How life-history traits and mating behaviour in female fall field crickets is influenced by macronutrient-ratio specific rearing diets

by

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Abstract

Nutrition can strongly influence life-history traits and behaviours related to sexual selection. An individual’s ability to acquire and allocate nutrients has proven to be a pertinent driver behind individual variation within populations and between sexes. Organisms must acquire the ratio of essential nutrients appropriate for growth, reproduction, and survival. I investigated whether high-protein versus high-carbohydrate rearing diet [3:1 versus 1:3 protein:carbohydrate (P:C), respectively] fed during development and into adulthood influenced various life history traits and female mating behaviours in *G. pennsylvanicus*. I determined that a high-protein diet promoted growth, fecundity, responsiveness to mate calling, and mounting behaviour at the cost of survival. Whereas a high-carbohydrate diet promoted survival at the cost of growth and reproduction. My research highlights the inability of *G. pennsylvanicus* to maximize all life-history traits on a single diet resulting in life-history trait trade-offs between reproduction and survival and the role macronutrient-ratios play on female mating behaviour.
Acknowledgements

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1. Introduction

The strategies and mechanisms that ultimately result in individuals choosing their reproductive partners have long been questioned by evolutionary biologists. These strategies exist in various forms including social interactions, physical ornamentation, or courtship displays to name a few, all of which have resulted in elaborate physical and behavioural characteristics over evolutionary time (Andersson & Simmons, 2006; Díaz-Muñoz et al., 2014; Tolentino & Anciães, 2020). The goals of reproduction often differ between males and females whereby males often want to mate with as many females as possible, while females often want to seek the male with the ‘best’ genes to allow her to produce offspring with high fitness (Tolentino & Anciães, 2020). Due to females having a higher associated cost of mating, females tend to become choosy when selecting a mating partner (Díaz-Muñoz et al., 2014; Pizzari et al., 2015). A large majority of research on sexual selection has focused on factors that influence male sexual traits. Less research however has focussed on factors influencing female mate choice, even though these decisions are what drive the evolution of male physical and behavioural characteristics (Andersson & Simmons, 2006; Darwin, 1871; Cotton et al., 2004).

As predicted by indicator models of sexual selection, elaborate sexual signals should serve as honest indicators of male condition where only those in good condition are able to incur the costs associated with signals that are both energetically costly to produce and maintain (Grafent, 1990). Females can use these honest indicators of male condition as signals representing both direct (immediate effects on fitness of the receiver) and indirect (enhancement of offspring fitness) mating benefits (Andersson & Simmons, 2006).

Female condition dependence may play a crucial role in mate choice due to the high energy requirements associated with seeking a mate; such that this behaviour requires time and
energy, along with placing the female at an increased risk of parasitism and predation. Females with strong mating preferences may be in higher condition and therefore more able to incur the costs of being choosy when selecting a mate compared to females in poorer condition (Cotton et al., 2004). Particularly, higher-condition females may be able to reap the benefits of devoting more time and energy towards assessing potential mates compared to low-condition females that may be unable to afford these costs (Cotton et al., 2004; Hunt et al., 2005). For example, female *Teleogryllus commodus* that experience food stress show weakened preferences for male phenotypes and were less likely to seek a mate and copulate (Hunt et al., 2005). Further, Judge et al. (2014) investigated how mating behaviour was impacted by female condition using *G. pennisylvanicus*. They showed that while high- and low-condition females (manipulated by altering adult diet) both exhibited preferences for higher calling effort, high-condition females exhibited higher latency to choose than low-condition females, suggesting high-condition females are choosier than low condition females.

The study of life history traits provides crucial information about development and behaviour due to changes in environmental and evolutionary factors. Life history traits broadly include body size, development time, growth rate, survival, mating behaviour, and reproduction (Ferguson, 2018; Hunt et al., 2005; Whitman, 2008). Nutrition can strongly influence life-history traits and behaviours related to sexual selection (Andersson & Simmons, 2006; Harrison, 2018; Harrison et al., 2013, 2014, 2017; Reifer et al., 2018). The ability of individuals to acquire and allocate these nutrients has been shown to be a pertinent driver behind individual variation within populations and between the sexes (Harrison, 2018). For example, high protein diets have been correlated with faster juvenile development in caterpillars (Roeder & Behmer, 2014), spiders (Mayntz & Toft, 2001), and birds (Adeyemo et al., 2012). Restricted availability of dietary
protein impairs disease resistance, immunity, and growth in southern leopard frog tadpoles (Venesky et al., 2012). Male sexual pheromone expression is maximized on a high carbohydrate diet in cockroaches (South et al., 2011). Furthermore, evidence from past research also suggests that female mate choice decisions in insects may be influenced by diet quantity and quality (e.g., Harrison, 2018; Hunt et al., 2005; Wilgers & Hebets, 2012).

Finding and acquiring quality food can pose a challenge in nature. If organisms are unable to acquire the ratio of essential nutrients that is appropriate for their growth, reproduction, and survival, they must either feed until satiated (resulting in under-consumption of the limited nutrient) or feed beyond satiation until they obtain enough of the limited nutrient to thrive (resulting in over-consumption of a subset of nutrients). Over-consuming food can have toxicity and obesity effects, while under-consuming food can result in malnutrition (Harrison, 2018; Raubenheimer & Jones, 2006). Until recently, much of the literature surrounding the effect of diet manipulation on reproduction and other life history traits did not carefully consider these nutrient ratios. Instead, studies often altered total diet quantity or manipulated a single nutrient. These approaches do not allow us to fully comprehend how nutrition influences individual life-history traits and behaviour as they do not take into consideration nutrient balance. When foods are imbalanced, allocation towards some life-history traits may be favoured over others, resulting in nutrition-related life-history trade-offs (Rowe & Houle, 1996).

Field crickets are omnivorous insects with a diet consisting of a wide array of plant and insect materials with variable nutrient content (Bertram et al., 2008). Because crickets have diets of variable nutrient composition, they must also have homeostatic regulatory systems that have evolved to compliment such variation (Raubenheimer & Jones, 2006). As a result, field crickets are an ideal study species for determining the effects of diet composition on life history traits and
behaviours due to their complex diet structures in nature likely making lab-manipulated diets ecologically relevant (Harrison, 2018). Though field crickets’ reproductive behaviours are complex, they are also easily quantifiable making them ideal study species for examining mating behaviour.

Harrison et al in (2018) determined sex-specific fitness trade-offs influenced by diet. Male *Gryllus veletis* maximized weight gain on a 3:1 protein: carbohydrate (P:C) diet whereas lifespan was maximized on a 1:3 P:C nutrient ratio. Most male acoustic signalling parameters increased with increasing protein or carbohydrates regardless of ratio (Harrison, 2018). Trade-offs in fitness traits were found in females as a high-protein diet maximized weight gain and egg production but reduced lifespan in comparison to a high carbohydrate diet. This research approach reveals an inability to maximize all fitness traits simultaneously and showcases the effects that diet composition has on fitness trait trade-offs (Harrison, 2018; House et al., 2016; Houslay et al., 2015; Hunt et al., 2005; Maklakov et al., 2008). Intriguingly, however, most nutrient balance studies to date have only been conducted on adults, so it is not obvious how dietary challenges during development influence adult behaviour.

In this thesis I used holistic diets to experimentally manipulate nutrient composition enabling me to investigate how carbohydrate and protein during development and into adulthood impacts life-history traits and female mate choice in the North American fall field cricket, *Gryllus pennsylvaniaeus*. *G. pennsylvaniaeus* is sexually dimorphic, and males rub their forewings together producing a pulse of sound. Females locate and differentiate between potential mates based on these acoustic signals (Ferguson, 2018; Wagner & Reiser, 2000). Females analyze various aspects of these calls to determine male quality including chirp rate, chirp duration, and signal amplitude (Figure 1; Wagner & Reiser, 2000). Prior research on male acoustic signalling
has revealed females to have stronger preference towards calls reflecting higher calling effort (Judge et al., 2014). Judge et al., (2014) manipulated female condition by changing adult diet, not juvenile diet, and did not carefully quantify differences in nutrient ratios. Judge et al., (2014) used oligitic diets by manipulating the amount of rabbit food to alter the protein levels. Unfortunately, this oligitic diet approach changes more than just protein content of the diet. Here I investigate the effects of developmental diet on life-history traits of females including: development time, body size, female responses to male mate attraction signalling, overall female mating behaviour, and total egg production. I hypothesized that females reared on the high-protein diet would have shorter development time, larger adult body size, and increased egg production compared to those reared on the high-carbohydrate diet.

I also hypothesized that diet would affect female mating behaviour. There are a few potential results I anticipate. (1) Females reared on the high-protein diet will become both more responsive due to having a shorter lifespan; and become choosier due to higher investment in egg production. (2) Females reared on the high-protein diet will become less responsive due to not having adequate energy reserves to expend in searching for a mate; and become less choosy due to producing more eggs there is less risk associated with making mate choice errors. (3) Females reared on the high-carbohydrate diet will become more responsive due to having more energy reserves to expend in searching for a mate; and become choosier due to having fewer eggs that there is a higher associated cost to making mate choice errors. (4) Females reared on the high-carbohydrate diet will become less responsive due to having a longer lifespan and therefore less pressure to quickly select a mate; and become less choosy due lower investment in egg production.
2. Methods

2.1 Cricket Care

*G. pennsylvanicus* were reared in communal colony bins (L x W x H = 64 x 40 x 42 cm) under a 14h:10hr light:dark photoperiod. Communal bins were maintained 3 times/week and given cardboard shelter (section of egg cartons), food (Harlan Tekland Inc. Rodent diet no. 8604M; hereby referred to as the ‘standard diet’) and water *ad libitum*. As soon as individuals developed wing-buds (when we are able to visually differentiate sexes; 5-6 molts complete out of 8-10), females were removed from the communal colony bins, weighed (OHAUS Pioneer Analytical Balance Model: Adventurer SL AS64; SE = 0.0001g) and placed into individual containers (540mL plastic containers with a mesh panel in the lid to allow for airflow) with cardboard shelter and *ad libitum* water. Each female then began being fed its assigned study diet (see section 2.3) and remained on this diet for the remainder of the study. Males were also removed from the communal colony bin at this time and placed in a male-only group container and provided with cardboard shelter and food and water *ad libitum*. Males continued to be fed the standard diet for the remainder of their testing. This approach ensured only virgin males and females were used throughout the experiments.

2.2 General Methods

I weighed each female as soon as her wing-buds developed (start weight) and then assigned the females to their diet(s). I then quantified the number of days it took until adult moult, weight at adulthood, and weight prior to the start of the behavioural trials. I then tested females for their mating preferences and/or mating behaviour when they were between 10-16 days post imaginal molt (adulthood). I selected this adult age for behavioural testing as female
cric
tes are sexually mature and exhibit the highest phonotaxis to acoustic mate attraction signals during this age range (Pacheco et al., 2013).

2.3 Diets

I created holitic experimental diets (diets following established protocols (Harrison, 2018; Simpson & Abisgold, 1985) and using powdered and liquid ingredients including a 3:1:1 mixture of vitamin-free casein, bacteriophage peptone, and egg albumen (protein source) and a 1:1 mixture of powdered sucrose and dextrin (carbohydrate source) (See Table 1 for full ingredient list). In total, I created four experimental diets: a high protein diet (3:1 (P:C)); a high carbohydrate diet (1:3); a pure-protein diet (1:0); and a pure-carbohydrate diet (0:1). Males utilized in the experiment (Section 2.5) remained on the standard diet.

Food dishes (petri dishes (50 x 9mm) with an upturned plastic lid (15 x 15mm) glued to the centre) were replenished weekly. Prior to replenishing, the food was dried in a drying oven (Precision Scientific; Mechanical Convection Incubator; Economy Model 4EM) for 7 days at 30°C to ensure removal of any excess moisture and then weighed. After diets were consumed and then removed from the individual homes, they were dried in a drying oven again for 7 days at 30°C and all faeces and debris were removed from the dishes using fine forceps before weighing. The difference in weight between the initial and later weighing comprised the mass of food consumed by the female.
Table 1. List and quantities of each ingredient used to create four different protein:carbohydrate ratio diets (High-protein (3:1), High-carbohydrate (1:3), Pure-protein (1:0), Pure-carbohydrate (0:1)). Quantities included were to create 900g of each diet to be used for the entirety of the study.

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2.3.1 If given a choice, what ratio of protein: carbohydrates will female crickets consume?

To determine the ratio of P:C to use for this study I, along with honours student Caterina Gasparini, first ran a preliminary experiment to quantify the spectrum of diet ratios females would consume when given the choice to self-select their food (N = 45). Two food dishes were placed in each individual’s container: 1 food dish contained the pure-protein (1:0) diet and 1 food dish contained the pure-carbohydrate (0:1) diet. These dishes were placed on opposite ends
of the individual’s container. Each female also received 2 water vials, one on each end of the container, to ensure that they were not selecting for 1 diet over the other simply due to the proximity to the water supply. The side these dishes were placed on were randomized for each cricket and the side each diet was on was switched weekly (when food dishes were replenished). This preliminary study showed that female *G. pennsylvanicus* would self-select a P:C ratio between 4:1 and 1:4. This preliminary research study along with prior studies on other field cricket species (e.g., Harrison, 2018 (*Gryllus veletis*); Maklakov *et al*., 2008 (*Teleogryllus commodus*)) supported the idea that my chosen diet ratios of 3:1 and 1:3 P:C lie within the normal range of proteins and carbohydrate levels that females might select.

**2.4 Does rearing diet influence female cricket responses to male mate attraction signalling?**

To test whether female *G. pennsylvanicus* reared on diets differing in their P:C ratio exhibit different responses to male calls I randomly assigned 40 females to each of the 2 diet treatments (see Section 2.3), resulting in a total of 80 females tested. I fed each female their assigned diet from wing-bud stage throughout adulthood and then tested each female for their response to male calling using a track-ball when she was 10-16 days old (post imaginal moult). Each female was used for 1 phonotaxis trial. These females were also used in a second experiment (see section 2.5) if they survived until test day; I therefore returned each female to their housing containers with shelter, *ad libitum* food (their assigned diet), and water.

**2.4.1 Artificial Signal Production**

Phonotaxis trials were conducted to examine female responses to male mate attraction signalling (see section 2.4.2) utilizing artificial male long-distance acoustic mate attraction
signals. Based on prior research showing (1) that chirp rate (the number of chirps per minute) varies with male condition (Wagner & Hoback, 1999; Whattam & Bertram, 2011) and (2) that females prefer males that signal at faster chirp rates (Wagner & Hoback, 1999; Wagner & Reiser, 2000), I selected chirp rate as the parameter to be altered in the calls made for phonotaxis trials (sensu Ferguson, 2018). I used artificial signals instead of natural signals to ensure that chirp rate was the only parameter of the male call that was altered; all other parameters were held constant (sensu Ferguson, 2018).

The artificial male calls used were produced and used by Dr. Genevieve Ferguson in her PhD work. Dr. Ferguson placed 36 lab-reared, virgin, 7-10 day old G. pennsylvanicus male crickets in the Electronic Acoustic Recording Systems (EARS) and recorded their acoustic mate attraction signaling behaviour (Figure 2 shows a spectrogram of a male call) over a four day period. She identified the population mean values for a variety of temporal and spectral calling components (Table 2). Using this information, as well as sound pulses representing the populations’ average carrier frequency and pulse duration produced using Wavtones (www.wavtones.com) an online tone generator, she produced artificial chirps that only differed in their chirp rates. Specifically, Dr. Ferguson created 15 artificial call signals. Using the chirp rate population mean and standard deviation [69.84 chirps/minute ± 19 standard deviation (SD)] call signals were produced that ranged from -3.5 SD to 3.5 SD in increments of 0.5 SD (0.5 SD = ± 9.5 chirps/minute; Table 2). Each call signal was 1 minute in duration.
Figure 1. Spectrogram of male call showing a series of pulses that are concatenated into chirps.

Most of the different temporal parameters quantified in the EARS are show
Table 2. Chirp rates of different calls used in phonotaxis trials. Calls differ only in their chirp rates shown below. All calls also consisted of the other following signalling components including their mean ± SD: Pulse (ms) 15.53 ± 2.64; Inter-Pulse Duration (ms) 36.42 ± 2.40; Pulses Per Chirp 3.25 ± 0.51; Chirp Duration (ms) 97.33 ± 16.66; Inter Chirp Duration (ms) 836.81 ± 302.75; Amplitude (db) 56.24 ± 8.09; Carrier Frequency (Hz) 4954.34 ± 244.27; Pulse Rate (#Pulses/sec) 19.32 ± 1.18.

<table>
<thead>
<tr>
<th>Chirp Rate (SD)</th>
<th>#Chirps/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>136.34</td>
</tr>
<tr>
<td>3</td>
<td>126.84</td>
</tr>
<tr>
<td>2.5</td>
<td>117.34</td>
</tr>
<tr>
<td>2</td>
<td>107.84</td>
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<tr>
<td>1.5</td>
<td>98.34</td>
</tr>
<tr>
<td>1</td>
<td>88.84</td>
</tr>
<tr>
<td>0.5</td>
<td>79.34</td>
</tr>
<tr>
<td>0</td>
<td>69.84</td>
</tr>
<tr>
<td>-0.5</td>
<td>60.34</td>
</tr>
<tr>
<td>-1</td>
<td>50.84</td>
</tr>
<tr>
<td>-1.5</td>
<td>41.34</td>
</tr>
<tr>
<td>-2</td>
<td>31.84</td>
</tr>
<tr>
<td>-2.5</td>
<td>22.34</td>
</tr>
<tr>
<td>-3</td>
<td>12.84</td>
</tr>
<tr>
<td>-3.5</td>
<td>3.34</td>
</tr>
</tbody>
</table>
2.4.2 Trackball

Two days prior to phonotaxis trials females were weighed and a dental elastic attached to their pronotum using Gorilla Super Glue Gel. Females were then returned to their individual container until their trial began.

Phonotaxis trials occurred using a trackball apparatus (Figure 2). The trackball is a spherical apparatus placed inside an acoustically isolated chamber to limit surrounding noise and other observer disturbances. The trackball is made up of 4 main components: a polystyrene ball floating on a cushion of air, a cowling that maintains airflow and houses the polystyrene ball, an electronic sensor to detect movement of the ball, and a software program that collects sensor readings and calculates movement of the ball (Pacheco et al., 2013). The chamber was illuminated using low intensity LED light sources with a red-light filter which filters out wavelengths sensitive to visual receptor cells of Orthoptera (Briscoe & Chittka, 2000). Two speakers were mounted to both the left and right side of the chamber to play the artificial mate calls. A GoPro HERO4 Silver camera was also mounted within the chamber to allow for trials to be monitored remotely (Figure 2).

Upon phonotaxis trials, I took each female from her individual container and placed her on top of the trackball. I secured the female by attaching the elastic on her pronotum to a tiny fishhook that was attached to a telescoping arm and centered above the trackball. This arm and hook apparatus allowed the female to turn freely, move up and down when she walked, and her walking allowed the Styrofoam ball to rotate with little to no resistance. The telescoping arm was adjusted to ensure it was not causing tension on the female thereby affecting her ability to walk naturally on the trackball. Once placed on the trackball, each female underwent their phonotaxis trial.
Each female’s phonotaxis trial was initiated with a 1-minute acclimatization period. I then played a 1 min average call (the 0 SD call, represented by the spectral and temporal properties shown in Table 2). The speaker side that played the first call was selected at random. Thereafter, I played all 15 artificial call signals (ranging from -3.5 SD to + 3.5 SD) in a random order. Each call signal was separated by 30 s of silence, and the side the speaker played from alternated. I played the 0 SD artificial call signal again at the end of the experiment. As a result, each female experienced 17 artificial call signals, three x 0 SD calls (beginning, end, and random) and 1 of each of the other 14 artificial call signals. Between the acclimatization time, the 17 artificial call signals, and the silent periods, each female was on the trackball and being analyzed for phonotaxis behaviour for ~25 minutes in total (sensu Ferguson, 2018). When a call was played, I quantified female movement on the trackball and then calculated the distance she moved directly towards the speaker that was playing (detailed below). After completion of their trial, if females were also participating in open arena trials, I removed each female’s elastic from the pronotum and placed the female back in her individual container. If females were not also participating in open arena trials they were euthanized via freezing for stored for body size and fecundity analyses later on.

I used an ANOVA to determine whether distance moved directly toward the speaker differed between the beginning, random, and end of the experiment playing of the 0 SD call. Responses to the population average call did not statistically differ when played at these different times confirming that females remained responsive throughout the entire trial.

The trackball quantifies total path length (total distance travelled) and net displacement (the distance travelled in a straight line from when the call started to be played and 1 minute later, when the call stopped playing, and the angle moved compared to the location of the
speaker). This net displacement distance and angle enabled me to calculate net vector score (total
distance travelled directly towards the speaker) using trigonometry for a right-angled triangle.
Throughout, for statistical analyses I use net vector score. I note, however, that I also ran these
results using total path length and had the same general results.

Figure 2. Graphic representation of trackball setup.
2.5 Does rearing diet influence female mating behaviour?

To test whether female mating behaviour (instead of just phonotaxis) was influenced by developmental diets, I quantified female mating behaviour with a male in an open arena. My sample size was 51 females fed the high-protein diet and 81 females fed the high-carbohydrate diet, resulting in a total of 132 trials. The sample size was biased towards high-carbohydrate because many of the individuals being fed the high-protein diet died prior to the open arena trials. Further, 2 trials were omitted from analyses due to technical errors, resulting in a total sample size of 130. I placed 1 experimental female in the open arena with 1 male (fed the standard diet) and then observed their mating behaviours (see section 2.5.1).

2.5.1 Open arena testing

I conducted all mating behaviour trials in a rectangular arena (L x W x H = 31.5cm x 16.3 cm x 21.5cm) made of clear acrylic panels. I secured white paper to the outside of the walls to ensure individuals could not see outside the arena as well as to minimize any reflections. The arena was placed inside an acoustic isolation chamber as outlined in Section 2.4.2. including both the LED lights and the GoPro to digitally record the trials for later analysis. The weight of both individuals was recorded prior to entering the arena. Clear plastic cups were turned upside down to enclose trial participants and allow for an acclimatization period of 60s prior to the beginning of the trial. The side that sexes were placed on at the beginning of this trial were randomly chosen to account for side bias. After this acclimatization period, the cups were pulled out of the arena and the trial began. This trial ran for 25 minutes after the acclimatization period. The following behaviours were collected during these open arena trials including: time the trial began (when the cups were lifted), antennation by 1 or both sexes, duration of male calling,
whether the female mounted the male, how long mounting occurred for, and aggression between 1 or both sexes. All parameters were extracted using video recordings from the GoPro and the behavioural analysis software BORIS (Friard & Gamba, 2016). After the male and female were removed, I cleaned the arena using 95% ethyl alcohol to remove any pheromonal and chemical signals. Trial sand at the bottom of the arena was also mixed after each trial and replaced weekly.

2.6 Fecundity and Body Size Analysis

All individuals were euthanized by freezing to allow for fecundity (females only) and body size analysis. Individuals were photographed using a Zeiss Stemi 305 Stereo Microscope and an Axiocam 208 camera. Size measurements (body and egg size) were conducted using the software ImageJ (Rasband, 2018). Using principal component analysis, overall measure of body size (PC1 Size) was calculated using the following measurements: head width (maximal distance from eye to eye), pronotum width (transverse distance across pronotum), and pronotum length (sagittal distance down pronotum) explaining 94.9% of the variation in these body measures (eigenvalue = 2.85; eigenvector loadings for head width, pronotum length, pronotum width: all between 0.57 to 0.58).

Females were dissected to isolate their eggs to quantify total number of eggs as well as the average size of 10 randomly selected eggs to analyze any correlation to diet.

2.7 Data Processing and Statistical Analysis

All computational and statistical analysis were conducted using JMP PRO 16.2. General linear models (LM) and nominal logistic regressions were fit using Akaike’s Information Criterion (AICc) with the following independent variables: diet, start weight, diet * start weight
(in the full saturated model). I included start weight to account for variation associated with body size and/or condition prior to the start of the experiment; diet was included as it was the main experimental treatment; I included the interaction between start weight and diet as smaller females may have responded differently to the diets than larger females. Other independent variables were occasionally also included in the full model, as specified below. These models enabled me to determine the factors influencing life history traits I measured including: development time, body size and weight at adulthood, juvenile survival, survival throughout the entire experiment, egg production, parameters related to female response to male mate attraction calls, and parameters related to mating behaviour when the female interacts directly with the male (discussed in sections 2.4, 2.5, and 2.6). For each dependent variable, I selected the best-fitting model from the candidate model set. Given that my experimental treatment was diet, I forced diet to remain in all models as a fixed factor independent variable. In all cases, the best-fitting model had an AICc value at least 2 below all other models in the candidate model set. Tables 3-7 outline best-fitting models used for all dependent traits.

I used nominal logistic regression to quantify female survival from wing bud to adulthood (yes/no) (N = 217 females), and to quantify female survival from wing bud to the conclusion of the experiment (whether they survived until testing in behavioural trials). For all individuals that survived until adulthood (N = 194), I used LMs to quantify development time, weight at adulthood, size at adulthood, and average daily food eaten (mg). I ran nominal logistic regressions to determine what influenced whether females produced eggs (yes/no; N = 194). For the subset of females that produced eggs (N = 156), I ran LMs to determine the factors influencing the number of eggs produced – the full models for these dependent variables also included average daily food eaten (mg).
For females that participated in the speaker based phonotaxis trials on the track-ball (N = 81), I ran a mixed model. To explore the factors influencing net vector score (cm), I forced chirp rate and diet type to remain as independent variables in the model. I included individual identification (ID) as a random effect to account for the fact that each female experienced multiple trials (one trial for each of the different chirp rates). For females that interacted with adult males in the open arena mating trials (N = 132), I ran nominal logistic regressions to determine what influenced: i) whether a female mounted the male (yes/no), and ii) whether a female was aggressive (yes/no). I also ran LMs to explore what influenced how long it took the female to mount [both latency from the start of the open arena trial to mounting (sec), and latency from first antennation to mounting (sec)]. The full models for these dependent variables also included male weight as a further covariate.
3. Results

3.1 Diet Choice: Preliminary Experiment

When given a choice to self-select their nutrient consumption the average nutrient consumption of females fell within the range of 1:4 - 4:1 P:C (Figure 3). The average ratio consumed was 1.14:1 P:C, a ratio that was significantly different than 1:1 (P = 0.0091). Therefore, when given the choice, females ate slightly more protein than carbohydrates on average/

![Histogram of P:C ratio consumed](image)

**Figure 3.** Average P:C ratio consumed by females in preliminary diet choice experiment (Mean = 1.14 ± 0.403 SD).
3.2 Survival

Of the 217 females used in this study, 194 survived to adulthood. Diet did not affect whether females survived to adulthood. However, weight at the beginning of the experiment influenced survival to adulthood (Table 3; \( P = 0.0005 \)): females that were lighter at the beginning of the experiment (either smaller or in poorer condition) were more likely to die before they reached adulthood than females that were heavier at the start of the experiment (Figure 4).

Diet influenced whether the females survived until behavioural testing (days 10-16 post imaginal moult). Specifically, 20% of the females reared on the high-carbohydrate diet did not survive until behavioural testing compared to 47% of females reared on the high-protein diet (Table 3; \( P < 0.0001 \)). Survival to behavioural testing was also influenced by start weight and an interaction between start weight and diet. Specifically, females fed the high carbohydrate diet were more likely to die during the experiment if they were smaller at the beginning of the experiment than if they were larger at the start of the experiment (Table 3; \( P = 0.0018 \)). Conversely, there was no effect of start weight on survival to behavioural testing for females that were fed the protein diet (Table 3; Figure 5).
Table 3. Nominal logistic regression output examining factors influencing female life-history traits including survival to adulthood, survival in experiment, and whether females produced eggs. Significant terms are bolded.

<table>
<thead>
<tr>
<th>Model</th>
<th>Fixed Effects</th>
<th>Estimate</th>
<th>Std Error</th>
<th>Chi-square</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Died Before Adulthood (Yes/No)</td>
<td>Intercept</td>
<td>-0.385</td>
<td>0.685</td>
<td>0.32</td>
<td>0.5738</td>
</tr>
<tr>
<td>$r^2 = 0.1015; N = 217$</td>
<td>Start Weight (g)</td>
<td>23.34</td>
<td>6.728</td>
<td>12.84</td>
<td>0.0005</td>
</tr>
<tr>
<td>Saturated Model:</td>
<td>Diet Type</td>
<td>0.0065</td>
<td>0.229</td>
<td>0</td>
<td>0.9775</td>
</tr>
<tr>
<td>Died Before Adulthood = Start Weight + Diet Type + Start Weight*Diet Type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Survival in Experiment (Yes/No)</td>
<td>Intercept</td>
<td>1.382</td>
<td>0.582</td>
<td>5.65</td>
<td>0.0175</td>
</tr>
<tr>
<td>$r^2 = 0.158; N = 200$</td>
<td>Diet Type</td>
<td>-0.896</td>
<td>0.209</td>
<td>18.46</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Saturated model:</td>
<td>Start Weight (g)</td>
<td>-19.6</td>
<td>5.347</td>
<td>13.44</td>
<td>0.0002</td>
</tr>
<tr>
<td>Survival in Experiment = Start Weight + Diet Type + Start Weight*Diet Type</td>
<td>Diet Type *Start Weight (g)</td>
<td>-16.71</td>
<td>5.347</td>
<td>9.77</td>
<td>0.0018</td>
</tr>
<tr>
<td>Produced Eggs (Yes/No)</td>
<td>Intercept</td>
<td>-0.499</td>
<td>0.641</td>
<td>0.61</td>
<td>0.436</td>
</tr>
<tr>
<td>$r^2 = 0.061; N = 194$</td>
<td>Diet Type</td>
<td>0.158</td>
<td>0.22</td>
<td>0.51</td>
<td>0.475</td>
</tr>
<tr>
<td>Saturated model:</td>
<td>Average Daily Food Eaten (mg)</td>
<td>0.112</td>
<td>0.038</td>
<td>8.72</td>
<td>0.0032</td>
</tr>
</tbody>
</table>
Figure 4. Influence of start weight on female survival to adulthood. Purple (triangle) markers represent females reared on the high-protein diet; orange (circle) markers represent females reared on the high-carbohydrate diet. The vertical black lines represent the line of best fit and grey rectangles represent the 95% confidence intervals.
Figure 5. Influence of start weight on female survival to behavioural testing. Purple (triangle) markers represent females reared on the high-protein diet; orange (circle) markers represent females reared on the high-carbohydrate diet. The vertical lines represent the lines of best fit, the lighter coloured rectangles represent the 95% confidence intervals.
3.3 Fecundity

While diet did not influence whether females produced eggs or not (yes/no), the average amount of food eaten per day (regardless of diet type) did (Table 3; \( P = 0.0032 \)). Females that consumed more food per day were more likely to produce eggs than females consuming less food per day (Table 3; Figure 6).

For the females that produced eggs, there was a significant effect of diet on the number of eggs produced (Table 4; \( P = 0.0281 \)). Females fed the high-protein diet produced more eggs than females fed the high-carbohydrate diet. Further, the number of eggs produced was also influenced by an interaction between daily food consumed and diet (Table 4; \( P = 0.0028 \)). Specifically, females fed the high-protein diet produced more eggs when they ate more food per day compared to females that consumed less food per day. Interestingly, this relationship between average daily food eaten and egg production did not hold for females reared on the high-carbohydrate diet (Figure 7).
Figure 6. Influence of average daily food eaten on whether females produced eggs. Purple (triangle) markers represent females reared on the high-protein diet; orange (circle) markers represent females reared on the high-carbohydrate diet. The vertical black lines represent the line of best fit and grey rectangles represent the 95% confidence intervals.
Figure 7. Influence of average daily food eaten on number of eggs produced. Purple (triangle) markers represent females reared on the high-protein diet and the purple line represents the line of best fit (significantly > 0); orange (circle) markers represent females reared on the high-carbohydrate diet and the orange line represents the line of best fit (significantly = 0).
Table 4. Linear model output for life history traits associated with development, weight, food consumption, and fecundity. Significant terms are bolded.

<table>
<thead>
<tr>
<th>Model</th>
<th>Fixed Effects</th>
<th>Estimate</th>
<th>Std Error</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Development Time</td>
<td>Intercept</td>
<td>15.731</td>
<td>0.705</td>
<td>22.33</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Saturated model: Development Time ~ Start Weight + Diet Type + Start Weight*Diet Type</td>
<td>Start Weight (g)</td>
<td>-24.424</td>
<td>5.277</td>
<td>-4.63</td>
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<tr>
<td></td>
<td>Diet Type</td>
<td>1.216</td>
<td>0.228</td>
<td>5.33</td>
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<tr>
<td>Adult Weight</td>
<td>Intercept</td>
<td>0.116</td>
<td>0.0122</td>
<td>9.56</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Saturated model: Adult Weight ~ Start Weight + Diet Type + Start Weight*Diet Type</td>
<td>Start Weight (g)</td>
<td>1.381</td>
<td>0.0909</td>
<td>15.18</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Diet Type</td>
<td>-0.0379</td>
<td>0.00394</td>
<td>-9.64</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Average Daily Food Eaten</td>
<td>Intercept</td>
<td>8.519</td>
<td>1.159</td>
<td>7.35</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Saturated model: Average Daily Food Eaten ~ Start Weight + Diet Type + Start Weight*Diet Type</td>
<td>Diet Type</td>
<td>-3.489</td>
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<td>-9.31</td>
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<tr>
<td></td>
<td>Start Weight (g)</td>
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<td>8.681</td>
<td>8.92</td>
<td>&lt;.0001</td>
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<tr>
<td>Total Egg Number</td>
<td>Intercept</td>
<td>22.992</td>
<td>11.913</td>
<td>1.93</td>
<td>0.0555</td>
</tr>
<tr>
<td>Saturated model: Total Egg Number ~ Start Weight + Diet Type + Start Weight<em>Diet Type + Average Daily Food Eaten + Diet Type</em>Average Daily Food Eaten</td>
<td>Diet Type</td>
<td>-8.985</td>
<td>4.0539</td>
<td>-2.22</td>
<td>0.0281</td>
</tr>
<tr>
<td></td>
<td>Average Daily Food Eaten (mg)</td>
<td>1.405</td>
<td>0.588</td>
<td>2.39</td>
<td>0.0180</td>
</tr>
<tr>
<td></td>
<td>Diet Type*Average Daily Food Eaten</td>
<td>-1.786</td>
<td>0.588</td>
<td>-3.04</td>
<td>0.0028</td>
</tr>
<tr>
<td>PC1Size</td>
<td>Intercept</td>
<td>-4.299</td>
<td>0.2387</td>
<td>-18.02</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Saturated model: PC1Size ~ Start Weight + Diet Type + Start Weight<em>Diet Type + Average Daily Food Eaten + Diet Type</em>Average Daily Food Eaten + Start Weight*Average Daily Food Eaten</td>
<td>Start Weight (g)</td>
<td>23.414</td>
<td>1.861</td>
<td>12.58</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Diet Type</td>
<td>-9.3172</td>
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<td>-3.94</td>
<td>0.0001</td>
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<tr>
<td></td>
<td>Average Daily Food Eaten (mg)</td>
<td>0.07714</td>
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<td>5.78</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Start Weight (g)*Average Daily Food Eaten (mg)</td>
<td>-9.5166</td>
<td>0.2146</td>
<td>-2.41</td>
<td>0.0171</td>
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</tbody>
</table>
3.4 Development

Development time was significantly influenced by diet type (Table 4; \( P < 0.0001 \)). Females reared on the high-protein diet developed into adults significantly faster (2.5 days on average) than females reared on the high-carbohydrate diet. Development time was also influenced by start weight (Table 4; \( P < 0.0001 \)) as heavier females at the start of the experiment reached adulthood faster than lighter females (Figure 8).

Adult weight was influenced by rearing diet (Table 4; \( P < 0.0001 \)). Females reared on the high-protein diet were 30% heavier, on average, than females fed the high-carbohydrate diet (e.g., average weight = 329 g vs 253 g). Adult weight was also influenced by start weight (Table 4; \( P < 0.0001 \)) whereby heavier females at the start of the experiment were heavier at adulthood and lighter females at the start of the experiment were lighter at adulthood (Figure 9). Similarly, females reared on the high-protein diet had significantly larger overall body size (PC1Size), on average, than females fed the high-carbohydrate diet (Table 4; Figure 11).

Rearing diet also significantly influenced how much food females consumed (Table 4; \( P < 0.0001 \)). Females reared on the high protein diet consumed 47% more food per day, on average, than females fed the high carbohydrate diet (e.g., average food consumed per day = 21.8 mg vs 14.8 mg, respectively). Average daily food consumed was also influenced by start weight (Table 4; \( P < 0.0001 \)). Females that were heavier at the start of the experiment consumed more food per day, on average, than females that were lighter at the start of the experiment (Figure 10; Table 4; \( P < 0.0001 \)). Females that consumed more food per day also grew larger than females that consumed less food per day (Table 4; Figure 12).
Figure 8. Influence of start weight on female development time. Purple (triangle) markers represent females reared on the high-protein diet; orange (circle) markers represent females reared on the high-carbohydrate diet and their respective lines of best fit.
Figure 9. Influence of start weight on female adult weight. Purple (triangle) markers represent females reared on the high-protein diet; orange (circle) markers represent females reared on the high-carbohydrate diet and their respective lines of best fit.
Figure 10. Influence of start weight on average daily food eaten. Purple (triangle) markers represent females reared on the high-protein diet; orange (circle) markers represent females reared on the high-carbohydrate diet and their respective lines of best fit.
**Figure 11.** Marginal effects plot with 95% confidence intervals showing how diet type influences overall female body size (PC1Size).
Figure 12. Marginal effects plot with 95% confidence intervals showing how start weight and average daily food eaten (mg) (low vs. high consumption) influences overall body size (PC1Size). Purple (triangle) markers represent females reared on the high-protein diet; orange (circle) markers represent females reared on the high-carbohydrate diet. Line of best fit and 95% confidence intervals are shown in black and grey, respectively.
3.5 Phonotaxis Trials

Females were more attracted to higher chirp rates (Table 5, P < 0.0001). Rearing diet significantly influenced females’ responsiveness to chirp rate (Table 5; P < 0.0001). Specifically, females fed the high-protein diet were more responsive (higher net vector scores) with increasing chirp rates than females fed the high-carbohydrate diet (Figures 13 and 14). As a result, females fed the high-protein diet had steeper preference functions than females fed the high-carbohydrate diet. Start weight also significantly influenced females’ response to the artificial calls (Table 5; P < 0.0001). Heavier females at the beginning of the experiment were more responsive (steeper preference function) compared to lighter females (Figure 15).

Table 5. Mixed model output for female response to artificial calls on the track ball that differed in chirp rate. Significant terms are bolded. The random effect (ID) was variance component was 0.0000 +/- 0.0001 SE, P = 0.8193, and accounted for 0.000% of the variation in the overall model.

<table>
<thead>
<tr>
<th>Model</th>
<th>Fixed Effects</th>
<th>Estimate</th>
<th>Std Error</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
</table>
| Net Vector Score (cm)  
$r^2 = 0.2457$; N = 81 | Intercept     | -1.544   | 0.869     | -1.78 | 0.076 |
| Diet Type | -1.834 | 0.282 | -6.50 | <.0001 |
| Saturated model:  
Net Vector Score ~ Start Weight + Diet Type + Start Weight*Diet Type + Chirp Rate + Diet Type*Chirp Rate + Start Weight*Chirp Rate + Diet Type*Start Weight*Diet Type + (1 | ID) | Start Weight (g) | 62.559 | 6.547 | 9.56 | <.0001 |
| | Chirp Rate (stdev) | 1.829 | 0.129 | 14.14 | <.0001 |
| | Diet Type*Chirp Rate (stdev) | -0.423 | 0.13 | -3.24 | 0.0012 |
| | Start Weight (g)*Chirp Rate (Stdev) | 14.925 | 3.006 | 4.96 | <.0001 |
Figure 13. Influence of chirp rate on net vector score. Purple (triangle) markers represent females reared on the high-protein diet; orange (circle) markers represent females reared on the high-carbohydrate diet and their respective lines of best fit.
Figure 14. Influence of chirp rate on net vector score highlighting variation between individuals. Purple lines represent each individual female reared on the high-protein diet; orange lines represent each individual female reared on the high-carbohydrate diet and the populations respective lines of best fit.
Figure 15. Marginal effects plot with 95% confidence intervals showing how start weight (small vs. large) and chirp rate (stdev) influences net vector score (cm). Purple markers represent females reared on the high-protein diet; orange markers represent females reared on the high-carbohydrate diet. Line of best fit and 95% confidence intervals are shown in black and grey, respectively.
3.6 Mating Behaviour

Whether or not a female mounted the male was influenced by an interaction between diet and start weight (Table 6; \( P = 0.0432 \)). Specifically, for females fed the high-protein diet, heavier females (at the start of the experiment) were more likely to mate than lighter females. However, for females fed the high-carbohydrate diet, start weight played a less significant role in influencing whether they mated (Figure 16). Male weight also influenced whether the female mounted (Table 6; \( P = 0.0079 \)) as females were more likely to mount heavier males than lighter males (Figure 17).

Latency to mount (from the start of trial and from the time of first antennation) was influenced by an interaction between diet type and start weight (Table 7; \( P = 0.0381 \) and \( P = 0.0058 \), respectively). Specifically, females that were heavier at the start of the experiment and fed the high-protein diet were quicker to mount a male than females that were lighter at the start of the experiment; conversely, start weight did not influence mounting latency in females fed the high-carbohydrate diet (Figure 19 and 20, respectively). Male weight also influenced latency to mount from the time of first antennation (Table 7; \( P = 0.0416 \)): females mounted heavier males quicker than lighter males (Figure 21).

Female aggression during the mating trials was influenced by both diet type and male weight (Table 6; \( P = 0.0205 \) and \( P = 0.0228 \), respectively). Specifically, 16/20 females that were aggressive towards a male in the open arena were those reared on the high-carbohydrate diet (Table 6; \( P = 0.0205 \)), and females were more aggressive towards lighter males (Table 6; \( P = 0.0228 \); Figure 18).
Table 6. Nominal logistic regression outputs for female behaviour in the open arena – specifically whether or not they mounted the male and whether or not they were aggressive to the male. Significant terms are bolded.

<table>
<thead>
<tr>
<th>Model</th>
<th>Fixed Effects</th>
<th>Estimate</th>
<th>Std Error</th>
<th>Chi-square</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mount (Yes/No)</td>
<td>Intercept</td>
<td>-4.659</td>
<td>1.149</td>
<td>16.43</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Diet Type</td>
<td>0.1837</td>
<td>0.2139</td>
<td>0.74</td>
<td>0.39</td>
</tr>
<tr>
<td>Saturated model:</td>
<td>Start Weight (g)</td>
<td>13.9</td>
<td>5.707</td>
<td>5.93</td>
<td>0.0149</td>
</tr>
<tr>
<td>Mount ~ Start Weight + Diet Type + Start Weight*Diet Type + Male Weight</td>
<td>Diet Type*Start Weight (g)</td>
<td>-11.81</td>
<td>5.842</td>
<td>4.09</td>
<td>0.0432</td>
</tr>
<tr>
<td></td>
<td>Male Weight (g)</td>
<td>6.155</td>
<td>2.318</td>
<td>7.05</td>
<td>0.0079</td>
</tr>
<tr>
<td>Aggression (Yes/No)</td>
<td>Intercept</td>
<td>0.979</td>
<td>1.151</td>
<td>0.72</td>
<td>0.395</td>
</tr>
<tr>
<td>Saturated model:</td>
<td>Diet Type</td>
<td>0.73</td>
<td>0.315</td>
<td>5.37</td>
<td>0.0205</td>
</tr>
<tr>
<td>Aggression ~ Start Weight + Diet Type + Start Weight<em>Diet Type + Male Weight + Total Duration Calling + Male Weight</em>Total Duration Calling</td>
<td>Male Weight (g)</td>
<td>-8.31</td>
<td>3.651</td>
<td>5.18</td>
<td>0.0228</td>
</tr>
<tr>
<td></td>
<td>Total Duration Calling (sec)</td>
<td>-0.00323</td>
<td>0.002</td>
<td>2.22</td>
<td>0.136</td>
</tr>
<tr>
<td></td>
<td>Male Weight (g)</td>
<td>6.155</td>
<td>2.318</td>
<td>7.05</td>
<td>0.0079</td>
</tr>
</tbody>
</table>
**Figure 16.** Influence of start weight on whether a female mounted a male during open arena trials. Purple (triangle) markers represent females reared on the high-protein diet; orange (circle) markers represent females reared on the high-carbohydrate diet and their respective line of best fit and 95% confidence intervals.
Figure 17. Influence of male weight on whether a female mounted a male during open arena trials. Purple (triangle) markers represent females reared on the high-protein diet; orange (circle) markers represent females reared on the high-carbohydrate diet. The vertical black lines represent the line of best fit and grey rectangles represent the 95% confidence intervals.
Figure 18. Influence of male weight on whether a female was aggressive towards the male during open arena trials. Purple (triangle) markers represent females reared on the high-protein diet; orange (circle) markers represent females reared on the high-carbohydrate diet. The vertical black lines represent the line of best fit and grey rectangles represent the 95% confidence intervals.
Table 7. Linear model outputs for females in the open arena interacting directly with males.

Specifically, their latency to mount from start of the trial and after first antennation. Significant terms are bolded.

<table>
<thead>
<tr>
<th>Model</th>
<th>Fixed Effects</th>
<th>Estimate</th>
<th>Std Error</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start to Mount Latency</td>
<td>Intercept</td>
<td>883.337</td>
<td>397.934</td>
<td>2.14</td>
<td>0.031*</td>
</tr>
<tr>
<td></td>
<td>Diet Type</td>
<td>123.195</td>
<td>81.402</td>
<td>1.52</td>
<td>0.135</td>
</tr>
<tr>
<td>Saturated model:</td>
<td>Start Weight (g)</td>
<td>-4544.508</td>
<td>2270.014</td>
<td>-2.00</td>
<td>0.051</td>
</tr>
<tr>
<td>Start to Mount Latency = Start Weight +</td>
<td>Diet Type*Start Weight (g)</td>
<td>5405.389</td>
<td>2233.292</td>
<td>2.42</td>
<td>0.021*</td>
</tr>
<tr>
<td>Diet Type + Start Weight*Start Type +</td>
<td>Male Weight (g)</td>
<td>1249.273</td>
<td>790.162</td>
<td>1.58</td>
<td>0.121</td>
</tr>
<tr>
<td>Male Weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First Antennation to Mount Latency</td>
<td>Intercept</td>
<td>550.967</td>
<td>345.237</td>
<td>1.6</td>
<td>0.111</td>
</tr>
<tr>
<td></td>
<td>Diet Type</td>
<td>78.117</td>
<td>70.622</td>
<td>1.11</td>
<td>0.271</td>
</tr>
<tr>
<td>Saturated model:</td>
<td>Start Weight (g)</td>
<td>-3933.098</td>
<td>1969.399</td>
<td>-2.00</td>
<td>0.051</td>
</tr>
<tr>
<td>First Antennation to Mount Latency =</td>
<td>Diet Type*Start Weight (g)</td>
<td>5643.786</td>
<td>1937.541</td>
<td>2.91</td>
<td>0.003*</td>
</tr>
<tr>
<td>Start Weight + Diet Type + Start</td>
<td>Male Weight (g)</td>
<td>1443.079</td>
<td>685.522</td>
<td>2.11</td>
<td>0.046*</td>
</tr>
<tr>
<td>Weight*Diet Type + Male Weight</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 19. Marginal effects plot with 95% confidence intervals showing how start weight influences latency to mount from start of the trial (start to mount latency (sec)). Purple (triangle) markers represent females reared on the high-protein diet; orange (circle) markers represent females reared on the high-carbohydrate diet with their respective line of best fit and 95% confidence intervals.
Figure 20. Marginal effects plot with 95% confidence intervals showing how start weight influences latency to mount after first antennation (latency antennate to mount (sec)). Purple (triangle) markers represent females reared on the high-protein diet; orange (circle) markers represent females reared on the high-carbohydrate diet with their respective line of best fit and 95% confidence intervals.
Figure 21. Marginal effects plot with 95% confidence intervals showing how male weight influences latency to mount after first antennation (latency antennate to mount (sec)). Purple (triangle) markers represent females reared on the high-protein diet; orange (circle) markers represent females reared on the high-carbohydrate diet. Line of best fit and 95% confidence interval shown in blue.
4. Discussion

Although 1g of protein and 1g of carbohydrate have equal metabolic energy, animals utilize these macromolecules differently. Carbohydrates are typically utilized as an energy source whereas proteins provide amino acids responsible for producing structural tissues, proteins, and enzymes (Clark et al., 2015). Using diets that differed in their protein to carbohydrate ratios (1:3 versus 3:1 P:C), I explored how macronutrient ratio and volume of food consumed during development and throughout adulthood impacted female life history traits and mating behaviour. I hypothesized that females reared on the high-protein diet would have shorter development times, larger body sizes and higher egg production due to the role of proteins in tissue development compared to carbohydrates. However, potential effects of diet on female mating behaviour remained unclear based on current literature. From my knowledge, my study is the first to examine female mating behaviour in crickets whereby the ratio and amount of protein and carbohydrate consumption was carefully monitored during development and into adulthood. My study therefore investigated the differing roles that macronutrients play in female *G. pennsylvanicus* life-history traits including: development time, body size, preference response to male mate attraction signalling, overall female mating behaviour and total egg production.

Females fed the holistic high-protein diet had shorter development times, were heavier at adulthood, had larger PC1Size scores, consumed twice as much food, produced significantly more eggs (especially with increasing food consumption), and were more responsive to artificial male mate attraction signalling than females fed the high-carbohydrate diet. Large juvenile females that were then fed the high-protein diet were also more likely to mount the male with shorter latency to mount than females that were smaller as juveniles. There were, however, costs associated with high-protein food consumption, given that nearly half of the females fed the
high-protein diet did not survive until testing (regardless of start weight) compared to only 20% of the females that died prior to testing when fed the high-carbohydrate diet.

Importantly and regardless of experimental diet, females that were lighter as juveniles were less likely to survive to adulthood, had longer development time, became smaller adults, consumed less food (on average), and were less responsive to artificial male mate attraction signalling. These start weight findings suggest that the quantity and quality of food consumed earlier in development is also important to a host of life-history traits. Below I discuss each of my findings, placing them into context with what is known about factors influencing mating behaviour.

Maklakov et al. (2008) determined that when given a choice, males consumed on average a 1:3 P:C ratio diet whereas females consumed slightly more protein than carbohydrates on average reflecting a sex-specific dietary preference. The results of our research support this finding as females also consumed slightly (but significantly) more protein, on average, than carbohydrates. This aligns with the notion that males utilize carbohydrates as an energy source to fuel their mate calling, whereas females utilize protein for growth and fecundity highlighting sex-specific reproductive differences in nutrient allocation (Maklakov et al., 2008).

The results support my hypothesis that females reared on the high-protein diet would minimize development time and maximize adult body size compared to females reared on the high-carbohydrate diet. These results also align with previous studies on the impacts of juvenile protein and carbohydrate consumption that also showed faster development time and larger body size at eclosion, though typically only focussing on males (Hunt et al., 2005; Idowu & Sonde, 2003; Kasumovic et al., 2011; Maklakov et al., 2008; Reifer et al., 2018; Roeder & Behmer, 2014).
Kasumovic et al. (2011) suggested that trait expression in adulthood may be affected by the amount of juvenile food consumed and subsequently the amount of resources available to allocate to different traits. In this study, females reared on the high-protein diet consumed significantly more food, on average, than females reared on the high-carbohydrate diet. These females also had shorter development times and larger body sizes, further providing evidence for the allocation of protein towards these fitness conferring traits.

Interestingly, I found no effect of diet on survival to adulthood. This may be explained by individuals not being fed their assigned diet for long enough to influence survival. Survival to adulthood was, however, influenced by initial weight. Small individuals may have already been at a survival disadvantage due to lower condition when compared to larger individuals. Further, they may not have had the energy stores available to handle an unbalanced diet, regardless of whether it was more heavily balanced towards proteins or carbohydrates.

Though I did not quantify life-time survival, I investigated the factors influencing survival until test day (10-16 days after adult eclosion). Nearly half of females fed the high-protein diet did not survive until test day compared to less than 1/5th of females fed the high-carbohydrate diet. These results align with other research (e.g., Harrison, 2018; Maklakov et al., 2008; Reifer et al., 2018; Simpson & Raubenheimer, 2009) showing that elevated protein availability negatively impacts survival whereas elevated carbohydrate availability positively impacts survival. Survival to test day was also influenced by an interaction between juvenile weight and diet. Females that were lighter and fed a high-carbohydrate diet were less likely to survive to testing compared to females that were heavier and fed a high-carbohydrate diet. In contrast, start weight played much less of a role in survival to testing when females were reared on a high-protein diet. These findings indicate that small individuals are vulnerable and that a
diet lacking adequate protein may not allow for critical growth stages to occur for these lighter individuals to become healthy adults. Whereas individuals consuming adequate protein are potentially receiving enough protein to overcome poorer (lighter) condition. Though, their long-term survival is still affected as these resources appear to continue to get used for growth and reproduction at the sacrifice of a longer lifespan. In my study, food dishes were switched weekly, therefore inhibiting our ability to accurately detail food consumption as juveniles versus adults (if they became adults halfway through the week, for example). A future study should ensure food is replaced when individuals become adults to allow for more robust data of overall food consumption before and after adulthood (similar to Reifer et al., 2018) and therefore may provide more information to support these results. Furthermore, based on the effects start weight played in many of the results of this study it may be interesting for a future study to include (1) only individuals within a tighter range of start weights to observe the impacts of diet on life-history traits more directly and/or (2) start the experiment much earlier in development as early development appears to have long lasting effects on other life history traits.

Though rearing diet did not influence whether females produced eggs or not, for those that produced eggs, females reared on the high-protein diet produced significantly more eggs than females reared on the high-carbohydrate diet. Furthermore, the more food these females consumed, the more eggs they produced. Similarly, Maklakov et al. (2008), determined that females reared on high-carbohydrate diets also produce significantly fewer eggs. The Maklakov study however looked at egg laying across the females lifetime, and did not examine a high protein diet, but an equal 1:1 P:C. Nonetheless, they found that those reared on the 1:1 P:C diet produced significantly more eggs than those reared on the high carbohydrate diet. These results (as well as others, e.g., Simpson & Raubenheimer, 2009) combined with my own findings show
that a reproductive trade-off occurs with higher carbohydrate ratios and that by lowering this ratio then energetic allocation towards fecundity is favoured.

The attractiveness of potential mates, known as mating preferences, are influenced by an individual’s responsiveness and choosiness. Whereby responsiveness is the chooser’s (females in this case) overall sexual receptivity, or motivation to mate; and choosiness (akin to ‘tolerance’ in Kilmer et al., 2017) is essentially the willingness of the female to mate with a narrow or wide assortment of males. Kilmer et al., (2017) defines this at the width of the preference function one-third of the way down from the peak preference. A smaller width represents the individual is very choosy (has narrow ‘tolerance’) and vice versa. These traits are also not mutually exclusive and therefore not all females have the same mating preferences. These differences in preferences may be driven by individual variation in female condition (Harrison, 2018) and likely the reasoning for the vast amount of variation across females in their preference functions.

Female *G. pennsylvanicus* exhibited strong preferences towards male mate attraction signals played at high chirp rates (both our study and e.g., Ferguson, 2018; Harrison et al., 2013). There was, however, a holitic diet effect, as females fed the high-protein diet were more responsive and more choosy overall (had higher and steeper preference functions) than the females fed the high-carbohydrate diet. These results align with 2 of our predictions: that females reared on the high-protein diet become both more responsive due to having a shorter lifespan (and therefore more pressure to quickly select a mate); and become choosier due higher investment in egg production compared to females reared on the high-carbohydrate diet. For the females reared on the high-carbohydrate diet, they would be expected to become less responsive as they have a longer lifespan (and therefore less pressure to quickly select a mate); they would become less choosy due to lower investment in egg production. Though these were expected
potential results, they were still somewhat surprising. Since carbohydrates are sources of energy, I expected that females with higher energy reserves would have been more responsive (higher net vector score) and more choosy towards faster chirp rates due to being able to expend more energy in searching for a mate. However, given females fed the high-carbohydrate diet had significantly fewer eggs, they may not be as choosy due to lower investment in egg production. Females reared on the high-protein diet, with less energy reserves but more eggs, may be more responsive towards a male with a high chirp rate because they are highly fecund and have less energy reserves to allocate towards searching for a mate. Judge et al., (2014) found that females also showed increased preference for higher calling effort during phonotaxis trials. However, there was no influence of female condition (high-condition reflecting females reared on a protein-rich diet) and in contrast to my research these high-condition females also exhibited longer latency to choose. This highlights potential variation between mating behaviours when G. pennsylvanicus is reared on a holitic versus oligitic diet. The contrast between research findings also underscores the need for more studies comparing how holitic versus oligitic diets impact life-history traits and behaviour.

When examining female mating behaviour in the open arena, heavier juvenile females that were fed the high-protein diet were more likely to mount a male and quicker to mount the male than lighter juvenile females and females fed the high-carbohydrate diet. No other studies have examined the influence of nutrient ratios on female mating behaviour in crickets. Females on the high-protein diet may be employing a ‘live-fast die-young’ method whereby they allocate protein towards growth and egg production at the cost of a longer lifespan thereby becoming more responsive (quicker latency to mount) due to having ample eggs that the pressure to ‘use them wisely’ isn’t as strong compared to those on the high-carbohydrate diet. Conversely,
females on the high-carbohydrate diet allocate their energetic stores to increasing lifespan at the consequence of growth and egg production. Due to this, females reared on the high-carbohydrate diet appear to be less responsive when selecting a mate likely being 1) they have the energetic stores to use in assessing a mate and 2) with such few eggs there is greater pressure to select a mate and provide her limited offspring with the best genes possible. Females were also significantly quicker to mount larger males than smaller males likely because larger males reflect their being in better condition (Harrison et al., 2013).

Regardless of diet, females were more aggressive towards smaller males than larger males. Importantly, females reared on the high-carbohydrate diet were also significantly more aggressive towards males than females reared on the high-protein diet. Adam & Hoy, (1995) examined agonistic behaviour in *Gryllus bimaculatus* and determined that males were more likely to exhibit aggressive behaviour than females. Interestingly, I did not observe males exhibiting aggressive behaviour towards the female, only vice versa, suggesting species level differences in male aggressive behaviour (Bertram & Rook, 2012). Adamo & Hoy (1995) showed that females initiated agonistic behaviour more often when they experienced food deprivation and competed for a food source. Adamo & Hoy (1995) determined that when resources were plentiful, *G. bimacilatus* females were less likely to be aggressive than when resources were scarce. In contrary, the females used in my study were not food deprived but exhibited significant aggressive behaviour towards males, particularly those fed the high-carbohydrate diet. All individuals utilized in the experiment were virgins so this aggressive behaviour could possibly be due to their lack of experience, but this would not explain the elevated aggression in females fed the high carbohydrate diet. Females fed the high carbohydrate diets may have been so protein limited (even though food was unlimited) that they were more
likely to be aggressive towards males, seeing them as a potential food source. In support of this hypothesis, is one anecdotal finding. In one trial a female (reared on the high-carbohydrate diet) aggressively latched on to a male and exhibited cannibalistic behaviour, eating the male’s exoskeleton of his abdomen for over 12 minutes and exposing the organs within.

Overall, my study shows that there is an inability to maximize all life-history traits on a single diet resulting in life-history trait trade-offs between reproduction and survival whereby reproduction is maximized on a high-protein diet and survival is maximized on a high-carbohydrate diet. My study provides initial evidence suggesting that the availability of dietary protein and carbohydrates does indeed influence female mating behaviour in *G. pennsylvanicus*. 
References Cited


Ferguson, G. L. (2018). How developmental and behavioural plasticity in the fall field cricket is influenced by the acoustic social environment and anthropogenic noise.


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