality did not significantly differ between the two treatments (LMM: chisq = 0.1, df = 0.1, p = 0.701).

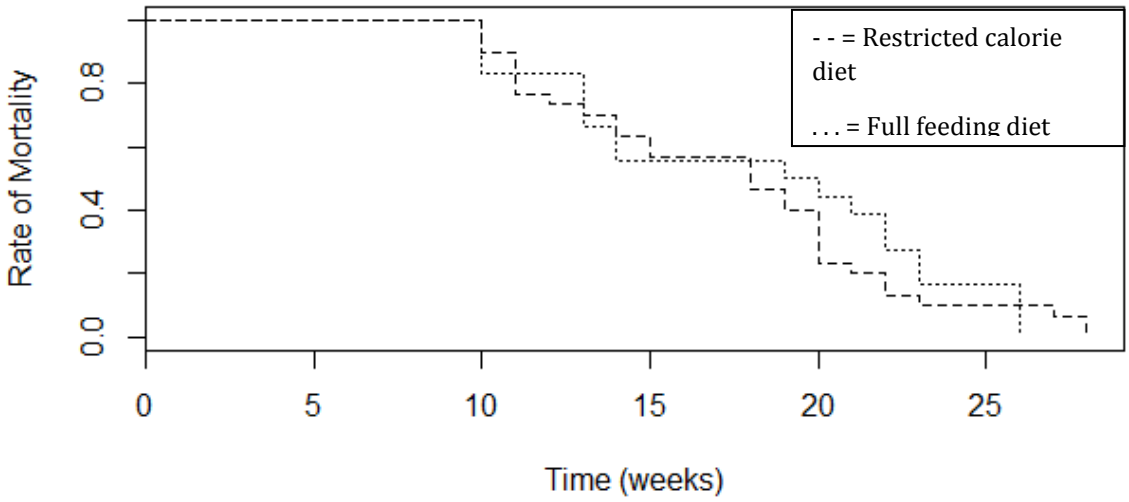

Fig 5: The proportion of adult female *A. keyserlingi* spiders alive over the experimental period. All spiders were alive for the first ten weeks, but after that spiders from both treatments started to die at a similar rate.

I also conducted the same experiment on adult male *Argiope keyserlingi* spiders. I randomly assigned them to either a restricted diet (n = 15, weight at maturity = 0.02127 ± 0.00462g) or an unrestricted diet (n = 15, weight at maturity = 0.01940 ± 0.00522g), making sure there was no size bias in the weight at maturity between the two treatments (Mann-Whitney U test: W = 915, n_1 = 15, n_2 = 15, p = 1.0). Overall, longevity in male spiders ranged from 56 days to 195 days (Fig 6). However, there was no overall effect of diet on longevity in terms of average lifespan or the rate of mortality (Mann-Whitney U test: W = 1276.0, n_1 = 15, n_2 = 15, p = 0.56 and chisq = 0.2, df = 1, p = 0.679 respectively).

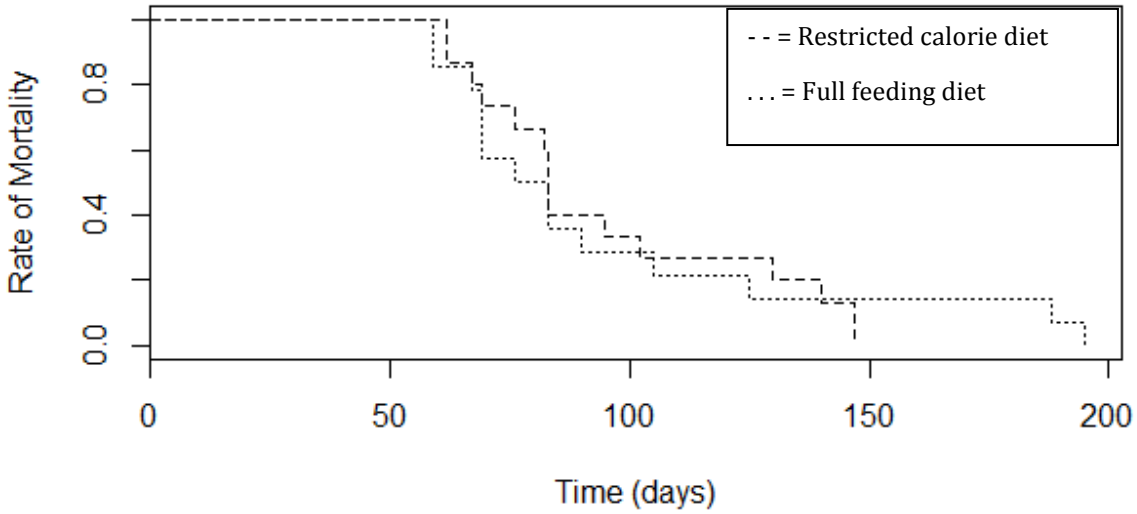

Fig 6: The proportion of adult male *Argiope keyserlingi* spiders alive over time. For the first 60 days, neither treatment yielded any deaths, however, a steep decline followed for both treatments, followed by a plateauing out, for the full feeding diet, while the restricted calorie diet just zeroed out.

Experiment 2: The effect of protein restriction on spiders' longevity and performance

Adult female *Argiope keyserlingi* spiders were randomly allocated to either a low protein treatment (n = 15, weight at maturity: 0.13196 ± 0.03837g) or a high protein treatment (n = 15, weight at maturity: 0.12992 ± 0.03298g), making sure there was no size bias in the weight at maturity between the two treatments (Mann-Whitney U test: W = 2575.0, n_1 = 25, n_2 = 27, p=0.7288). The longevity of the spiders in this experiment ranged from 12 days to 119 days. But there was no significant effect of the amount of protein in the spiders' diet on their longevity (LMM: Chisq = 0.2, df = 1, p = 0.679).

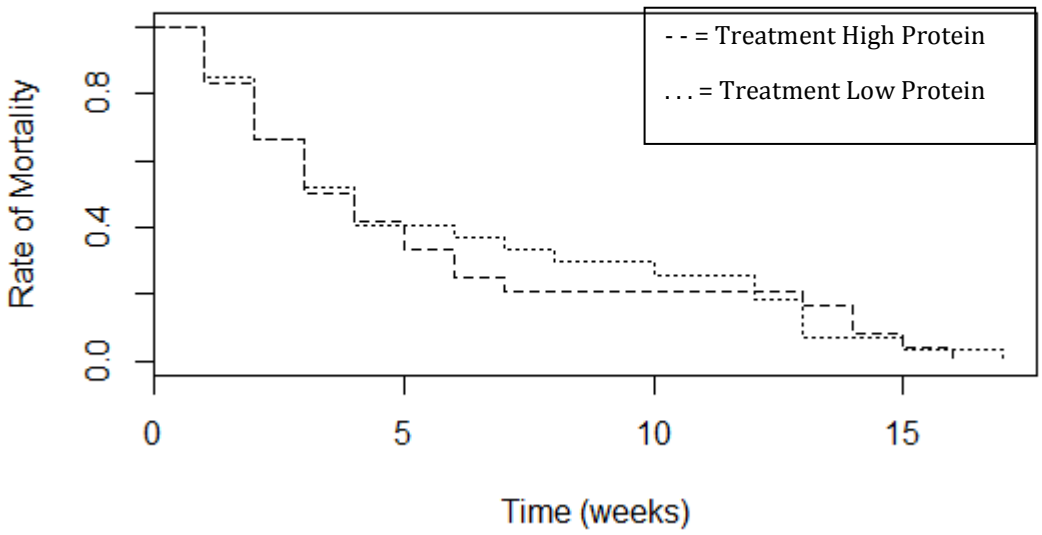

Fig 7: The proportion of adult female spiders alive in response to either a high protein (n = 25) or a low protein (n = 27) treatment, represented by survival curves showing time (in weeks) against the rate of mortality. The proportion of protein in the spiders diet did not affect their longevity, with both curves following similar patterns.

Web area was measured every week, on a fresh web. There was a significant decline in the web area for both treatment groups of spiders (LMM: chisq = 5.588, df = 1, p = 0.018). Individuals on the high protein diet tended to build bigger webs overall (LMM: chisq = 5.558, df = 1, , p = 0.018) (Fig. 8). But the longer the spider lived in this experiment, the smaller the web became in either treatment, as seen in Figure 8. However, there was no significant interaction between diet and the size of their webs (LMM: chisq = 11.048, df = 14, , p = 0682).

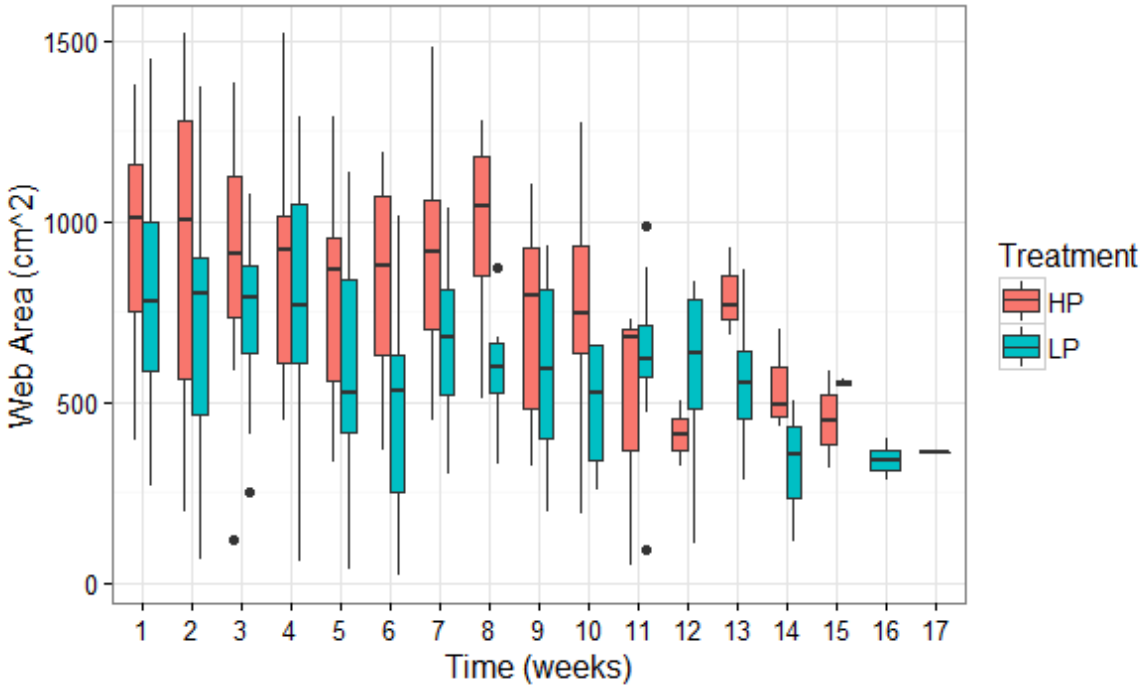

Fig 8: Web area of adult female *Argiope keyserlingi* spiders per week in response to two different treatments (HP: high protein; LP: low protein) represented by boxplots showing the median, quartiles and non-outlier range (black circles indicate outliers).

Every week, the spiders were recorded running from the centre of the hub to the last horizontal spiral thread. Up until week 8, spiders both treatment groups were running at consistent speeds. From week 8 however, the spiders appeared to increase and not decrease their running speed (Fig. 9). There was a significant interaction between diet and age (in weeks) (LMM: chisq = 29.813, p = 0.008, df = 14). The significant diet and week interaction suggests a treatment effect on the change in running speed with age. However, there was no overall clear pattern of how diet affects running speed. In some week's spiders on the high protein diet ran faster than the spiders on the low protein diet and in other weeks the running speed was the reverse (Fig 9).

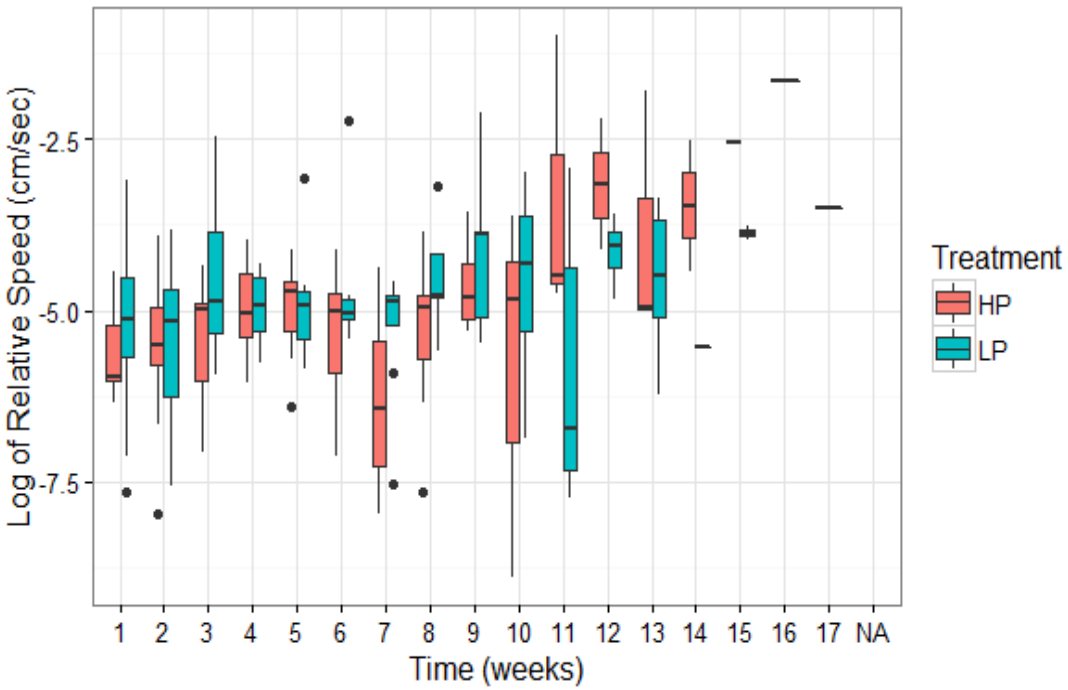

Fig 9: This graph shows the relative running speed of adult female *Argiope keyserlingi* spiders from the centre hub of the web to last spiral thread in a horizontal direction. This relative running speed is represented by boxplots showing median, quartiles and non-outlier range (outliers are highlighted with black dots). HP: high protein diet, LP: low protein diet.

There was a significant effect of week on the condition of the spider (Fig 10), with spider weight varying from week to week (LMM: chisq = 29.504, p = 0.020, df = 16). However, there was no clear increase or decrease with time. The amount of protein in their diet did not affect the condition (LMM: chisq = 1.129, df = 1, p = 0.288), and the interaction between diet and week was also not significant (LMM: chisq = 8.443, df = 14, p = 0.865) Fig 10).

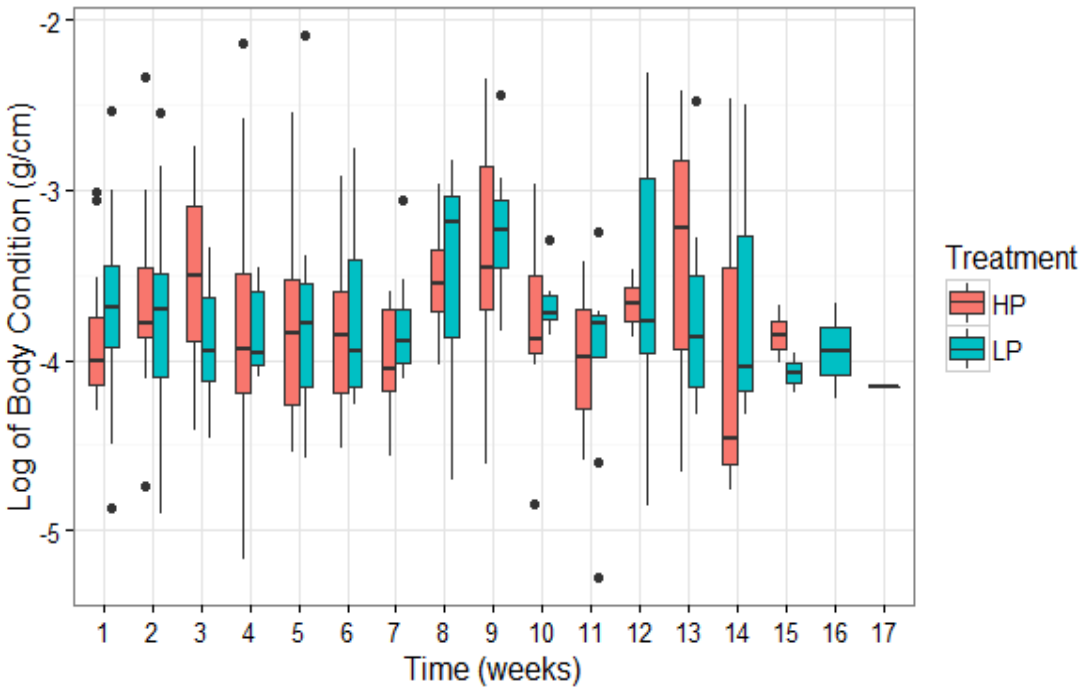

Fig 10: Log body condition per week in boxplots showing median, quartiles and non-outlier range (outliers are highlighted with black dots). HP: high protein diet, LP: low protein diet.

The response time to the vibratory stimulus varied over the experiment in both feeding treatments (Fig 11). There was a significant interaction between diet and week (LMM: chisq = 30.372, p = 0.006, df=14) (Fig 11) but like with running speed, no overall pattern emerged: some weeks spiders on the high protein diet reacted faster, while on other weeks the spiders on the low protein diet reacted faster.

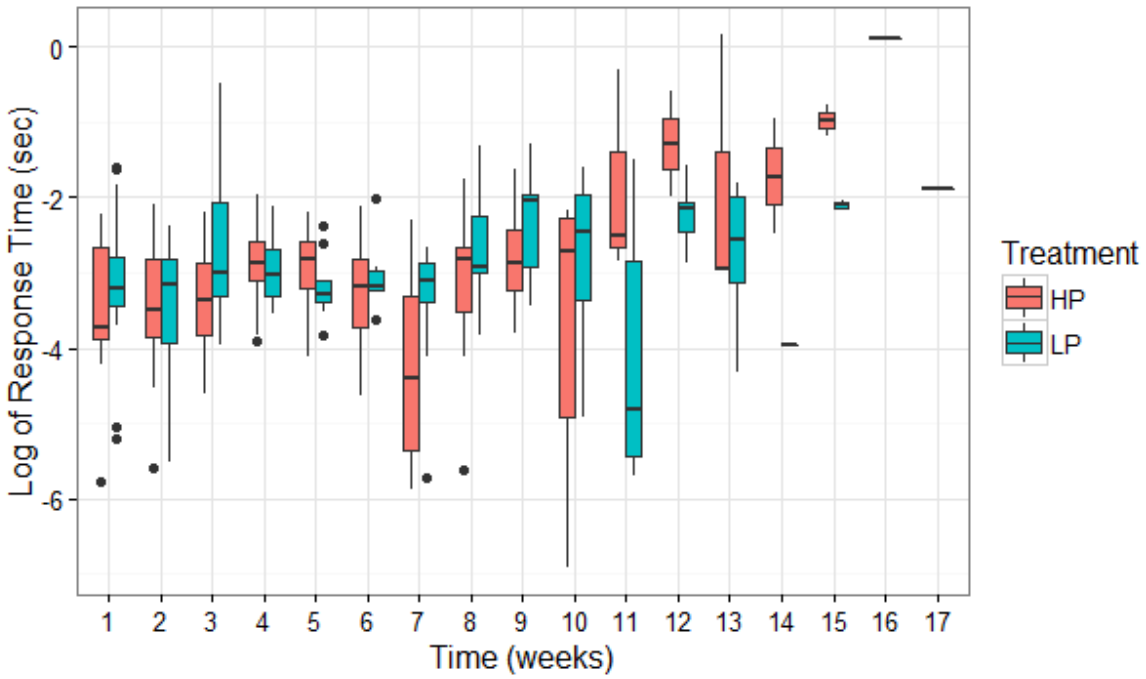

Figure 11: The log of the response time over the number of weeks the spiders were measured. Boxplots showing median, quartiles and non-outlier range (outliers are highlighted with black dots). HP: high protein diet, LP: low protein diet.

Table 3: Summary of the Statistics for Protein experiment with 4 performance parameters

Response Variable	Df	X^2	P
Web Area			
Treatment: weeks	14	11.048	0.682
Treatment	1	5.588	0.018
Weeks	16	33.159	0.007
Relative Running Speed			
Treatment: weeks	14	29.813	0.008
Body Condition			
Treatment: weeks	14	8.443	0.865
Treatment	1	1.129	0.288
Weeks	16	29.504	0.020
Response Time			
Treatment: weeks	14	30.372	0.006

Discussion

Experiment 1

During dietary restriction, there is a point at which calorie intake can be lowered, for a life extending effect, but any further reduction in the calorie intake, starvation causes a decline in longevity (Gaetani & Virgili 2010). Here, I predicted that male and female *A. keyserlingi* spiders that received less food would live for longer. However, contrary to my prediction, dietary restriction had no effect on longevity in this wild species.

There could be several reasons as to why my results did not support my hypothesis. First, the available calories between the two treatments may not have been different enough or possibly there was too much restriction. Mathison et al. (2012) stated that caloric restriction refers to 30% reduction in the food intake (Mattison et al. 2012). However, in experiment 1, I reduced the feeding frequency of the spiders, by 50%. Experiment 1 did not find an effect, unlike the result in Mathison et al. (2012), which found a life extending effect with only a 30% calorie restriction in the Rhesus monkey (*M. mulatta)* (Mattison et al. 2012). Food intake was reduced by 50%, as in my experiment, might be too much of a restriction to cause a life extending effect.

The excessive reduction of calories could also be the reason behind Niitepold et al. (2014) non-effect of life extending effect on the two species of butterflies. This study examined the effects of dietary restriction on longevity and reproduction in two species of butterfly (*Coliaseurytheme* and *Speyeria mormonia*) (Niitepold et al. 2014). Niitepold et al. (2014) restricted their caloric diet by 50%, like my experiment, while leaving another group on a full feeding diet. They concluded that dietary restriction reduced the body mass and the fecundity of the butterflies, but had no life extending effect (Niitepold et al. 2014). Therefore, there is a limit to the precent of calories that can be restricted from the diet, to extend that species' life span, and further reduction would just lead to a decline in longevity (Gaetani & Virgili 2010).

The relationship between diet and longevity can be seen indirectly when examining the reproductive output of well-fed spiders. This has been demonstrated in several studies including Kasumovic et al. (2009) who tested dietary restriction on another species of spider, the males of the Australian redback spider (*Latrodectus hasselti)*

(Kasumovic et al. 2009). This study concluded that although male survival depended on body condition, there was no increase in longevity (Kansumovic et al. 2009). Kasumovic et al. (2009) also showed that there is a competitive trade-off, meaning that males who were in the presence of females paid the survival cost for reproducing (Kansumovic et al. 2009). In my study, I used unmated females that tended not to produce any eggs during the experiment. Future studies should consider the reproductive rate of the adult female spiders, to examine whether this relationship between diet, aging and longevity reduces the rate of fecundity in favour of survival, as predicted by Nakagawa et al. (2012).

Experiment 2

In this experiment, I aimed to understand the relationship between diet, longevity and aging. To do this, I manipulated the amount of protein intake in the diet of the live prey, *Drosophila melanogaster* that were then fed to an orb web spider, *Argiope keyserlingi*. I predicted the adult female spiders that received less protein in their diet would live longer, but spiders that received more protein in their diet would age faster. This is illustrated in several studies, including one conducted by Chen et al. (2013) who tested the effects of dietary restriction on the oriental fruit fly (*Bactocera dorsalis)*. This species was subjected to constant feeding treatments that differed in amounts of the protein: carbohydrate ratio for approximately 45 days (Chen et al. 2013). Chen et al. (2013) concluded that as the protein to carbohydrate ratio decreased, the individuals aged faster, resulting in dying earlier (Chen et al. 2013). Another study that illustrates my predicted hypothesis is Wang et al. (2013) who examined the effects of protein and lipid intake upon life span in the fruit fly *Drosophila melanogaster.* What these authors found was that a cranberry diet that is high in sugar and low in protein extended the life span of adult females but a low sugar and high protein diet reduced the longevity of the flies (Chen et al. 2013). However, contrary to my prediction and the results of similar studies, protein restriction had no effect on longevity *in Argiope keyserlingi*.

Longevity in *Argiope keyserlingi*, tested here, was not influenced by the protein intake, contradicting evidence of another study Solon-Biet et al. (2014), that fed mice on one of 25 diets varying along the protein:carbohydrate ratio. In mice, longevity was enhanced when protein was traded with carbohydrates (Solon-Biet et al. 2014).

The clear difference in these two experiments are the type of species used, and as I have previously stated, model species, such as mice are more likely to show an effect with dietary or protein restriction than spiders or other non-model species (Nakagawa et al. 2012). Also, these experiments use very different species, with very different biologies. This can also be generalised to other species, for instance, the Queensland fruit fly *(Bactrocera tryoni).* Fanson et al. (2006) examined how both calories and nutrients affected longevity and fecundity in the Queensland fruit fly (Fanson et al. 2009). Q-flies were fed on 28 different diets, that varied in both carbohydrate:protein ratios and concentrations (Fanson et al. 2009). These authors found that as they increased the C:P ratio, while keeping the calorie count constant, there was a positive life extending effect on these flies (Fanson et al. 2009). In general, life span was reduced as the calorie count decreased (Fanson et al. 2009). The Q-fly diets used yeast as a medium for the flies (contained at least 45% protein, 24% carbohydrates etc.), which differs from my experiment as I only used a maximum of 2.5% protein, which raises the possibility that in my experiment I did not use enough protein to generate a life extending effect.

The response variable web area, was significant not between the interactions but between the number of weeks and the treatment the spider was fed. Spiders who were fed the high protein treatment built larger webs than the spiders fed the low protein treatment. Protein is essential for silk production, particularly aciniform silk, which is used for making the cross-like decoration arms in the centre hub of the web (Blamires et al. 2009). This infers that adult spiders that are fed higher amounts of protein in their diets, allocate their excess protein reserves towards building bigger webs than spiders on low protein diet (Blamires et al. 2009). This in turn, allows them to continue building larger webs creating a positive feedback cycle as larger webs are able to capture more prey thus accessing more protein in their diet (Blamires et al. 2009). However, in that study, the spiders on the low protein treatment had less protein reserves to waste on web building but rather used their limited resources in somatic maintenance (or body repair) (Adler & Bonduriansky 2014). Therefore, the low protein spiders appeared to be engaging in the resource re-allocation hypothesis, stated in Adler and Bonduriansky (2014) because under dietary restriction the limited resources into somatic maintenance rather than reproduction to conserve energy, and promote survival (Adler & Bonduriansky 2014).

In my study, the response variable running speed was engaging in resource re-allocation (Adler & Bonduriansky 2014). I found a significant interaction between speed and treatment as well as speed and the number of weeks that the spider was alive. Specifically, spiders on both treatments varied a lot more at old age, both within their treatment groups. The high protein spiders, however, maintained a faster speed than the low protein spiders, as a decline in performance began to set in. However, there are not many studies that have looked at this performance parameter in the literature. Running speed is an important factor for the spider when examining their aging and diet, patterns, as I have discovered it is significantly linked. Future studies on this parameter could focus on why speed is affected by diet: is it because excess protein in the species' bodies can lead to higher body mass, but less energy to run? Maybe spiders, feel fuller than the spiders who are fed a low protein diet which in turn affects their behaviour.

Body condition was not affected by diet in this experiment, but spiders did not maintain a similar weight from week to week, but weight fluctuated from week to week. This performance parameter was like a parameter that Blamires et al. (2009) measured in their experiment. However, unlike in my experiment, they found that spiders grew larger on the high protein treatment than on the low protein treatment (Blamires et al. 2009). Why did Blamires et al. (2009) find contradictory results to my experiment? There are three possible reasons: 1) the two studies used different types of protein and standard mix as treatment options. Whereas my experiment used potato mix, sugar and Nature's Way protein supplement (100% natural soy protein isolate), Blamires et al. (2009) used yeast, semolina, agar and a lecithin-based protein (also a soy protein isolate but only 68%) (Blamires et al. 2009). 2) The delivery of the protein is unlikely to be a substantial reason as to why these different results occurred, as we both used fruit flies as the live prey and cultivated them on the different treatments for one week prior to feeding them to the spiders. 3) Finally, the amount of protein supplement that was added to the treatment options may have resulted in the different outcomes. Blamires et al. (2009) was not specific about how much lecithin-based protein was added and was consequently available to the spiders. But it is likely that subtle differences in diet can have significant effects on body condition. In general, body condition is affected by the amount of protein in the

diet. Jenson et al. (2011) restricted the protein:lipid ratio of juvenile wolf spiders (*Pradosa prativaga*). While this study looked at the nutritional regulation in wolf spiders, rather than the longevity of the spiders, they did find a significant difference in the body condition of the juveniles (Jenson et al. 2011). The spiders fed a high protein:lipid diet grew larger than the spiders who were fed a high lipid:protein diet (Jenson et al. 2011). A final example of a study that found a significant effect between diet and body condition is that of Wilder (2013). Wilder (2013) examined the growth of juvenile spiders and how it is affected by the interaction between the clutch, reproduction and diet (Wilder 2013). As with Blamires et al. (2009), Wilder (2013) also used a different type of protein to my experiment. Instead of adding a protein supplement, Wilder (2013) added 40% dog food to an instant potato flake medium (Wilder 2013). Presumably, this resulted in a higher protein availability compared to my own experiment and could be the reason behind a significant affect on growth size, as the spiders on the high nutrient diet (high protein) had a greater growth rate, in terms of mass and body size and condition (Wilder 2013).

Finally, the response variable response time was measured, which reflects the time taken by the spider to get to the stimulus. This result yielded a significant interaction between treatment and weeks in terms of response time, against the distance that the spider had to run: high protein spiders ran slower than low protein spiders. . This result could be because the spiders fed the high protein treatment were eating fewer flies, because they are reaching their target sooner and with age became less motivated, running at a slower speed. Another reason as to why the high protein spiders ran slower than the low protein spiders as they grew older, is their weight. Heavier spiders do seem to suffer from the physical constraints of having to lift a heavier abdomen and change the web architecture accordingly (Herberstein & Heiling 1999). However, there was no significant difference between the treatments in the spider's body condition. There are not many studies that have looked at response time relative to the age of the individual and to fully understand this relationship requires more detailed experiments .

In my study, I used unmated adult female spiders that tended not to produce any eggs/cocoons during my experiment and thus my experiment could not detect any trade-off between survival and reproduction. For example, Chen et al. (2013) aimed

to test the effects of dietary restriction on life span using both unmated and mated males and females, and concluded that unmated males and females lived significantly longer than their mated counterparts, and females also lived twice as long as the males (Chen et al. 2013).

Conclusion

In conclusion, my experiments do not broadly support my hypotheses, however, they still revealed that spiders show patterns of aging. This was not obvious in my caloric restriction experiment, as I focused on measuring the spider's longevity and not the performance of spiders with age. However, the protein intake experiment showed that despite the treatment not influencing longevity, web area, speed or body condition, spider performance changes with age. Nevertheless, the results from my experiments support the conclusions of Nakagawa et al. (2009)'s meta-analysis, arguing that the life extending effects of dietary restriction are most prominent in model species, but less common in non-model species, which gives way to the idea of a laboratory artefact. Typical laboratory environments relax natural selection away from predators, parasites and diseases, commonly found in the wild (Zuk et al. 2014).

To further this research topic, there are several options I would take in redesigning this study. Firstly, I would mate the adult females to determine whether reproduction reduces longevity. Secondly, I would recommend using diets that differ more in protein content. Thirdly, it was suggested that any follow up experiments, use a larger species of fly (e.g. the house fly, *Musca domestica)* for their entire larval period to ensure the adult flies differed significantly in macronutrient content which should also be measured to confirm that the prey treatment differed. Finally, it would be interesting to add new/extra treatments into the experiment: for instance, a control treatment (using the standard mix), and either a high lipid, or high carbohydrate treatment, as similar studies have found that altering protein to carbohydrate ratio alters a species' chance of living longer.

Literature Cited

Adler, M. I. & Bonduriansky, R. 2014: Why do the well-fed appear to die young? Bio Essays **36**, 439-450.

Anoteux, M., Marchal, J., Chaline, N., Desquilbet, L., Leborgne, R., Gilbert, C. & Pasquet, A. 2012: Aging alters spider orb web construction. Animal Behaviour. **84**, 1113-1121

Arking, R. 2006: The Biology of Aging Oxford University Press.

Bates, D., Machler, M. M., Ben, B. & Walker, S. 2015: Fitting Linear mixed-effects models using lme4. RStudio **67**, 1-48.

Blamires, S. J., Hochuli, D. F. & Thompson, M. B. 2009: Prey protein influences growth and decoration building in the orb web spider *Argiope keyserlingi*. Ecological Entomology **34**, 545-550.

Bonsall, M. B. & 2006: Longevity and aging: appraising the evolutionary consequences of growing old. . Philosophical Transactions of the Royal Society **361**.

Chen, E.-H., Wei, D., Wei, D.-D., Guo-Rui & Wang, J.-j. 2013: The effect of dietary restriction on longevity, fecundity and antioxidant responses in the oriental fruit fly, *Bactrocera dorselis* Journal of Insect Physiology **50**, 1008-1016.

Cooper, T. M., Mockett, R. J., Sohal, B. H., Sohal, R. D. & Orr, W. C. 2004: Effect of caloric restriction on life span of the housefly, *Musca domestica.* . the FASEB journal **15**, 1591-1593.

Dinesh Rad, Ken Cheng & Herberstein, M. E. 2007: A natural history of web decorations in the St Andrews Cross spider Australian Journal of Zoology **55**, 9-14.

Fanson, B. G., Weldon, C. W., Perez-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.

Gaetani, S. & Virgili, F. 2010: Sideways glance: does dietary restriction promote longevity, through impairing fecundity? Not necessarily, if the diet has a correct nutrient balance. Genes Nutr **5**, 183-187.

Harman, D. 1968: Free Radical Theory of Aging: Effect of free Radical reaction inhibitors on the mortality rate of male LAF mice. . Journal of Gerontology **23**, 476-482.

Hawley, J., Simpson, S. J. & Wilder, S. M. 2014: Effect of prey macronutrient content on body composition and nutrient intake in a web building spider. PLOS ONE **9**.

Herberstein, M. E. & A.M., H. 1999: Web asymmetry in spider orb-webs (Araneidae): a result of physical constraints? . Animal Behaviour **58**, 1241-1246.

Herberstein, M. E., Craig, C. l., Coddington, J. A. & Elgar, M. A. 2000a: The functional significance of silk decorations of orb-web spiders: a critical review of empirical evidence Biological Reviews **78**, 649-669.

Herberstein, M. E., Craig, C. L. & Elgar, M. A. 2000b: Foraging strategies and feeding regimes: web decoration investment in Argiope keyserlingi. Evolutionary Ecology Research **2** 69-80.

Hine, C., Hartputlugil, E., Zhang, Y., Ruckenstuhl, C., Lee, B. C., Brace, L., Longchamp, A., Trevino-Villarreal, J. H., Mejia, P., Ozaki, C. K., Wang, R., Gladyshev, V. N., Madeo, F., Mair, W. B. & Mitchell, J. R. 2015: Endogenous hydrogen sulfide production is essential for dietary restriction benefits. Cell **160**, 132-144.

Hine, C. & Mitchell, J. R. 2015: Calorie restriction and methionine restriction in control of endogenous hydrogen sulfide production by the transsulfuration pathway. Experimental Gerontology **68**, 26-32.

Jenson, K., Mayntz, D., Toft, S., Raubenheimer, D. & Simpson, S. J. 2011: Nutrient regulation in a predator, the wolf spider *Pardosa prativaga*. Animal Behaviour **81**.

Kansumovic, M. M., Brooks, R. C. & Andrade, M. C. B. 2009: Body condition by not dietary restriction prolongs lifespan in a semelparous capital breeder. Biology Letters **5**, 636-638.

Kasumovic, M. M., Brooks, R. C. & Andrade, M. C. B. 2009:
Body condition but not dietary restriction prolongs lifespan in a semelparous capital breeder. Biological Letters **5**.

Kohl, K. D., Coogen, S. C. P. & Raubenheimer, D. 2015: Do wild carnviores forage for prey or for nutrients? Bio Essays **37**.

Le Couteur, D. g., Wilder, S. M., Cabo, R. d. & Simpson, S. J. 2013: The evolution of research on ageing and nutrition Journal of Gerontology **69**, 1-2.

Lee, K.P., Simpson, S.J., Clissold, F.J., Brooks, R., Ballard, J.W.O., Taylor, P.W., Soren, N. & Raubenheimer, D. 2007: Lifespan and reproduction in *Drosophila:* New insights from nutritional geometry. PNAS. **106:7,** 2498-2503

Masoro, E. J. 1988: Food restriction in rodents: an evaluation of its role in the study of ageing Journal of Gerontology: BIOLOGICAL SCIENCE **43**, 59-64.

Mattison, J. A., Roth, G. S., Beasley, T. M., Tilmont, Handy, A. M., Herbert, R. L., Longo, D. I., Allison, D. D., Young, J. E., Bryant, M., Barnard, D., Ward, W. F., Qi, W., Ingram, D. K. & de Cabo, R. 2012: Impact of caloric restriction on health and survival of rhesus monkeys from the NIA study. Nature **489**.

Mayntz, D., Raubenheimer, D., Salomon, M., Toft, S. & Simpson, S. J. 2005: Nutrient-Specific foraging in invertebrate predators. Science **307**.

McCay, C. M., Crowell, M. F. & Maynard, L. A. 1935: The effect of retarded growth upon the length of life span and upon ultimate body size The Journal of Nutrition

McCormick, M. A., Tsai, S.-y. & Kennedy, B. K. 2011: TOR and ageing: a complex pathway for a complex process. Philosophical Transactions of the Royal Society **366**, 17-27.

McDonald, R. B. & Ruhe, R. C. 2011: Aging and Longevity: why knowing the difference is important to nutrition research Nutrients **3**, 274-282.

Nakagawa, S., Lagisz, M., Hector, K. L. & Spencer, H. G. 2012: Comparative and meta-analytic insights into life extension via dietary restriction. Aging Cell **11**, 401-409.

Niitepold, K., Perez, A. & Boggs, C. L. 2014: Ageing, lifespan and energetics under adult dietary restriction in Lepidoptera Physiological and Etochemical Zoology **87**, 684-694.

Piper, M. D. W., Partridge, L., Raubenheimer, D. & Simpson, S. J. 2011: Dietary restriction and aging: a Unifying perspective Cell Metabolism **14**.

Rao, D., Cheng, K. & Herberstein, M. E. 2007: A natural history of web decorations in the St Andrew's Cross spider (*Argiope keyserlingi)*. Australian Journal of Zoology **55**, 9-14.

Raubenheimer, D. & Simpson, S. J. 1999: Integrating nutrition: a geometrical approach Entomologia Experimentalis et Applicata **91**, 67-82.

Raubenheimer, D., Simpson, S. J. & Mayntz, D. 2009: Nutrition, ecology and nutritional ecology: toward an integrated framework. . Functional ecology **23**, 4-16.

RStudio. 2015: RStudio: Intergrated Development for R. RStudio, Inc.,, 0.99.879 edn, Boston, MA

Simpson, S. J. & Raubenheimer, D. 2007: Caloric restriction and aging revisited: The need for a Geometric analysis of the nutritional bases of aging
Journal of Gerontology: BIOLOGICAL SCIENCE **62A**, 707-713.

-. 2009: Macronutrient balance and lifespan AGING **1**.

-. 2011: the nature of nutrition: a unifying framework. Australian Journal of Zoology **59**, 350-368.

Solon-Biet, S. M., McMahon, A. C., Ballard, J. W. O., Ruohonen, K., Clogger, V. C., Warren, A., Huang, X., Pichaud, N., Melvin, R. G., Gokam, R., Le Couteur, D. g. & Simpson, S. J. 2014: The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, ageing, and longevity in ad libitum fed mice. Cell Metabolism **19**, 418-430.

Therneau, T. M. & M., G. P. 2000: Modeling Survival data: Extending the Cox Model. R Studio.

Wickham, H. 2009: ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.

Wilder, S. M. 2011: Spider Nutrition: An integrative perspective Advances in insect physiology **40**.

Wilder, S. M. 2013: Variation among clutches in the response of spiders to prey nutrient content. Journal of Arachnology **41**, 53-58.

Wilder, S.M., Le Couteur, D.G. & Simpson, S.J. 2013: Diet mediates the relationship between longevity and reproduction in mammals. AGE **35,** 921-927

897 Willcox, D. C., Willcox, B. J., Todoriki, H., Curb, J. D. & Suzuki, M. 2006: Caloric restriction and human
898 longevity: what can we learn from the Okinawans? . Biogerontology **7**, 173-177.
899 Zschokke, S. & Herberstein, M. E. 2005: Laboratory methods for maintaining and studying web-
900 building spiders Journal of Arachnology **33**, 205-213.
901 Zuk, M., Garcia-Gonzalez, F., Herberstein, M. E. & Simmons, L. W. 2014: Model systems, taxonomic
902 bias and sexual selection: beyond Drosophila. Annual Review of Entomology **59**, 321-338.
903
904

Supplementary Material

Appendix 1 (Linear mixed model)

Linear mixed model – Web area vs. Treatment

```
model1<-lmer (Web.area~Treatment+weeks+Treatment: weeks+(1|id),
data=webarea)
model2<-lmer(Web.area~Treatment+weeks+(1|id),data=webarea)
anova(model1,model2)
summary(model1)
model3<-lmer(Web.area~+weeks+(1|id),data=webarea)
anova(model2,model3)
model4<-lmer(Web.area~Treatment+(1|id),data=webarea)
anova(model2,model4)
```

Linear mixed model – relative running speed vs. treatment

```
model15 <-
lmer(ln.relative~Treatment+weeks+Treatment:weeks+(1|id),data=relativespeed)
model25<-lmer(ln.relative~Treatment+weeks+(1|id),data=relativespeed)
anova(model15,model25)
model3<-lmer(ln.relative~+weeks+(1|id),data=relativespeed)
anova(model25,model3)
model4<-lmer(ln.relative~Treatment+(1|id),data=relativespeed)
anova(model25,model4)
```

Linear mixed model – body condition vs. treatment

```
model12<-lmer(ln.ratio~Treatment+weeks+Treatment:weeks+(1|id),data=bodysize)
model22<-lmer(ln.ratio~Treatment+weeks+(1|id),data=bodysize)
anova(model12,model22)
model32<-lmer(ln.ratio~+weeks+(1|id),data=bodysize)
anova(model22,model32)
model42<-lmer(ln.ratio~Treatment+(1|id),data=bodysize)
anova(model22,model42)
```

Appendix 2 (Survival curves and longevity)

```
survivalcurve2 <- survfit(Surv(Time,Event)~Treatment)
survivalcurve2
plot(survivalcurve2, xlab="Time (weeks)", ylab="Rate of Mortality",lty=2:3)
survivalcurve2<-survdiff(Surv(Time,Event)~Treatment)
survivalcurve2
```

Appendix 3 (Results of Linear mixed models)

Data: webarea

Models:

model3: Web.area ~ +weeks + (1 | id)

model2: Web.area ~ Treatment + weeks + (1 | id)

```
       Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
model3  4 3898.4 3912.9 -1945.2   3890.4
model2  5 3894.9 3913.0 -1942.4   3884.9 5.5136      1    0.01887 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: webarea
Models:
model4: Web.area ~ Treatment + (1 | id)
model2: Web.area ~ Treatment + weeks + (1 | id)
       Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
model4  4 3914.9 3929.3 -1953.4   3906.9
model2  5 3894.9 3913.0 -1942.4   3884.9 21.969      1  2.771e-06 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: relativespeed
Models:
model4: ln.relative ~ Treatment + (1 | id)
model25: ln.relative ~ Treatment + weeks + (1 | id)
        Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
model4   4 727.22 740.88 -359.61   719.22
model25  5 711.27 728.35 -350.63   701.27 17.952      1  2.265e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Data: bodysize
Models:
model42: ln.ratio ~ Treatment + (1 | id)
model22: ln.ratio ~ Treatment + weeks + (1 | id)
        Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
model42  4 430.63 445.21 -211.31   422.63
model22  5 424.33 442.56 -207.16   414.33 8.2977      1   0.003969 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```