

**Are you what you eat? How protein and carbohydrate availability during  
development influence cricket cognition and other fitness conferring traits in  
adulthood**

by

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A thesis submitted to the Faculty of Graduate and Postdoctoral Affairs in partial  
fulfillment of the requirements for the degree of

Master of Science

in

Biology

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## **Abstract**

The conditions experienced during development may influence adult cognition and fitness, especially when conditions are stressful. To test the developmental stress hypothesis, I manipulated Texas field crickets (*Gryllus texensis*) juvenile diet and observed its effects on cognition (Chapter 1) and other fitness-related traits; I also examined whether diet influenced trade-offs between traits (Chapter 2). Individuals improved their cognition scores with training, but not during the test trial, suggesting developmental diets were stressful, hindering spatial cognition. Female fecundity was greatest in larger females that grew quickly. Males that developed quickly and performed well cognitively had a high probability of adopting a caller strategy; these males also exhibited high calling effort, suggesting high overall condition. For all others, adopting a caller strategy and exhibiting high calling effort only occurred with trade-offs, either via slower development, poorer performance in the cognitive task, or both. Diet did not predict cognitive and reproductive trade-offs.

## **Acknowledgements**

I am immeasurably grateful to my supervisor, Dr. Susan Bertram, for her support, guidance, and encouragement over the course of completing my Master's degree. I want to thank her for the huge involvement in my research, completion of my thesis, and meetings at Mike's Place that left my brain refreshed and my stomach full. Through the many times over these two years when life outside of academia made it seem too hard to continue, her unwavering reassurance, optimism, and belief in my abilities kept me going, and for that I am forever thankful.

I am also extremely grateful to the Biology Department at Carleton University for making my graduate experience such a memorable one. Questions were never left unanswered and I always felt supported, even while being in the lab every morning at 5a.m. To my thesis advisory committee members, Dr. Tom Sherratt and Dr. Julie Morand-Ferron, thank you for your thought-provoking questions and genuine advice over the last two years.

A ginormous thank you is needed for all members of the Bertram lab for their support with animal care, experimental assistance, and keeping me company during those incredibly early lab mornings, including Michelle Leveillee, Alyssa Froome, Wes Partington, Jory Curry, Jillian Sims, Angelica Ramos-Camacho, and Kathryn Hunt. I want to especially thank Mykell Reifer and Dr. Sarah Harrison for helping me get my experiment up and running and answering what probably felt like never-ending questions, Dr. Genevieve Ferguson for being there for brainstorming sessions, a constant support when 'thesis-ing' was overwhelming, and being my grad student inspiration, and Donovan Tremblay, for being my confidante through graduate school and someone who was always willing to help me with my frequent computer troubles- the laughs, board games, and beer helped me get through the last two years.

The completion of my master's thesis would not have been possible without the support of my family and friends. To my partner, Zack Partington, for the endless support, creative cricket experimental carpentry, homemade pasta, and personal cheerleading, I thank you to the ends of the earth. To my dad (Mike), sister (Alanna), AJ, and grandparents, I truly would have never gotten through this program without your love, willingness to listen to me stress over the phone, and gift cards to Loblaws (to fuel my brain power). To my mom, I wish you were here to have seen how hard I worked to make you proud, I hope I have. And to everyone else who knows a little too much about crickets because of me (Alicia and Lauren especially), you are troopers and I thank you for still wanting to be my friend.

Funding for my research presented in this thesis was provided through research grants awarded to my supervisor, Dr. Susan M. Bertram, from the Natural Sciences and Engineering Research Council of Canada Discovery Grants, the Canadian Foundation for Innovation, the Ontario Research Fund, and the Carleton University Research Fund. I was directly supported by Departmental Scholarships from Carleton University.

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## General Introduction

The conditions an animal experiences during critical periods of development can strongly influence their reproduction and survival, and thus their overall fitness (Bonaparte *et al.*, 2011). Cognitive ability is thought to be associated with fitness, as an animal's ability to acquire, store, and process information from their environment can have important repercussions for adult reproductive success and their ability to survive (Shettleworth, 2010; Henry and Stoner, 2011; Shaw *et al.*, 2015; Sugimachi *et al.*, 2016). Cognitive research on visual learning in mammals and birds has been extensive, but within invertebrates, cognition research has often been limited to honey bees (*Apis mellifera*; von Frisch, 1967; Matsumoto *et al.*, 2013; *Bombus terrestris audax*; Woodgate *et al.*, 2016) and fruit flies (*Drosophila melanogaster*; Ofstad *et al.*, 2011). To help combat this taxon specific focus for invertebrates, for my Master's research I investigated inter-individual variation in cognitive abilities using the Texas field cricket (*Gryllus texensis*) as my model organism.

While crickets have been extensively studied in a behavioural ecology setting, far less is known about their cognitive abilities. To my knowledge, there have been only four studies to date that have investigated field cricket spatial cognitive abilities; all suggest that they use spatial memory to return to important locations. Hale and Bailey (2004) showed that juvenile Australian raspy crickets (Orthoptera: *Gryllacrididae*) use visual cues to orient toward their burrow. Mangan and Webb (2009) revealed that Southern field crickets (*Gryllus bimaculatus*) can learn to return to a target in an experiment analogous to the Morris Water Maze used for rats, suggesting that their cognitive abilities may be matched to that of a larger, seemingly more sophisticated species. Collett *et al.* (2013) found that male Southern field crickets (*Gryllus bimaculatus*) use spatial memory to remember cool spots in an otherwise hot experimental arena.

Lastly, for my undergraduate honours thesis research, I showed that female Texas field crickets, *Gryllus texensis*, use their spatial cognitive abilities to remember the location of a food reward in a six-arm radial arm maze (RAM). Specifically, Texas field cricket females decreased the number of errors that they made, their distance travelled, and their latency to find the food reward across training trials (Doria et al., 2019). Females learned well enough that even after I removed the food reward and all olfactory cues, females could still accurately orient to the correct location (Doria et al., 2019). My finding suggests evidence for spatial cognitive abilities in Texas field crickets as female cricket learning was not solely contingent on olfactory cues. Intriguingly, some females learned quickly and maintained this learning over the course of the trials, others learned more slowly, and some did not seem to learn at all. I also found that learning was influenced by individual thigmotactic behaviour, which was repeatable within individuals but largely variable across them, further adding to the notion that large individual variation exists within this species' learning abilities. Together, these cricket studies all suggest that crickets are an appropriate model organism for investigating why individual variation persists in cognitive behaviour.

One hypothesis put forth to explain individual variation in cognitive behaviour is the developmental stress hypothesis. The developmental stress hypothesis proposes that stress experienced during development can influence cognitive ability in adulthood (Spencer and MacDougall-Shackleton, 2011). Much support for this hypothesis has come from imposing developmental dietary stressors on songbirds (*Melospiza georgiana*; Nowicki et al., 2002; Bonaparte et al., 2011) and rodents (Bloxham et al., 2014), and observing the adverse effects it has on adult song-learning and memory. To my knowledge, my first data chapter presents the first comprehensive study of the developmental stress hypothesis in invertebrates.

Given that developmental diet influences cognitive abilities across many taxa and crickets show evidence of cognition, developmental diet has the potential to impact cricket cognition. Research suggests that diet profoundly impacts cricket development. For example, a study by Whattam and Bertram (2011) showed that male Jamaican field crickets (*Gryllus assimilis*) fed unlimited amounts of food during development were larger and signalled longer and more loudly than their food restricted peers, providing evidence for the idea that developmental stress is something that can impact cricket fitness in adulthood. Diet quality can also be important, as cricket nutritional status ebbs and flows according to what macronutrients are important for development and reproduction (Fukumara and Nagata, 2017). For example, cricket development time is faster when they are fed a high protein diet (Hunt *et al.*, 2004; Kelly and Tawes, 2013; Harrison *et al.*, 2014). Proteinaceous diets over carbohydrate-biased diets also lead to larger body size at adulthood (Hunt *et al.*, 2004; Kelly *et al.*, 2013; Cordoba-Aguilar *et al.*, 2016; Han and Dingemanse, 2017; Harrison *et al.*, 2014). Dietary protein is especially important for female egg production (Maklakov *et al.*, 2008; Zajitschek *et al.*, 2012; Harrison *et al.*, 2014; Han and Dingemanse, 2017). Lastly, dietary carbohydrates are important for fueling energetically demanding activities such as male acoustic mate attraction signalling (Maklakov *et al.*, 2008; Bertram *et al.*, 2009; Thomson *et al.*, 2014). Clearly, food quality during development can profoundly impact adult life history traits. It is possible, therefore, that food quality during development may also impact adult cognitive abilities. It is feasible, then, that the developmental stress hypothesis may be supported in crickets, as nutritional stress during development may affect brain development, and subsequent development of cognitive ability important for fitness traits such as male signaling and female fecundity (Morand-Ferron *et al.*, 2016; Peters *et al.*, 2014). The first data chapter of my thesis therefore tests the developmental stress hypothesis.

Specifically, I fed developing crickets diets that were rich in either proteins or carbohydrates and then asked how these developmental diets influenced cricket spatial cognitive abilities.

For my second data chapter, I investigated how cricket spatial cognitive abilities traded off with other life history traits and together how they influenced fitness. Fisher (1930) proposed that an ideal life history which maximizes both reproduction and survival will be constrained by the costs of reproducing, leading to trade-offs. Early work on trade-offs suggested that a high reproductive rate led to shortened lifespan (Rose and Charlesworth, 1981; Partridge and Fowler, 1992), but this is not always the case. Reguera et al. (2004) compared virgin and mated stalk-eyed fly (*Cyrtodiopsis dalmanni*) females and found no relationship or trade-offs between longevity and lifetime fecundity. Buffenstein (2008) found that trade-offs between longevity and fecundity exist in dwarf hamsters (*Phodopus sungorus*), but not in mole voles (*Ellobius talpinus*). Research to date on cricket trade-offs has been largely consistent in that trade-offs do exist between fitness-related traits. A study of male decorated crickets (*Grylloides sigillatus*) highlighted a common trade-off between immunity and reproduction (Kerr *et al.*, 2010). An area of research that is currently gaining traction involves investigating the mechanisms by which animals decide what functions are more and less important to trade-off when dealing with unfavourable developmental conditions, such as poor nutrition (Bonaparte *et al.*, 2011). A study that manipulated dietary nutrients in fruit flies (*Drosophila melanogaster*) found that reproductive rate and lifespan can be maximized if dietary nutrients are optimal for the species (Grandison *et al.*, 2009). Conversely, female crickets tend to experience trade-offs with nutrient intake, as protein is ideal for egg production but not longevity (Clark *et al.*, 2013; Harrison *et al.*, 2014). It is clear that evidence for trade-offs across different species is non-uniform and is influenced by developmental diet. These findings led me to investigate how cognition fits into the equation. As my previous research showed that female Texas field crickets have the ability to

learn over consecutive training and test trials, and the above research suggests that many cricket species' physiology and fitness-related traits are influenced by diet, I investigated whether trade-offs occur between adult cognition and fitness conferring traits. Do individuals with higher cognitive abilities produce fewer eggs or signal less often because they are utilizing nutritional resources for brain power? Or do individuals with higher cognitive abilities simultaneously produce more eggs and signal more often for mates, because they are of overall superior quality? I address these questions in the second data chapter of my thesis.

*Chapter 1: How protein and carbohydrate availability during development  
influence cricket cognition in adulthood*

## Abstract

The conditions an animal experiences during critical developmental periods may influence their adult phenotype and subsequent fitness. Stressful developmental environments, particularly those involving limited food quantity or quality, have been shown to be particularly impactful to animal adult cognition. As a result, researchers have introduced the developmental stress hypothesis, which suggests that variation in cognitive ability is partly dependent on stress levels experienced during development. To test the developmental stress hypothesis in an invertebrate system, I raised Texas field crickets (*Gryllus texensis*) on a protein- or carbohydrate-rich diet from their penultimate juvenile molt to adulthood and then quantified the impact of these developmental diets on adult cognition. To test cognitive abilities, I trained and tested individuals to locate a food reward at the end of one arm in a radial arm maze. To obtain duration- and accuracy-based measures of cognition, I quantified each individual's latency to find the reward and number of complete entries made. Both cognitive measures showed significant improvement across four training trials. Surprisingly, juvenile diet did not significantly predict adult cognitive ability. Also surprisingly, performance greatly worsened during the final test trial, when no food reward was present, a finding that conflicted with my previously published research. Crickets appeared to rely more heavily on olfactory based cues rather than visual cues for learning in my current study compared to my previous study. The fundamental thing that differed across these two studies was the developmental diet, suggesting that reductions in either proteins or carbohydrates during development may result in a reduction in cricket cognitive abilities in adulthood. Together my findings lend tangential support to the developmental stress hypotheses, while simultaneously highlighting the need for more dietary controls in subsequent studies.

## Introduction

The mechanisms by which animals acquire, process, store, and act on information from their environment is known as cognition (Shettleworth, 2010). Bird cognition studies have provided great insights into the cognitive abilities of non-human animals, revealing that spatial cognition is often used to remember food caches. For example, Western-scrub jays (*Aphelocoma californica*) scatter-hoard their insect food supply. Salwiczek et al. (2010) showed that jays can accurately locate their cache sites even after months without returning to that location. Likewise, magpies (*Pica pica*) can hoard or retrieve food from filled trays, showing evidence of what-where-when memories and indicating a strong spatial memory for food stores (Zinkivskay *et al.*, 2009). Invertebrates also display spatial cognitive abilities. For example, crayfish (*Orconectes rusticus*) can remember the location of their shelters and an exit door (Tierney and Andrews, 2013). Equally, many insect species use spatial cognitive abilities to locate food sources. For example, Cartwright and Collett (1983) found that honeybees (*Apis mellifera*) do not use olfaction to spatially orient, but instead continuously compare their retinal image with the ‘snapshot’ of their location and adjust their flight path accordingly.

### *Inter-individual Variation in Cognition*

More recently, many researchers have begun to quantify inter-individual variation in cognitive abilities. This approach enables us to begin to identify the factors influencing variation in cognitive performance at the individual level, and how this variation correlates with an individual’s overall fitness (Morand-Ferron *et al.*, 2016). For instance, Sonnenberg et al. (2019) found that black-caped chickadees (*Poecile atricapillus*) living in harsher climatic environments showed greater food caching, spatial learning and memory. Individuals with increased food caching, learning and memory abilities should experience enhanced reproduction over individuals with poorer abilities. Woodgate et al. (2016) suggested that interindividual variation

in behaviour should be a direct predictor of problem-solving ability, after they observed vast variation in bumblebees' memory of flight paths to foraging locations. Several of these studies suggest that inter-individual variation in cognitive performance may be related to developmental differences.

### *Developmental Stress Hypothesis and Implications for Cognition*

The developmental stress hypothesis suggests that variation in cognitive ability is partly dependent on the stress level experienced during development (Kelly and Tawes, 2013; Spencer and MacDougall-Shackleton, 2011). Stressful environments such as limited food quantity or poor nutritional quality can lead to organisms taking longer to mature, maturing at a smaller body size, and maturing in poorer condition. This, in turn, would be costly to adult fitness as individuals are underdeveloped and malnourished, negatively influencing their ability to cognitively process their surroundings, decreasing their reproductive success and increasing mortality (Kelly and Tawes, 2013).

There is substantial experimental support for the developmental stress hypothesis. Nowicki et al. (2002) experimentally imposed stressors on swamp sparrows (*Melospiza georgiana*) during different stages of development and found that these stressors impaired brain regions controlling song as well as song development. A review by Morand-Ferron et al. (2016) also reinforced the idea that environmental factors such as nutrition and parasitism can heavily influence an adult bird's ability to cognitively perform and reproduce successfully. Further, a review by Peters et al. (2014) suggests that developmental stress may provide an explanation for an association between cognition and song-learning in birds. Evidence of nutritional stress on cognition is not limited to birds. Benton et al. (2003) investigated the effect of a low versus high carbohydrate breakfast on cognitive performance in laboratory Wistar rats (*Rattus norvegicus domestica*); rats undergoing operant conditioning tests displayed better learning performance

after a low-carbohydrate breakfast than their high-carbohydrate counterparts (Benton *et al.*, 2003). Further, Woo *et al.* (2013) placed Sprague Dawley rats on a 13-week high fat diet and significantly reduced their cognitive function. Positive effects on learning capacity was only restored after the rats were placed on an 8-week routine of diet and exercise. These studies provide support for the idea that nutritional stress influences inter-individual variation in cognition.

To date there has been limited research on how nutritional stress influences invertebrate cognitive abilities. It is, however, well known that the balance of carbohydrates and proteins in the diet impacts other life history traits. For example, forest tent caterpillars (*Malacosoma disstria*) and field crickets (*Gryllus veletis* and *Gryllus assimilis*) that are fed a protein-biased diet develop faster and grow bigger compared to individuals that are fed a carbohydrate-biased diet (Despland and Moseworthy, 2006; Harrison *et al.*, in prep; Reifer *et al.*, 2018). Female crickets (*Teleogryllus commodus* and *Gryllus veletis*) fed diets rich in protein also experience greater egg production than crickets fed diets rich in carbohydrates (Zajitschek *et al.*, 2012; Harrison *et al.*, 2014; Hans and Dingemanse, 2017). Conversely, male field crickets often require carbohydrate-rich diets in adulthood to compensate for the energetically demanding act of signalling for mates (Zajitschek *et al.*, 2012; Hans and Dingemanse, 2017; Reifer *et al.*, 2018). These studies reveal that male and female field crickets (*Gryllus veletis*) appear to utilize nutrients differently for reproduction (Harrison *et al.*, 2014; Han and Dingemanse, 2016). Han and Dingemanse (2017) recently found that when given a choice, male Southern field crickets (*Gryllus bimaculatus*) consumed significantly more carbohydrates, but less protein, than their female counterparts, furthering the notion that this difference exists to fuel their different reproductive strategies. Given nutrient availability impacts all of the aforementioned life history traits, nutrient availability also has the potential to impact invertebrate cognitive abilities.

### *Field Cricket Cognitive Ability*

While crickets have been extensively studied in a behavioural ecology setting, far less is known about their cognitive abilities. To my knowledge, there have been four studies to date on field cricket spatial cognitive abilities. Hale and Bailey (2004) showed that juvenile Australian raspy crickets (Orthoptera: *Gryllacrididae*) use visual cues to orient toward their burrow. Mangan and Webb (2009) revealed that Southern field crickets (*Gryllus bimaculatus*) can learn to return to a target in an experiment analogous to the Morris Water Maze used for rats. Collett et al. (2013) found that male Southern field crickets (*Gryllus bimaculatus*) use spatial memory to remember cool spots in an otherwise hot experimental arena. Most recently, my undergraduate honours thesis research showed that female Texas field crickets, *Gryllus texensis*, use their spatial cognitive abilities to locate a food reward in a six-arm radial arm maze (RAM). Specifically, females improved in their ability to find the food reward, decreasing the number of errors made, their distance travelled, and their latency to find the food reward across training trials and subsequent test trials without the food reward present (Doria *et al.*, 2019). My study also revealed individual variation in cognitive abilities, suggesting that field crickets are an appropriate model organism for an invertebrate based test of the developmental stress hypothesis.

### *Focus of Research and Predictions*

To test the developmental stress hypothesis, I manipulated the balance of proteins to carbohydrates in the diet during male and female field cricket development and then examined how dietary differences impacted inter-individual variation their cognitive abilities. I tested the hypothesis that the developmental diet influences cricket learning ability and cognition ( $H_1$ ). I predicted that (1a) crickets reared on a protein-rich diet should have better developed spatial cognitive abilities than crickets reared on a carbohydrate-rich diet, as dietary protein is known to

be important for the maintenance and manufacturing of tissues, which should include the brain (Bertram et al., 2009; Srygley, 2017). Alternatively, I predicted that (1b) crickets reared on a carbohydrate-rich diet should have better developed spatial cognitive abilities than crickets reared on a protein-rich diet because carbohydrates are important for fueling energetically costly activities, which should include cognitive processing (Bertram et al., 2009; Srygley, 2017). Because of my uncertainty about whether protein or carbohydrate availability would have a greater impact on cognitive abilities, my alternative predictions should be considered to be mutually exclusive and competing.

I simultaneously investigated whether sex influences cricket learning ability and cognition (H<sub>2</sub>). I expected that there could be sex differences because of the range size hypothesis, which suggests that spatial cognition differences occur between sexes when one sex in a species must remember more landmarks and the relationships between them to hold larger territories (Gaulin and Fitzgerald, 1986). I predicted that (2a) females should have better developed spatial cognitive abilities than males because they must traverse across many territories to find mates, as well as remember the location of those males (Ecuyer-Dab and Robert, 2004; Guigeuno *et al.*, 2014). Conversely, I predicted that (2b) males should have better developed spatial cognitive abilities than females as they actually hold territories (Perdue *et al.*, 2011). Because of my uncertainty about which sex should have greater cognitive abilities because of differences in their behaviour, my alternative predictions should be considered to be mutually exclusive and competing.

My experimental design also enabled me to examine whether there was an interaction between sex and diet on cricket cognitive abilities (H<sub>3</sub>). The literature surrounding how diet might influence cognition in invertebrates is sparse, resulting in multiple contrasting predictions. My predictions were dependent on the findings for H<sub>1</sub>. Support for prediction 1a, that protein

enhances cognition, would suggest that there should also be evidence to support prediction 3a, that males should have better spatial cognitive abilities than females when they are fed protein-rich diets. Under this scenario, females should need to use more of the protein for egg production, leaving less available for enhancing cognitive abilities. Conversely, males should be able to direct a larger portion of the protein into building brain mass and enhancing their subsequent cognitive abilities (Hunt et al., 2004; Kelly and Tawes, 2013). Support for prediction 1b, that carbohydrate enhances cognition, would suggest that there should also be evidence to support prediction 3b, that females should perform better than males when they are fed carbohydrate-rich diets. Under this scenario, males should use the excess carbohydrates to fuel their mate signalling and territorial fights, while females should be able to use the excess carbohydrates to enhance their cognitive function (Maklakov et al., 2008; Zajitschek et al., 2012).

## **Methods**

### *Experimental Population*

I ran my experiment from March to October 2018 using Texas field crickets (*Gryllus texensis*). The crickets I used in my experiment were descendants of adults collected September 2014 from Smithville, Texas (30.0086° N, 97.1594° W). To obtain my experimental population, I placed 50-60 newly hatched individuals together into a group rearing bin (64 x 40 x 42 cm) that contained *ad libitum* standard diet (Teklad Rodent diet #8604, Envigo, Madison WI, USA), water, and cardboard egg cartons for shelter. I housed this experimental population in a greenhouse under a 14:10 hour light:dark cycle at  $28 \pm 2$  °C. To obtain my experimental individuals, I removed newly eclosed penultimate juvenile males and females using a thrice weekly (Monday, Wednesday, Friday) schedule. I chose to start the experiment at the

penultimate juvenile stage because this is when I could easily distinguish males from females. To ensure a constant supply of experimental individuals, I continually replenished the experimental population with newly hatched individuals, ensuring a rearing bin density of 50-60 individuals.

### *Experimental Individuals*

I weighed each newly eclosed experimental individual using an electronic balance (OHAUS Pioneer Analytical Scale). I then transferred it into its own individual container (11.5 x 11.5 x 7.5 cm) containing the experimental diet (high-protein or high-carbohydrate, detailed below), a water cup, a crumpled sheet of paper towel for shelter, and a lid with screening for air flow. Individual containers were kept in the greenhouse under the same abiotic conditions as the population in the group rearing bin. Experimental individuals were fed their assigned diet *ad libitum* and were monitored daily until they reached adulthood. Upon adult eclosion, each experimental individual was placed back on the standard diet (Teklad Rodent diet #8604, Envigo, Madison WI, USA). Experimental individuals were then trained and tested following the protocols outlined below. In total, I trained and tested 77 males (39 on high-protein, 38 on high-carbohydrate) and 77 females (38 on high-protein, 39 on high-carbohydrate) for their spatial cognitive abilities.

### *Diets*

I created protein- and carbohydrate-rich diets in December 2017 and again in August 2018 (to replenish my supplies) following established protocols (*sensu* Simpson & Abisgold, 1985; Harrison et al. 2014). I randomly assigned experimental individuals to a protein-rich (3:1 protein:carbohydrates) or carbohydrate-rich (1:3 protein:carbohydrates) diet. I only varied the protein and carbohydrate components in these diets. Specifically, the protein sources I used were casein (262.34g versus 87.45g), peptone (87.45g versus 29.15g), and albumen (87.45g versus 29.15g) for the high-protein versus high-carbohydrate diets, respectively. The carbohydrate

sources I used were sucrose (72.87g versus 218.62g) and dextrin (72.87g versus 218.62g) for the high-protein versus high-carbohydrate diets, respectively. All diet ingredients were weighed using an electronic balance (OHAUS Pioneer Analytical Scale) to the nearest 0.001g. I ensured that the protein- and carbohydrate-rich diets (P:C 3:1 and 1:3 diets, respectively) contained identical amounts of vitamins and minerals, including cellulose (650g), vitamin mix (TD. 10963; 0.689g), choline chloride (1.651g), cholesterol (7.150g), mineral mix (18.200g), L-ascorbic acid (3.575g) and lipids.

### *Food Deprivation and Reward Introduction*

I began the process of preparing to quantify cricket cognitive abilities when they were 1 to 7 days post adult eclosion. Crickets varied in age because I was only able to begin quantifying cricket cognition on Mondays (hereafter Day 1), due to a limitation in the number of 6-arm radial mazes (RAMs) and space to run the cognition trials (described below). On the following Wednesday (Day 3), I removed the standard diet from each individual's container to initiate a 24hr food deprivation period. This food deprivation period increased the likelihood that the experimental individuals were motivated by hunger during subsequent training and experimental phases. On the next day (Thursday; Day 4), I placed two cubes (4 x 4 x 2mm) of Royal Gala apple dusted in the standard diet into each individual's container as a 'food reward'. I introduced the experimental individuals to these food rewards prior to any training to ensure that experimental individuals were acquainted with this novel food in a familiar environment. On the Friday (Day 5) I assigned each individual to their own RAM that they used for their Discovery Trial (Days 5-7), Training Trials (Days 8-11), and Test Trial (Day 12), all described below.

### *Radial Arm Mazes (RAMs)*

My experiment utilized 10 different RAMs. The RAMs were built out of white plastic by CANUS Plastics in Ottawa, Ontario. Each RAM consisted of six arms that radiated away from

the central platform in a star shape (Supplementary Figure 1). The central platform was 15 x 6 cm (d x h), each arm was 15 x 5 x 6 cm (l x w x h) and had uniquely identified markings on the walls and/or end of the arm, as well as a unique colour at the end of each arm (green, yellow, blue, orange, white, and red). Wood crickets have shown to have a perceptual range of at least 105 cm (Goulet *et al.*, 1981), so field crickets are likely able to distinguish these markings. Further, my previous research showed that females can be trained in these RAMs (Doria *et al.*, 2019). I placed a metal sunken dish at the end of each arm, flush with the floor of the RAM. Because it is possible that experimental individuals might utilize visual cues that are external to the RAMs to aid their spatial memory, I placed four distinctively coloured and patterned fabric on the walls surrounding the outside of the RAMs. Additionally, this fabric acted as a blind, preventing the crickets from seeing me during the trials. The position of each RAM within the setup never changed, such that each of the RAMs and their reward dishes were always placed in the exact same location each time they were used.

### *Discovery Trials*

Several studies have shown that initial familiarization with test devices or arenas can reduce confounding effects of novelty responses on performance in cognitive tests (Morand-Ferron *et al.*, 2016). Further, in accordance with these other studies, my previous work revealed that a 48 hr Discovery trial was necessary for female Texas field crickets to show evidence of learning in a RAM (Doria *et al.*, 2019). Therefore, on Friday (Day 5) of the cognition experiment, I haphazardly scattered 12 pieces (2 x 1 x 1 mm) of Royal Gala apple dusted in standard diet throughout each RAM. I also added a cup with water to each RAM. I then placed each experimental individual into their assigned RAM for a 48 hr Discovery Trial (Days 5-7). Each experimental individual remained under their translucent cup for a 5 min acclimatization period before being released by carefully raising the cup by pulling it up using a string, after

which the cricket could freely roam the RAM. Each individual was alone in their RAM throughout the Discovery, Training, and Testing Trials. On Sunday (Day 7), after the 48 hr period, I recorded the number of pieces of Royal Gala apple that had been consumed by each experimental individual to obtain individual measures of satiation. I then returned each individual to their individual container with water (no food).

### *Training Trials*

Starting on the following day (Monday; Day 8), I trained all experimental individuals (N=154) using four consecutive Training Trials (one training session per day, on Days 8-11). Each individual's Training Trials were run in the morning (07:00 to 12:00 h), approximately 24 hours apart, to minimize time of day effects. Using a random number generator, I randomly assigned one of the dishes to be the individuals 'baited' (reward) dish; that reward dish remained the same for all of that individual's consecutive Training Trials. I placed a food reward that consisted of one piece (4 x 4 x 2 mm) of Royal Gala apple dusted in standard diet into the arm's reward dish. Given the sunken nature of all the dishes, the food reward was presumably not visible to the individual until they were adjacent to the reward dish. Each experimental individual remained under their translucent cup for a 5 min acclimatization period before being released by carefully raising the cups by pulling them up on a string. Each cricket was given a 30 min period to find the reward dish. I initiated this 30 min period as soon as each individual willingly left the acclimatization zone (area under the translucent cup). I video recorded each Training Trial from above and later quantified each individual's latency to leave the acclimatization zone, their latency to find the reward dish, the distance each individual travelled to find the reward dish (path length), the number of complete arm entries each individual made prior to finding the reward dish, and the number of errors each individual made prior to finding the reward dish (details provided in Scoring Videos, below). I removed each experimental

individual from its RAM after it had either (1) found and consumed the food reward and left the arm that had contained the food reward or (2) after the 30 min period expired if it did not find the food reward. I then returned each cricket to their individual container and provided them with another Royal Gala apple food reward (4 x 2 x 2 mm) dusted in standard diet. After each Discovery, Training and Test Trial, I thoroughly cleaned each RAM using 95% ethanol and a paper towel to ensure chemical and food cues were absent.

### *Test Trial*

On Friday (Day 12), following the four consecutive Training Trials, I ran each experimental individual through a Test Trial. The Test Trial was conducted in the same manner as Training Trials, with the only exception being that the previously baited sunken food dish no longer contained a food reward. My goal for the Test Trial was to determine whether my four measures of cognition changed now that there was no food reward or food cues present in the dish that originally contained the food reward. On Day 12, following the Test Trial, I weighed each experimental individual with an OHAUS electronic balance and euthanized them by freezing.

### *Scoring Videos*

I watched all video recorded trials using the Behavioral Observation Research Interactive Software (BORIS). For each Training and Test trial, I used an ethogram I created within BORIS to quantify all behaviours and time measurements to the nearest 0.001 seconds. For all Training and Test trials, I recorded the individual's latency to emerge from under the acclimatization cup, as well as four cognition measures:

- (1) Latency to locate the reward dish after exiting from the acclimatization zone; the reward dish contained the food reward in the Training Trials but not the Test Trial.

- (2) The distance travelled before locating the reward dish, calculated by summing the distance travelled in each complete arm entry (15 cm), and partial arm entry (5 cm) before locating the reward dish. A complete arm entry consisted of the cricket's head reaching the sunken dish at the end of the arm. A partial arm entry consisted of a cricket entering the arm but leaving before their head reached the sunken dish.
- (3) The number of complete arm entries made prior to locating the reward dish.
- (4) The number of working memory errors, which consisted of the number of unique arm entries that the individual made prior to locating the reward dish.

I coded trials where the individuals did not find the reward dish as missing data (81/717= 11.3%).

### *Statistical Analysis*

I performed all statistical analyses using JMP version 14.0. My previous findings from my honours thesis revealed that the four cognitive measures (reward latency, path length, complete entries, and working memory errors) were highly correlated (Doria *et al.*, 2019). Further, my initial analysis of the findings in the present study also showed high correlations across all four of these cognition measures. As such, I decided to omit path length and working memory errors from my analysis; path length is highly correlated with other cognition measures, and working memory errors more closely tests short-term memory within each specific trial as opposed to cognition over consecutive trials. I focused instead on reward latency and number of complete entries, because reward latency is a commonly used duration-based measure of cognition while number of complete entries tests accuracy-based cognitive abilities. This approach enabled me to investigate cognition in two ways.

To quantify whether spatial cognitive ability was influenced by developmental diet (H<sub>1</sub>), sex (H<sub>2</sub>), or their interaction (H<sub>3</sub>), I ran general linear mixed models (GLMM). My dependent

variables were reward latency and number of complete entries. My independent variables were sex, diet, trial number, and all possible interactions. I included individual IDs as a random effect as the same individuals were tested multiple times. I included the following variables as covariates in my GLMMs: the amount of food consumed during the Discovery trial as a measure of satiety and motivation (included as the number of pieces consumed out of 12); the colour of the individual's reward arm; latency to emerge from under their acclimatization zone; and whether or not there was acoustic mate attraction signalling that occurred during the Test Trial. These measures were included to account for possible extraneous factors influencing cricket performance during the trials and tests. I  $\log_{10}$  transformed the residuals of reward latency and number of complete entries to meet assumptions of normality, and checked these assumptions using normal quantile (QQ) plots. I utilized projection-based model reduction techniques when the interactions were non-significant. I ran Tukey's HSD post-hoc tests for trial number to ascertain where the significant differences lay.

## **Results**

### *Cognitive Performance*

Reward latency and number of complete entries cognition scores were negatively influenced by training trial number (Table 1; Figure 1a,c). This finding suggests an individual's ability to locate the food reward improved with training, given that lower scores suggest individuals found their reward dishes faster, and made fewer complete entries as they experienced more training.

### *How Sex and Diet Influence Cognitive Performance*

My experiment tested whether developmental diet influenced an individual's spatial cognitive performance in adulthood ( $H_1$ ), whether males and females differed in their cognitive

abilities (H<sub>2</sub>), and whether there was an interaction between sex and diet (H<sub>3</sub>). There was minimal support for H<sub>1</sub>, as juvenile diet did not significantly influence spatial cognitive performance (Table 1). There was, however, a non-significant trend that individuals fed a carbohydrate-rich diet had lower number of complete entries compared to individuals fed a protein-rich diet (Table 1;  $p=0.0798$ ). Figure 1 highlights the cognitive performance of each sex separated by diet for both cognition measures (Figure 1a, c). There was no support for H<sub>2</sub>, as the sexes did not differ in their cognitive performance. Moreover, there was no significant interaction between juvenile diet or sex, providing no support for H<sub>3</sub>. Because of the lack of significant interactions, I present the simplified models (full models available in Supplementary Table 1). Given there was no significant effects of diet, sex, and their interactions, I combined all treatments to show how the overall cognitive performance of crickets changed across their training and test trials (Figure 1b,d; Table 1).

Table 1. Relationship between experimental variables with two log<sub>10</sub> transformed cognition measures for male and female Texas field crickets

Cognition measure	Experimental variables	df	F	P
Reward Latency	Sex	1, 130	0.0332	0.8557
	Diet	1, 129	0.2033	0.6529
	Sex*Diet	1, 130	0.0694	0.7926
	<b>Trial number</b>	<b>4, 503</b>	<b>3.2787</b>	<b>0.0114</b>
	Discovery motivation	1, 129	0.1004	0.7518
	Colour	5, 130	1.3125	0.2626
	Emerge latency	1, 561	0.0027	0.9586
	Chirp during test	1, 134	0.9935	0.3207
	# of Complete Entries	Sex	1, 132	0.5257
Diet		1, 131	3.0251	0.0843
Sex*Diet		1, 132	0.1886	0.6648
<b>Trial number</b>		<b>4, 507</b>	<b>7.1427</b>	<b>&lt;0.0001</b>
Discovery motivation		1, 130	1.3848	0.2414
Colour		5, 132	1.5159	0.1891
<b>Emerge latency</b>		<b>1, 541</b>	<b>5.3200</b>	<b>0.0215</b>
Chirp during test		1, 137	1.7878	0.1834

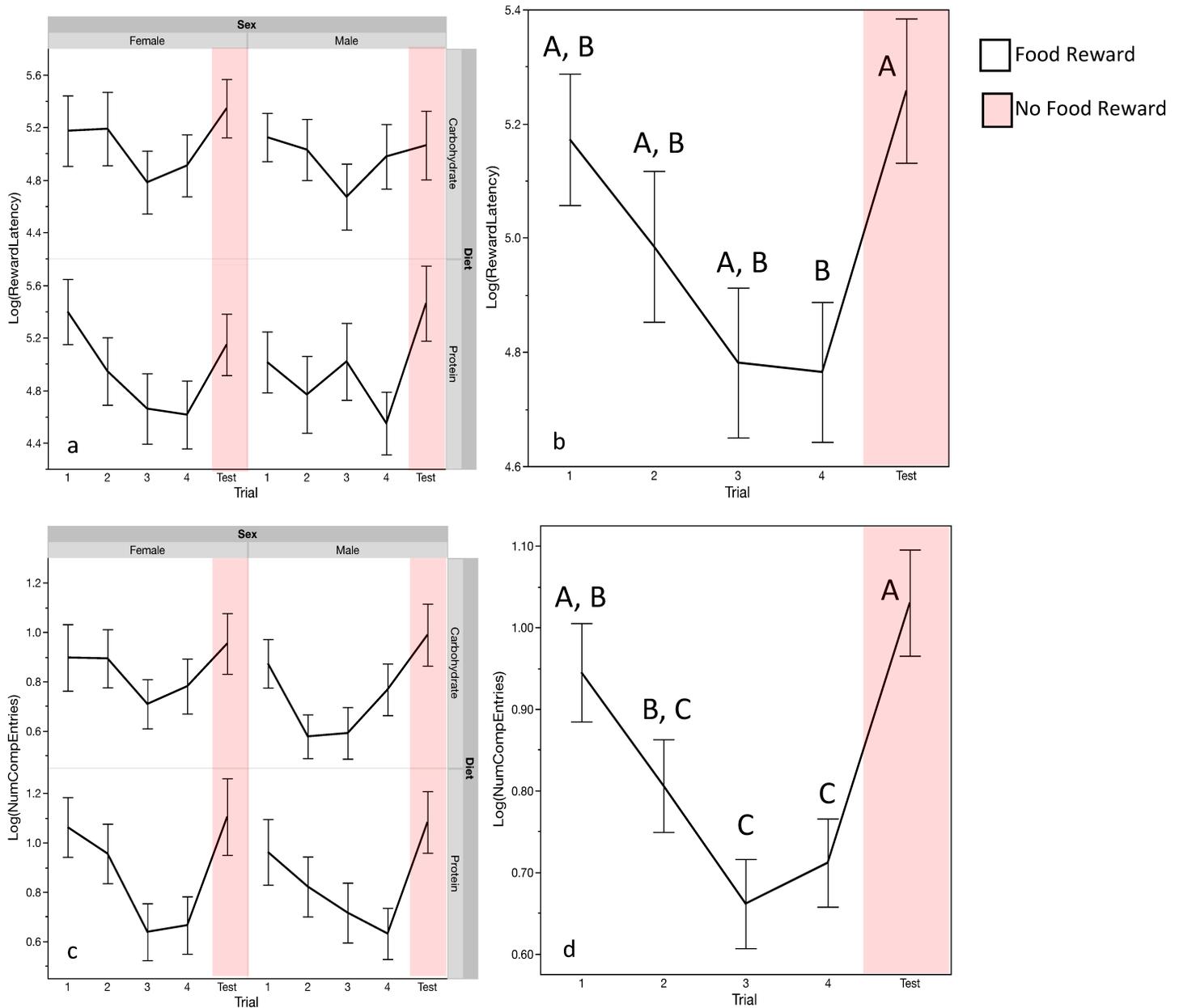


Figure 1. General linear mixed model (GLMM) showing how sex, juvenile diet and training number influence reward latency scores (a) and number of complete entries (c) in *Gryllus texensis* field crickets when a food reward is and is not present. Concatenated GLMM showing overall reward latency performance (b) and number of complete entries (d) of individuals regardless of sex and diet, as they were not shown to be significant predictors of cognitive performance. Levels not connected by the same letter are significantly different (b, d).

### *Test Trial*

Both cognition scores dramatically worsened when individuals participated in the Test trial (Figure 1, pink section). Specifically, both reward latency and number of complete entries observed in the Test trial were significantly higher than in training trial 4 ( $\alpha= 0.050$ ,  $Q= 2.7378$ ), and in training trials 2-4 ( $\alpha= 0.050$ ,  $Q= 2.7376$ ), respectively. This finding suggests that when food and their associated olfactory cues are removed, the crickets do not perform at the same level as they did during training.

### *Predictors of Cognitive Performance*

Reward colour, discovery motivation and chirping during the test trial were not significant predictors of cognitive performance for Texas field crickets (Table 1). Latency to emerge from under the acclimatization zone did, however, significantly predict spatial cognitive performance for number of complete entries, as individuals who took more time to emerge from the acclimatization zone entered significantly fewer arms before finding the food reward compared to those that emerged quickly ( $p= 0.0231$ ; Figure 2).

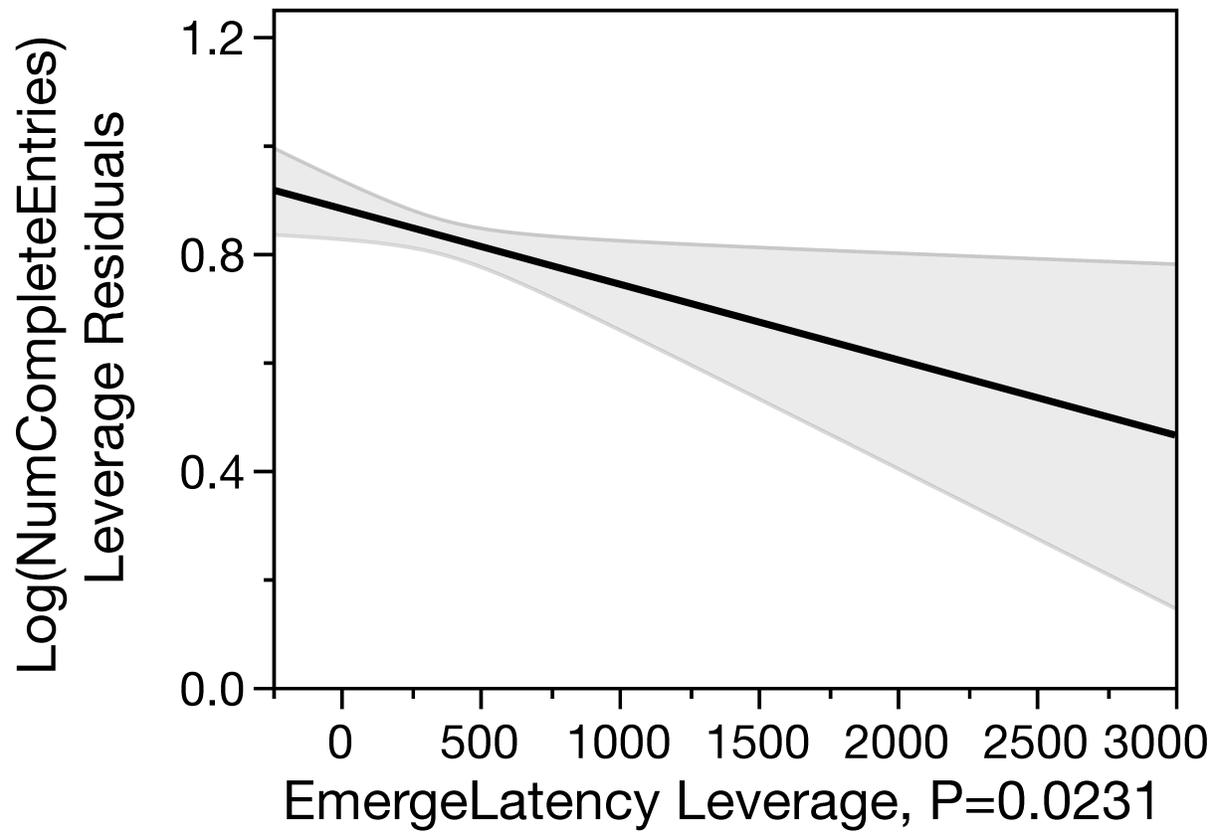


Figure 2. Leverage plot indicating that male and female Texas field crickets that took longer to emerge entered significantly fewer arms before finding the food reward

## Discussion

Crickets learned over the course of their four training trials, as trial number significantly influenced reward latency and number of complete entries cognition scores. This finding was congruent with my previous research, which showed that over consecutive training trials, females significantly decreased their latency to find the reward, distance travelled, arms entered, and errors made (Doria *et al.*, 2019). Additional studies on fruit flies (*Drosophila melanogaster*, Matsumoto *et al.* 2003; Matsumoto and Mizunami, 2004) and crickets (*Gryllus bimaculatus*, Nakatami *et al.*, 2009), have also found that training sessions enable individuals to learn and retain memory from both aversive and appetitive conditioning trials.

Interestingly, my current study suggests that cognitive performance gravely worsened during the Test trial compared to the previous Training trials. In fact, for both reward latency and number of complete entries, responses in the Test trial did not differ significantly from the first Training trial. These results suggest that even after four consecutive days of training and exposure to the RAM, crickets were unable to find the location of the food reward once the food reward and its associated olfactory cues were removed (Figure 1b,d). This finding lies in direct contrast to my previous research which showed that high cognitive performance was maintained during the Test trial (Doria *et al.*, 2019). The only factor that changed between the training and test trial was the removal of the food reward, and with that the olfactory cues associated with it. Awata *et al.* (2015) obtained evidence that the discrepancy between actual and perceived reward determines whether learning occurs. As such, it could be that individuals associated the olfactory cue during training with the perception of a guaranteed reward, and once that was removed, they were not motivated to locate the previously baited arm, resulting in worse performance. If motivation is linked to decreased movement, a way to support whether this was linked to poorer performance could be to quantify the difference between the area of the RAM an individual traversed during training trial 4

and the test trial. If an individual moved significantly less after the food reward was removed, this may indicate that motivation was a factor in the decreased cognitive performance.

There are many alternative explanations that could aim to explain why these individuals did not show evidence of learning when the food reward was removed on the test trial, though there is evidence for these not being supported. For instance, it could be proposed that crickets could view the reward from a further distance than being adjacent to it. If this was the case, individuals should have located the reward in the first training trial, and many failed to do so. Their typically poor performance at the start of the experiment negates this idea. Next, it could have been that with time individuals were less stressed in the RAMs and could process their environment more soundly. However, a reduction in stress should have resulted in increased performance as trials progressed, including the test trial.

#### *Diet and Cognition*

Counter to my first hypotheses, I found that the diet that juveniles experienced during late stages of development did not significantly predict cognitive performance. Specifically, my results revealed that neither crickets reared on carbohydrate-rich nor protein-rich diets had better developed spatial cognitive abilities over one another. I found a non-significant trend that individuals raised on a carbohydrate-rich diet made slightly fewer complete entries before finding their reward than individuals raised on a protein-rich diet, regardless of sex ( $p= 0.0798$ ). This trend might have occurred because carbohydrates are important for fueling energetically costly activities, which should include cognitive processing (Bertram et al., 2009; Srygley, 2017). High-carbohydrate foods are also known to alter brain regions associated with learning, and when consumed in large quantities promote food-seeking behaviours (Relchelt et al., 2017). This information hints at the idea that the carbohydrate fed individuals were more inclined to seek out the food reward with slightly more accuracy than their protein-biased counterparts.

Crickets are known to prefer carbohydrates over protein (Fukumura and Nagata, 2017; Harrison *et al.*, 2014). Given this result, individuals fed protein-rich diets may have been more motivated to locate the carbohydrate dense food reward, as they were more deprived of this macronutrient throughout development. This would have resulted in the trend for slightly better cognitive performance during the trials with a carbohydrate-based food cue present than their carbohydrate-rich fed counterparts.

Overall, it was generally surprising that diet did not significantly influence cognitive ability, given bird and rodent studies suggest otherwise. Studies on juvenile European starlings (*Sturnus vulgaris*, Buchanan *et al.*, 2003) and zebra finches (*Taeniopygia guttata*, Spencer *et al.* 2003), have shown that limited food availability and nutritional restriction lead to reduced song repertoire size, phrase duration, and song complexity compared to their unrestricted counterparts. These studies show evidence of the fact that diet should have profound impacts on cricket adult cognitive performance, though studies of other species show this is not always the case. One such study using zebra finches showed that food restriction during early development resulted in lower song frequency but had no effect on song complexity (Zann and Cash, 2008). Another such study using school-aged children found that cognitive performance on a computer test was not impacted significantly by breakfast diet (Brindal *et al.*, 2012). Though a comparison of humans and invertebrates such as crickets is not ideal, these studies suggest that the evidence is somewhat inconclusive surrounding how and if developmental diet influences cognition.

All this being considered, I believe I have found tangential support for the developmental stress hypothesis when the results of my current study are compared to the results of my previous research (Doria *et al.*, 2019). For my undergraduate honour's thesis, female Texas field crickets showed sustained learning over trials with and without a food reward (Doria *et al.*, 2019). The same experimental protocols as the current study were used in my previous research, with the

exception of the females' juvenile diet *not* being manipulated. My previous research showed learning in the test trials, but once I manipulated the juveniles' diet, they showed no evidence of learning in the test trials. This difference between the studies suggests that any kind of nutrient manipulation may be stressful, especially when the nutritional levels do not fall within their optimal range, and as a result cognitive performance suffers. Cognitive performance without olfactory cues in the test trial may simply not have been within the realm of their ability for individuals who experienced either the reduced protein / elevated carbohydrate treatment or the reduced carbohydrate / elevated protein treatment.

### *Sex and Cognition*

I found no support for H<sub>2</sub>, that sex influences cricket learning ability and cognition, nor H<sub>3</sub>, that there was an interaction between sex and diet on cricket cognitive abilities. Though I was surprised to find a lack of sex differences in cognitive ability, literature shows mixed results as to whether these differences actually exist. While it is well documented that males often outperform females on spatial cognition tasks (Perdue *et al.*, 2011), there is also conflicting evidence. For example, Perdue *et al.* (2011) found that in an eight-arm radial arm maze, male giant pandas (*Ailuropoda melanoleuca*) made significantly fewer errors than females, but there was no difference between male and female small-clawed otters (*Aonyx cinereus*) that performed the same task. Similarly, two different studies on spatial ability in meadow voles (*Microtus pennsylvanicus*) found sex differences in one (Gaulin and Fitzgerald, 1986), but not the other (Sawrey *et al.*, 1994). Ultimately, it could be that within this cricket's mating system, the range size held by males and traversed by females for mating are not diverse enough to evoke superior spatial ability in either sex, in which case no sex differences would be expected (Perdue *et al.*, 2011).

### *Predictors of Cognitive Performance*

Emergence latency significantly influenced cognitive performance across trials, as slow emergers found the food reward with fewer arm entries than fast emergers (Figure 2). This finding is similar to that of Udino et al. (2017), who found that more active carpenter ants (*Camponotus aethiops*) were slower learners than inactive individuals. It could be that individuals who are slow to emerge pay more attention to their surroundings and analyze where the food reward is before beginning each trial. This does not, however, help in explaining why individuals would then worsen so drastically during the Test trial if they were relying on visual cues before emerging. Based on the fact that they performed so poorly during the Test trial, crickets may use a combination of visual cues and olfaction to locate the food reward, but the processes behind this are not clear cut. This idea stems from my previous work, which showed no significant relationship between emergence latency and cognition during the test trial, when no food reward and thereby olfactory cues were present (Doria et al., 2019). Olfaction may not have played a significant role in my previous research because juvenile diet was not manipulated in those females, and as such they experienced a less stressful development. With my current research, if the excess of either protein or carbohydrates in the juvenile diet was too stressful, it may mean that individuals needed both visual and olfactory cues to cognitively perform at the same level as my previous individuals, who seemingly only used visual cues to locate the correct arm. If this is the case, individuals with better cognitive ability would have taken longer to emerge from their acclimatization zone upon the realization that there was no olfactory cue to aid them in navigating the RAM. These individuals would be the slower emergers that performed better than their quick-to-react counterparts, who may have been poorer learners in general, though this was not tested.

*Future Directions for Research*

My current study is limited by the inability to wholly grasp what is influencing cricket spatial cognitive performance. It remains unclear why diet and sex do not impact cognition, but ultimately the largest discrepancy between my current study and my previous study comes down to diet and the test of the developmental stress hypothesis. If extreme diets, in general, are stressful for individuals during the juvenile stage, I could infer that no one macronutrient is the answer to superior cognitive abilities in adulthood. It may be the combination of both protein and carbohydrates in optimal quantities is necessary for strong cognitive function. There is a widely held assumption that consuming food with excess nutrient content is okay as long as it is the right kind of nutrient (Boersma and Elser, 2006). This might not be the case, however, and Raubenheimer and Simpson (1997) have developed a framework specifically showing that an excess of both proteins or carbohydrates can decrease fitness. If excess nutrients decrease fitness, then I believe I have found tangential evidence to support the developmental stress hypothesis. That said, I must admit that I erred in my lack of including a treatment where juvenile diet was not manipulated. To test this idea, researchers could rear individuals on one of three diets: protein-rich, carbohydrate-rich, and control diet during development and look at successive learning and cognition outcomes in adulthood.

My findings suggest that individuals cannot maximize their cognitive ability when either protein or carbohydrates are limited in development. This finding suggests that individuals may be unable to maximize all other life history traits on any one extreme diet. This idea led me to investigate potential trade-offs between fitness proxies (acoustic mate attraction signalling in males and fecundity in females) and how fast individuals develop, how large they grow, and their cognitive abilities. Specifically, I was interested in determining whether developmental diet influences how these individuals trade off fitness proxies with cognition or other life history traits. Could it be that more fecund females or higher effort signalling males have greater

cognitive ability? Conversely, can individuals not simultaneously maximize fitness with cognitive ability? I explore whether there are trade-offs between cognition and reproductive based fitness proxies in Chapter 2.

*Chapter 2: Do adult crickets exhibit trade-offs between cognitive ability and other fitness conferring traits?*

## Abstract

Diet and nutritional uptake can greatly influence fitness related traits in animals, including insects. Because traits often cannot be maximized utilizing the same macronutrients in the diet, trade-offs between various traits associated with reproduction and survival can ensue. Little is known about whether fitness conferring traits trade-off with insect cognitive ability. I raised juvenile Texas field crickets (*Gryllus texensis*) on either protein- or carbohydrate-rich diets until adulthood and quantified their development time (number of days on the high-protein or high-carbohydrate diet), adult body size, cognitive ability, fecundity, and signalling behaviours. I asked whether (1) trade-offs exist between female fecundity or male signalling effort (fitness proxies) and development time, body size, or cognitive ability, or (2) whether individuals who exhibit high fitness proxies simultaneously exhibit elevated fitness conferring traits (good genes hypothesis: high condition individuals should be able to afford the costs). Female fecundity was influenced by an interaction between development time and body size, as larger females that grew more quickly contained more eggs. The probability that a male adopted a caller strategy was significantly influenced by a three-way interaction between development time, body size, and cognitive performance ( $p=0.0164$ ). Males that developed quickly and performed well in the cognitive task had a high probability of adopting a caller strategy; these males also exhibited high calling effort. For all other males, adopting a caller strategy and exhibiting high calling effort only occurred when they either developed slower, performed poorly in the cognitive task, or both. Surprisingly, diet did not predict these trade-offs between cognitive and reproductive abilities. Together these findings lend support for the idea that only a subset of seemingly high conditioned individuals can afford the costs of displaying multiple fitness conferring traits at once; to maximize their fecundity or signalling effort, all others must trade-off reproduction with other fitness conferring traits.

## Introduction

Fitness can be heavily influenced by the quality and quantity of nutrients an individual can obtain (Bertram *et al.*, 2009; Clark *et al.*, 2013; Harrison *et al.*, 2014); as a result, an individual's reproductive success and lifespan are frequently affected by nutrient availability (Maklakov *et al.*, 2008). Further, fitness conferring traits often cannot be maximized using the same nutrients. Given many nutrients in nature have limited distribution, individuals are often challenged when attempting to sustain an appropriate balance of nutrients in their diet (Bazazi *et al.*, 2016). As a result, trade-offs between various fitness-related traits can ensue (Zajitschek *et al.*, 2012; Costa *et al.*, 2016).

### *Trade-offs Between Fitness Conferring Traits Across Species*

There is a lot of evidence that both supports and refutes the idea that fitness conferring traits exhibit trade-offs. For example, a study of neotropical ponerine ants (*Platythrea punctate*) showed a positive relationship between reproducing ants and longevity, such that those who reproduced offspring also lived longer (Hartmann and Heinze, 2003). This is most commonly seen in eusocial insects with one main reproducer, and may result from the fact that these reproducers are often larger, which can also influence life span (Prothero and Jurgens, 1987). Positive relationships between longevity and reproductive success, and thereby a lack of trade-offs, has been shown in the Brazilian and Japanese ant (*Cardiocondyla obscurior*; Heinze and Schrempf, 2012; Kramer *et al.*, 2015) and bumblebees (*Bombus terrestris*; Blacher *et al.*, 2017). Recently, this positive relationship has also been shown in some eusocial rodents. Schmidt *et al.* (2013) showed that female Damaraland mole-rats (*Fukomys damarensis*) reproducers, or 'queens', live significantly longer than their non-reproductive subordinates. This is not, however, the typical case in most species, as limited resources often require individuals to invest into many traits, which often cannot all be maximized simultaneously (Busso *et al.*, 2017). For instance, in

another eusocial species, the burying beetle (*Nicrophorus vespilloides*), wounded females will trade-off social immunity to invest in personal reproductive success (Cotter *et al.*, 2013). Black scavenger flies (*Sepsis thoracica*) trade off immune function and growth (Busso *et al.*, 2017), such that they cannot concurrently grow large and be unaffected by disease, though both are important for reproduction and survival. Northern and Southern hemisphere birds differentially trade-off fecundity and survival, such that Northern hemisphere birds tend to reduce risk to their offspring with greater risk to themselves, while Southern hemisphere birds will trade-off their offspring for reduced mortality risk (Ghalambor and Martin, 2001). Another study on birds, using the Siberian jay (*Perisoreus infaustus*), showed that females trade off reproduction and longevity so as not to risk mortality (Griesser *et al.*, 2017). A complex trade-off between age, size, and fecundity exists in female eastern grey kangaroos (*Macropus giganteus*), in that older females will trade-off small size in favour of reproduction (Quesnel *et al.*, 2018). More recently, a study of Siberian dwarf hamsters (*Phodopus sungorus*) and water voles (*Arvicola amphibious*), showed that they trade off longevity and reproductive output (Novikov *et al.*, 2019), indicating that they cannot both live long *and* experience elevated reproduction.

#### *Dietary Influence on Fitness Conferring Traits Across Species*

Substantial invertebrate research indicates that diet can strongly impact life history traits important to fitness. Kelly *et al.* (2014) revealed that juvenile environment, particularly poor nutrition, negatively impacts adult survival, immune function, and reproduction related traits in the Texas field cricket (*Gryllus texensis*). More explicit research that investigates the balance of carbohydrates and proteins in the diet has also supported the idea that carbohydrate and protein availability strongly impacts life history traits. Kaspi *et al.* (2002) found that male and female medflies (*Ceratitis capitata*) reared on high protein diets developed more quickly and were larger than their high carbohydrate counterparts. Further, carbohydrates strongly impacted adult

male sex pheromones in cockroaches, making them more attractive to females (South *et al.*, 2011). South *et al.*'s (2011) finding suggests carbohydrates are key to elevated fitness in adult roaches. Further supporting the notion that diet can play a pivotal role in fitness, Maklakov *et al.* (2008) found that male *T. commodus* called more when fed a high-carbohydrate diet than when fed an even protein:carbohydrate ratio. Harrison *et al.* (2014) manipulated diet in adult field crickets (*Gryllus veletis*) and found that lifespan, weight gain, acoustic mate signaling and egg production were maximized on diets with different protein:carbohydrate (P:C) ratios. Another study found that males thrive best on carbohydrate-rich diets as signaling is energetically costly, whereas females require more protein for egg production, showing that the macronutrient requirements optimizing fitness in *Teleogryllus commodus* crickets are sex-specific (Zajitschek *et al.*, 2012). Together these results suggest the potential for trade-offs between fitness conferring traits and other life history traits, especially under different nutritional regimes.

#### *Trade-offs Between Cognition and Other Fitness Conferring Traits*

One piece of the puzzle that has, until recently, largely been missing from this question of how fitness conferring traits trade-off with each other is cognition. That said, recent research on the subject is gaining traction. We now know that an organism's ability to learn can increase their fitness but simultaneously reduce allocation of resources to other traits important for survival and reproduction (Dukas and Bernays, 2000; Del Giudice and Crespi, 2018). For example, fruit flies (*Drosophila melanogaster*) showed a 40% reduction in learning ability when they were long-lived (Burger *et al.*, 2008), and cabbage white butterflies (*Pieris rapae*) with higher cognitive abilities exhibited delayed reproduction (Snell-Rood *et al.*, 2011). However, a study on parasitoid wasps (*Nasonia vitripennis*) found no evidence that learning caused trade-offs in longevity or fecundity, counter to many other studies out there (Liefting *et al.*, 2019). To

further complicate the story, to my knowledge, researchers have yet to examine how diet fits into this equation.

### *Trade-offs in Cricket Fitness Conferring Traits*

Though some say a cognitively adept individual and reproductive efforts, for example, should go hand in hand (Sonnenberg *et al.*, 2019), more research suggests that crickets cannot have the best of both worlds. For example, Anderson and Kasumovic (2017) showed in Australian black field crickets (*Teleogryllus commodus*) that investment in cognitive ability was associated with increased development time, which can negatively influence reproductive efforts if an individual takes a long time to reach sexual maturity. Other research appears scarce on whether trade-offs exist in crickets between cognitive ability and other fitness related traits. There is, however, a substantial amount of research revealing other trade-offs exist between fitness conferring traits. For example, a study that investigated immune function in house crickets (*Acheta domesticus*) found that individuals had a tendency to trade-off adult muscle mass and body weight with immune priming, both of which are vital to effective reproductive efforts and survival. Lailvaux *et al.* (2010) found that male crickets (*Teleogryllus commodus*) exhibit trade-offs between burst performance with jumping ability and endurance performance for signalling, suggesting that locomotion and reproductive success cannot be simultaneously maximized traits. In two different studies, male decorated crickets (*Grylloides sigillatus*) with increased spermatophore production exhibited inferior immunity, revealing a fundamental and commonly seen trade-off between reproduction and immune function (Kerr *et al.*, 2010; Gershman *et al.*, 2010). A study using Mediterranean field crickets (*Gryllus bimaculatus*) also found that individuals pay a cost for their allocation of resources, such that individuals who develop quickly die younger, possibly because of a lower quality soma (Santostefano *et al.*, 2017).

Not only is there evidence of trade-offs in fitness-related traits in crickets, some studies have also shown that diet can affect these trade-offs. When manipulating protein intake throughout the lifespan to mimic lifetime differences in resource acquisition on field crickets (*Teleogryllus commodus*), there was a trade-off between longevity and male signalling. More specifically, males on a high-protein diet developed quicker and with elevated signalling earlier in life, but died sooner than males fed a low-protein diet (Hunt *et al.*, 2004; but see Judge *et al.*, 2008). In the wing-polymorphic cricket (*Gryllus firmus*), females should prefer proteinaceous foods that aid in egg production, but with that comes shorter life expectancy (Clark *et al.*, 2013). A recent study of decorated crickets (*Gryllodes sigillatus*) showed that male encapsulation rate increased with protein intake, while signalling effort was maximized by ingesting carbohydrates (Rapkin *et al.*, 2018). Females, on the other hand, did not experience trade-offs between their immunity and egg production (Rapkin *et al.*, 2018) when diet was manipulated. It is evident through the aforementioned studies that crickets often experience trade-offs in their fitness related traits, and diet can influence these trade-offs. Research is still divided, though, between studies showing positive or negative correlations between fitness-related traits and whether they trade-off with one another. The good genes hypothesis, for example, posits that males with more elaborate traits should be healthier and thereby more likely to attract females and sire more progeny than males with less elaborate traits (Zahavi, 1975; Hamilton and Zuk, 1982). If traits such as a larger body size, louder signalling, and higher overall signalling effort are considered elaborate (which has been shown with Jamaican field crickets (*Gryllus assimilis*; Bertram *et al.*, 2016)), selection should favour a lack of trade-offs, and prefer coherence between traits associated with good condition leading to effective reproduction and survival. I therefore wanted to determine whether trade-offs exist between cognitive ability and fitness traits related to reproduction, or whether they act harmoniously.

### *Focus of Research and Predictions*

To quantify whether trade-offs exist between fitness-related traits and cognitive ability, I used the Texas field cricket as my model organism. I manipulated the balance of proteins to carbohydrates in the diet during male and female field cricket juvenile development as a developmental stress, and then investigated whether fitness proxies traded off with development time, body size, or cognitive ability. The key hypothesis I explored was that juvenile diet influences trade-offs between adult cognitive ability and fitness conferring traits ( $H_1$ ). Because females typically require protein for egg development (Maklakov *et al.*, 2008), I predicted that (1a) females reared on a protein-rich diet would utilize more of these resources for egg production and subsequently have reduced cognitive abilities than females reared on a carbohydrate diet. Because males typically require carbohydrates to fuel signalling (Bertram *et al.*, 2009; Maklakov *et al.*, 2008; Thomson *et al.*, 2014), I predicted that (1b) males reared on a carbohydrate-rich diet would utilize more of these resources to mate attraction signalling and thereby experience reduced adult cognitive function.

## **Methods**

### *Experimental Population*

The experimental individuals I used in this chapter were the same individuals used in my first data chapter. Given this, below I provide only a brief overview, as details about how I created the experimental population, the diets, how I reared the experimental crickets, and how I quantified cognition can be found in my first data chapter. I ran my experiment from March to October 2018 using Texas field crickets (*Gryllus texensis*). Crickets were descendents of adults collected in September 2014 from Smithville, Texas (30.0086° N, 97.1594° W). I placed 50-60 newly hatched individuals into a group rearing bin containing *ad libitum* standard diet (Teklad

Rodent diet #8604, Envigo, Madison WI, USA), water, and shelter, and housed them under a 14:10 hour light:dark cycle at  $28 \pm 2$  °C. My experimental individuals were newly eclosed penultimate juvenile males and females. I weighed each newly eclosed penultimate juvenile using an electronic balance (OHAUS Pioneer Analytical Scale). I then transferred each juvenile into its own individual container (11.5 x 11.5 x 7.5 cm) and randomly assigned it to one of two powdered, holidic diets (carbohydrate rich and protein rich). I quantified the time it took to develop to adulthood. Upon adult eclosion, I reweighed each experimental individual and placed them back on the standard diet (Teklad Rodent diet #8604).

To test whether there were trade-offs between cognitive performance and other life-history traits that influence fitness, I quantified cognitive behaviour and reproductive behaviour. I trained individuals to locate a food reward in a radial arm maze (RAM) over four consecutive days. On the fifth day, I retested the individual to observe its behaviour without the presence of the food reward and associated odours. I quantified two measures of cognition, a duration-based measure (reward latency), and an accuracy-based measure (number of complete entries). Reward latency is the amount of time it took an individual to locate the food reward after emerging from its acclimatization cup. Number of complete entries is the number of times an individual walked down an arm to the food dish before locating the food reward.

To quantify female fecundity, I euthanized the females and then photographed them for body size measurements. I dissected each female's ovaries (n=77) to obtain egg counts *sensu* Danya Yaremchuk's Female cricket dissections protocol in the Bertram Lab Experimental Manuals. I photographed each females' eggs and used ImageJ software (<http://image.j.nih.gov/ij/>) to obtain accurate egg counts (Supplementary Figure 2). To quantify male reproductive behaviour, I measured male acoustic mate attraction signalling behaviour. I recorded male signalling using the Electronic Acoustic Recording System (EARS;

Supplementary Figure 3) over a two-week period starting when males were 1-7 days post adult-molt. Experimental males (n=77) spent 24 hours/day in the EARS with the exception of the Discovery trial (48 hours) and the cognitive training and testing (2 hours/day) on days 5-12. The EARS allowed me to record multiple parameters of male signals, including: number of pulses, pulse duration (ms), number of interpulses, pulse period (ms), pulses per chirp, number of chirps, number of interchirps, chirp period (ms), number of calls, time spent calling, mean amplitude, and mean frequency (Figure 3; McAuley and Bertram, 2016). After 14 days, I weighed the males and then euthanized them by freezing. I later photographed them for body size measurements as described below.

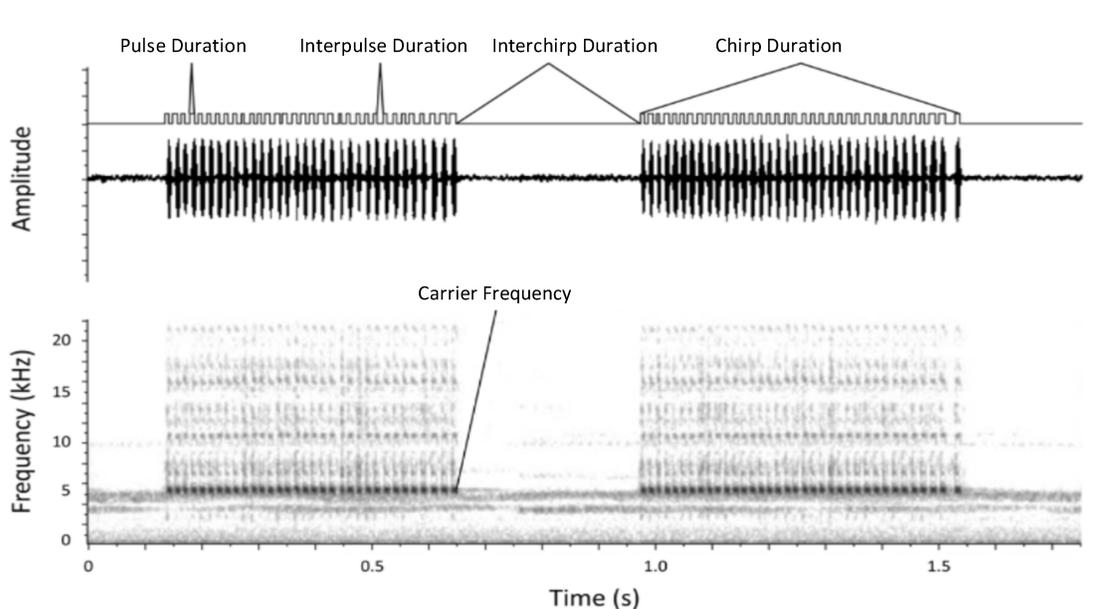


Figure 3. Waveform of two chirps from a male Texas field cricket (*Gryllus texensis*) mate attraction signal displaying some parameters I quantified; pulse duration, interpulse duration, chirp duration, interchirp duration, and carrier frequency.

To obtain adult body size measures, I photographed each individual's body, dorsal side up next to a ruler using a LUMIX digital camera on 0.2X zoom, with the camera attached to a tripod (Supplemental Figure 4). I used ImageJ software to obtain three morphological measures: maximum pronotum length along the anterior-posterior axis, maximum pronotum width and maximum distance between the outer edges of the eyes. I ran a principal component analysis to obtain an overall measure of body size for each sex. The first principal component (PC1 Size) for females explained 67.5% of the variation in pronotum length, pronotum width, and head width (eigenvalue = 2.0238; eigenvector loadings: 0.48-0.63 for the three size variables). The first principal component for males explained 77.1% of the variation in pronotum length, pronotum width, and head width (eigenvalue = 2.3140; eigenvector loadings: 0.55- 0.61 for the three size variables).

#### *Statistical analyses*

I performed all statistical analyses using JMP version 14.0. I investigated the factors influencing the fitness proxies (female fecundity and male time spent calling). I ran a general linear model (GLM) for females. My independent variables included developmental diet, development time, body size and adult cognitive performance, and all possible interactions. Cognitive performance for both reward latency and number of complete entries was based on training trial 4, as this was the trial across most individuals where cognitive performance was considered the highest. Training trial 4 across all treatments (sex and diet) was statistically significant from the test trial in which no evidence of learning, and poor cognitive performance was observed. I therefore considered the last training trial to be the best indicator of cognition to use in this subsequent analysis. I  $\log_{10}$  transformed the dependent variable (egg count) for females to normalize the distribution of data. For males, I split my analysis into two parts. First, I used a nominal logistic regression model to ascertain the factors influencing whether a male

called or not. I categorized 'silent' individuals as those whose Daily Time Spent Calling (TSC) score was less than one minute ( $26/67 = 38.8\%$ ). My independent variables were the same as those described above. Second, I ran a GLM for only the males that called, quantifying how developmental diet, development time, body size, and adult cognitive performance influenced overall signalling effort. I  $\log_{10}$  transformed the dependent variable (time spent calling) for calling males to normalize the distribution of data. The normal quantile (QQ) plots met the assumptions of normality. When the interactions were not significant, I utilized projection-based model reduction techniques and include the simplified version of the models. The full models can be found in the supplementary tables.

## **Results**

### *Factors Influencing Female Fecundity*

My experiment investigated the factors influencing female fecundity in Texas field crickets. Specifically, I examined whether developmental diet, developmental time, body size, cognitive performance (either reward latency or number of complete entries), and their interactions influenced the number of eggs a female carried. I present two reduced models (full models are presented in the supplementary figures) one for each measure of cognition, because both cognition measures were highly correlated and therefore resulted in multicollinearity if I included them in the same model. Female egg count was significantly influenced by development time, as females who matured to adulthood faster carried more eggs (Table 2). Further, there was a significant interaction between body size and development time (Table 2; Figure 4). Specifically, the negative relationship between development time and egg number was much steeper for larger females than it was for smaller females (Figure 4). Female fecundity was not, however, explained by their cognitive behaviour [either reward latency (Table 2a) or number

of complete entries (Table 2b)]. Further, female fecundity was not influenced by the developmental diet that they were reared upon, or any interactions with developmental diet. This result suggests that developmental diet does not influence the steepness of trade-offs between fitness proxies and other cognitive or life history traits.

Table 2. Factors influencing female cricket fecundity. Model (a) includes reward latency as the cognition variable while model (b) includes number of complete entries as the cognition variable. I was unable to include both reward latency and number of complete entries in the same model due to multicollinearity.

Model	Source	df	F	P
a) Reward Latency	Diet	1	0.218	0.6423
	PC1 Size	1	0.261	0.6116
	<b>Development Time</b>	<b>1</b>	<b>4.303</b>	<b>0.0428</b>
	<b>PC1Size*Development Time</b>	<b>1</b>	<b>4.685</b>	<b>0.0349</b>
	Reward Latency	1	0.006	0.9365
b) # Complete Entries	Diet	1	0.229	0.6343
	PC1 Size	1	0.287	0.5941
	<b>Development Time</b>	<b>1</b>	<b>4.754</b>	<b>0.0336</b>
	<b>PC1Size*Development Time</b>	<b>1</b>	<b>4.371</b>	<b>0.0413</b>
	Number Complete Entries	1	0.143	0.7069

\*Indicates a significant effect, where  $p < 0.05$

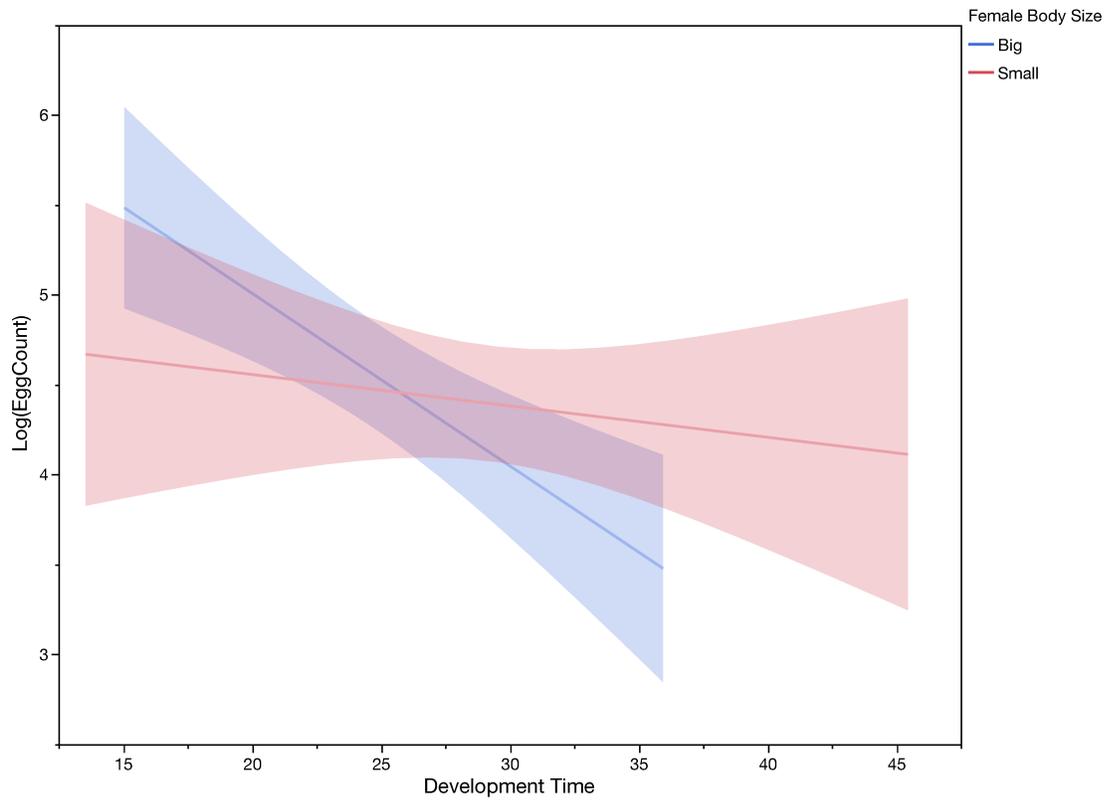


Figure 4. Female Texas field cricket fecundity based on body size classification and development time.

### *Factors Influencing the Probability that a Male Signals for Mates*

My experiment also investigated the factors influencing the probability that a male would adopt a caller strategy and signal for mates, versus adopting a non-caller (silent) satellite strategy. Specifically, I investigated whether developmental diet, developmental time, body size, cognition (either reward latency or number of complete entries), and their interactions influenced the likelihood that male field crickets would adopt a caller versus a non-calling mate attraction strategy. I present two reduced models (full models are presented in the supplementary figures) one for each measure of cognition, because both cognition measures were highly correlated and therefore resulted in multicollinearity if I included them in the same model. The probability that a male adopted a caller strategy was not directly influenced by development time, body size, or cognition variables. However, when I used reward latency as my cognition measure, my nominal logistic regression revealed that the probability of adopting a caller strategy was influenced by a three-way interaction between male size, development time, and cognition (Table 3; Figure 5). Specifically, larger males were more likely to become callers than smaller males, but this was influenced by development time and cognitive performance. Larger males that developed quickly and performed well in the cognitive task had a high probability of becoming a caller. To have a high probability of becoming a caller, all other individuals had to exhibit trade-offs. For example, larger males had to take longer to develop and perform poorly in the cognitive task to have a high probability of becoming a caller. Similarly, smaller males had to develop quickly (regardless of their cognitive performance) or if they exhibited higher cognitive performance, they had to trade this off with a longer development time. These complex interactions suggest trade-offs exist between the fitness related measures of signalling strategy, development, body size, and cognition in all but the largest, fastest developing, and seemingly brightest males. The probability that a male would adopt a non-calling silent strategy versus a caller strategy was not

influenced by the developmental diet that they were reared upon, nor any interactions between developmental diet and other life history traits. This result suggests that developmental diet does not influence the steepness of trade-offs between fitness proxies and other cognitive or life history traits.

Table 3. Nominal logistic regression model investigating the factors influencing the probability that a male will adopt a caller or a non-calling satellite strategy. Model (a) includes reward latency as the cognition variable while model (b) includes number of complete entries as the cognition variable. I was unable to include both reward latency and number of complete entries in the same model due to multicollinearity.

Model	Source	ChiSquare	P
(a) Reward Latency	PC1Size	0.6603	0.4165
	Diet	0.6238	0.4296
	Development Time	0.1285	0.7200
	Reward Latency	2.2359	0.1348
	PC1Size*Development Time	0.0083	0.9274
	PC1Size*Reward Latency	1.1834	0.2767
	<b>Development Time*Reward Latency</b>	<b>4.0500</b>	<b>0.0422</b>
	<b>PC1Size*Development Time*Reward Latency</b>	<b>5.7644</b>	<b>0.0164</b>
(b) # Complete Entries	PC1Size	1.0259	0.3111
	Diet	0.1172	0.7320
	Development Time	0.0159	0.8998
	Number Complete Entries	0.3569	0.5502

\*Indicates an interaction between variables

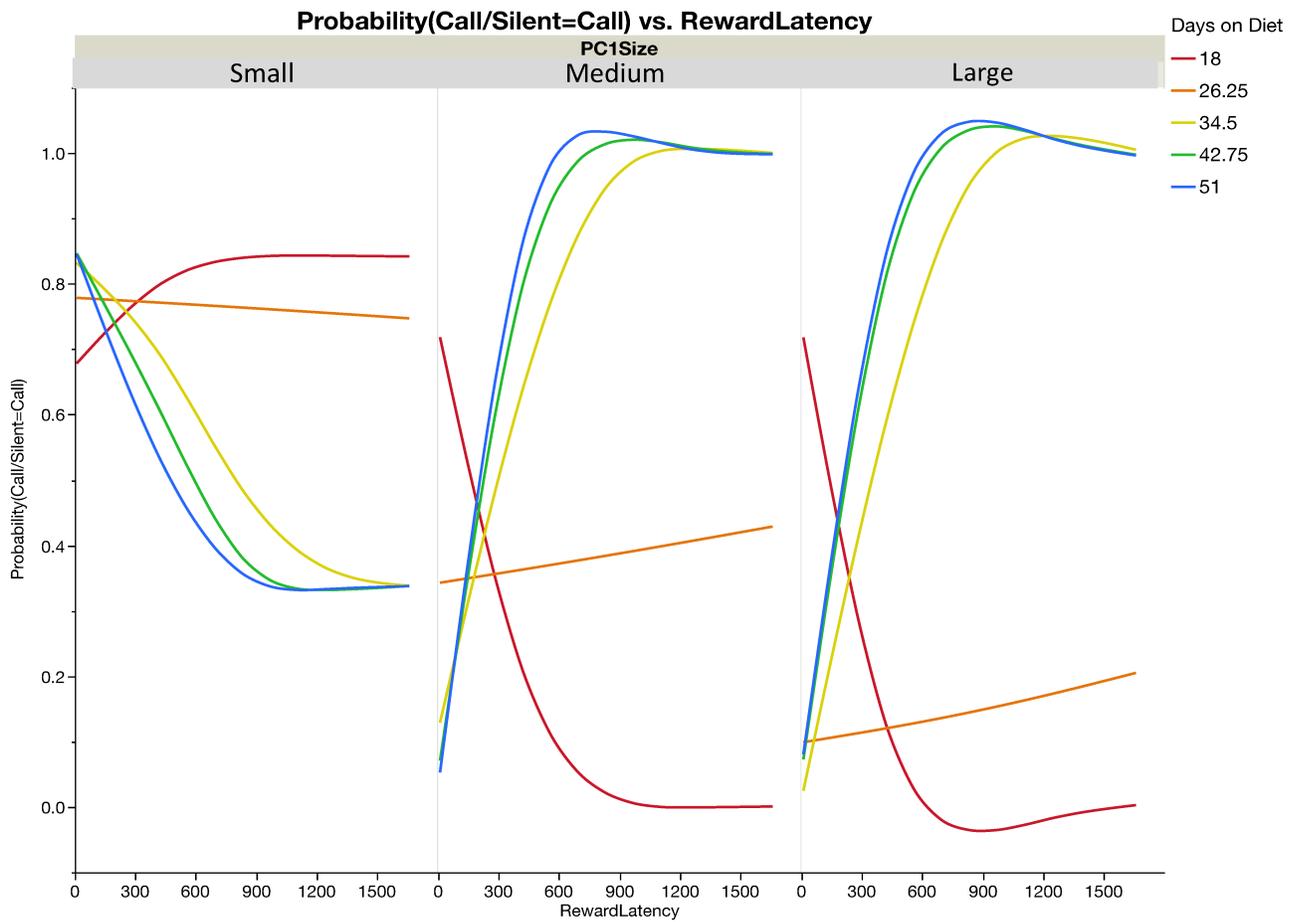


Figure 5. Nominal logistic regression model displaying a male field cricket’s probability of calling or staying silent relative to their size, development time, and latency to find a food reward in cognition training.

### *Factors Influencing Time Spent Calling for Males that Adopted a Caller Strategy*

For males that adopted a caller strategy (those who signalled for at least a minute a day), I ran general linear models to investigate the factors influencing how much time they spent calling. I used time spent calling as my proxy for fitness, as calling time is a strong proxy of reproductive success in Texas field crickets (Cade and Cade, 1992). Specifically, I investigated how time spent calling was influenced by developmental diet, development time, body size, and cognition variables (reward latency or number of complete entries). I present two reduced models (full models are presented in the supplementary figures) one for each measure of cognition, because both cognition measures were highly correlated and therefore resulted in multicollinearity if I included them in the same model. A trade-off occurred between male signalling effort and cognitive ability, but this relationship was dependent on other variables. For example, time spent signalling was significantly influenced by an interaction between the latency to find the food reward in the maze and male development time (Table 4). Specifically, slow developing males spent more time signalling only if they were poor cognitive performers. Fast developing males spent more time signalling if they were also strong cognitive performers (Figure 6a). Time spent signalling was also significantly influenced by an interaction between number of complete entries and body size. Specifically, larger males only signalled with high effort when they were fairly poor at locating the food reward (Table 5; Figure 6b). Time spent calling for smaller males was not contingent on their cognitive performance.

Similar to my aforementioned analyses, time spent calling was not influenced by the developmental diet that males were reared upon, nor any interactions between developmental diet and other life history traits. This finding suggests that developmental diet does not influence the steepness of trade-offs between fitness proxies and other cognitive or life history traits.

Table 4. Reduced general linear model displaying Time Spent Calling (TSC) over male Texas field cricket's lifetime and how it is influenced by size, diet, development time, reward latency, and their interactions.

Source	df	F	P
Diet	1	0.2504	0.6225
PC1Size	1	2.0948	0.1641
Development Time	1	2.5695	0.1254
Reward Latency	1	0.0001	0.9933
Diet*PC1Size	1	3.4728	0.0779
Diet*Development Time	1	2.4002	0.1378
PC1Size*Development Time	1	0.0827	0.7768
Diet*Reward Latency	1	0.1455	0.7071
PC1Size*Reward Latency	1	4.3740	0.0502
<b>Development Time*Reward Latency</b>	<b>1</b>	<b>5.1721</b>	<b>0.0347</b>
Diet*PC1Size*Reward Latency	1	4.0132	0.0596
Diet*Development Time*Reward Latency	1	4.0464	0.0587

Table 5. Reduced general linear model displaying Time Spent Calling (TSC) over male Texas field cricket's lifetime and how it is influenced by size, diet, development time, number of complete entries, and their interactions.

Source	df	F	P
Diet	1	1.9703	0.1758
PC1Size	1	1.4336	0.2452
Development Time	1	0.8851	0.3580
Number Complete Entries	1	1.7311	0.2032
Diet*PC1Size	1	0.0782	0.7827
Diet*Development Time	1	0.0049	0.9450
PC1Size*Development Time	1	0.4279	0.5205
Diet*Number Complete Entries	1	2.7192	0.1148
<b>PC1Size*Number Complete Entries</b>	<b>1</b>	<b>4.4848</b>	<b>0.0469</b>
Development Time*Number Complete Entries	1	0.1302	0.7220
Diet*Development Time*Number Complete Entries	1	1.1611	0.2941

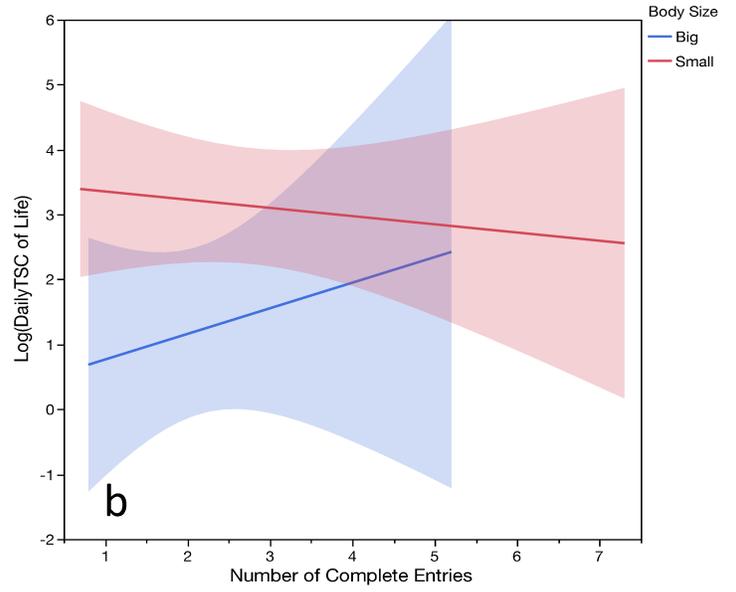
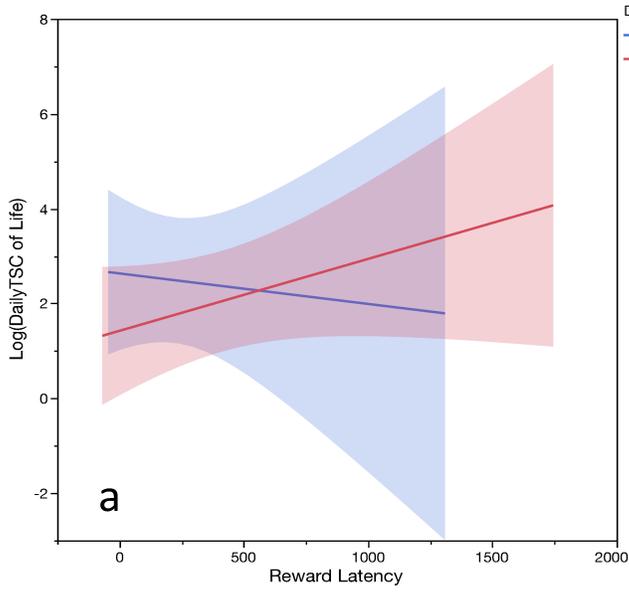


Figure 6. How the interaction between (a) development time and reward latency, and (b) size and number of complete entries influence male Texas field cricket time spent calling (TSC).

## Discussion

### *Female Fecundity*

Female fecundity was influenced by both development time and body size. Fast developers contained more eggs than slow developers, but this relationship was more pronounced in larger females (Table 2; Figure 4). These results lead me to believe that females of this species exhibit a positive relationship between many fitness-related traits, as regardless of size, if they grew faster, they were more fecund. Surprisingly, female fecundity was not influenced by the developmental diet. Given that chapter 1 suggests that both the high carbohydrate and the high protein developmental diets may have been stressful, females who were able to develop quickly on these diets may have had the resources available to produce more eggs. Fecundity was not influenced by female cognitive ability. Because both juvenile diets appeared stressful, females may have been forced to allocate the majority of their resources to developing quickly and producing eggs at the expense of their cognitive ability. Conversely, it may be that female reproductive performance is not affected by learning. In support of this idea, a recent study on male and female nematodes (*Caenorhabditis remanei*) found that female reproductive performance was not affected by learning performance (Zwoinska *et al.*, 2016).

### *Male Calling Strategies and Signalling Effort*

The probability of a male adopting a caller strategy was influenced by a three-way interaction between body size, development time, and reward latency (Table 3; Figure 5). Males who developed quickly and performed well in the cognitive tasks were more likely to adopt a caller strategy. However, other males had to exhibit trade-offs between fitness conferring traits to have a high probability of adopting a caller strategy. These results suggest that a small number of individuals may be able to do it all: grow fast, be large at adulthood, perform well on cognitive tasks, and adopt a caller strategy. This is synonymous with the good genes hypothesis, which

states that only males in good condition can afford the costs of elaborate ornamentation required to attract mates (Zahavi, 1975). This hypothesis suggests that the cost of producing larger, more elaborate secondary sexual traits will be marginal for males that are of higher quality (Zahavi, 1975; Iwasa and Pomiankowski, 1999). Given females are more attracted to callers than non-calling satellite males, the caller strategy typically results in elevated fitness.

It is important to note, however, that the vast majority of males seemed to exhibit trade-offs among their fitness conferring traits, such that they could not simultaneously maximize adopting a caller strategy with shorter development times, larger body sizes and higher cognitive abilities. Smaller males may have utilized juvenile diet nutrients to grow fast and fuel signalling at the expense of cognitive performance. Conversely, larger males may have grown slowly to fuel signalling behaviours, possibly because they already had a size advantage over smaller males. This idea is intriguing as Kelly et al. (2013) suggests that often times animal fitness benefits from a quick transition from the juvenile to adult life stage, and at a larger size. However, a study from our lab by Harrison et al. (2014) showed that in the spring field cricket (*Gryllus veletis*), it did not matter what carbohydrate:protein ratio in the diet males consumed, signalling parameters were robust as long as they consumed a lot of the diet they were given. Although this work by Harrison et al. (2014) is referring to male signalling and not just adopting a caller versus non-caller strategy, I infer that the likelihood of a male calling would also be related to factors like development time and body size.

For callers, overall signalling effort was influenced by an interaction between either development time or body size and cognitive ability. Males that developed quickly and exhibited strong cognitive performance also spent more time signalling for mates (Figure 6). This finding lends further support to the hypothesis that high condition individuals can afford the costs of bearing elaborate traits. However, to maximize signalling effort, all other individuals exhibited

trade-offs between signalling effort and cognitive effort, body size, or both. Male black scavenger flies (*Sepsis thoracica*) exhibit trade-offs between size and immunity, such that smaller males are healthier but larger males likely have higher reproductive success (Busso *et al.*, 2017). These results are interesting because they suggest that trade-offs may be dependent on development time, as slow developing males appear to trade-off fitness traits while fast developing males have a positive relationship between them, possibly revealing that males allocate resources to fitness-related traits differently depending on their ability to process nutrients. These results tie into the good genes hypothesis in that only good quality males should be able to afford to heavily invest in elaborate ornamentation (Hamilton and Zuk, 1982). Slowly developing males may have sacrificed a healthy soma on top of poorer cognition in order to maximize their signalling effort in comparison to their fast developing counterparts, showing that maybe some individuals can ‘have it all’, while others appear to struggle. Our lab supported this idea in a study that utilized Jamaican field crickets (*Gryllus assimilis*). We showed that females preferred to mate with males that had overall better condition: they were larger, heavier, and had more elaborate sexual signals (Bertram *et al.*, 2016). Prior to this, another study from our laboratory on the same species found that when feeding males either unlimited or limited amounts of food during the juvenile life stage, males that were fed unlimited food had a higher mass and signalled longer and louder (Whattam and Bertram, 2011).

### *Diet Effects*

Surprisingly, diet did not significantly impact fecundity. I was surprised by this result because protein availability in adult females is crucial to egg production (Harrison *et al.*, 2014). These differences might have occurred because my crickets received their dietary treatment only during development while Harrison *et al.*'s (2014) crickets received their dietary treatment only during adulthood. However, in opposition to this idea, another study using Mormon crickets

(*Anabrus simplex*) found no association between the number of eggs oviposited and dietary treatment, regardless of the life-stage the diet was presented to females (Srygley, 2017), so there is a degree of inconclusivity surrounding how diet influences female reproductive traits. It is important to note, however, that many of these studies observe the effects of a proteinaceous diet during the adult life stage of invertebrates, as it is proposed that egg production occurs during adulthood, and not prior to.

Diet also did not significantly influence whether a male adopted a non-calling satellite strategy or a caller strategy. This result was somewhat perplexing, as a plethora of other studies have shown otherwise (e.g., Kaspi *et al.*, 2002; Hunt *et al.*, 2004; Bertram *et al.*, 2006, Bertram *et al.*, 2009). However, Gray and Eckhardt (2001) similarly found that male song was unaffected by dietary treatment in an experiment using the Texas field cricket. Harrison *et al.* (2014) also found that protein versus carbohydrate intake during adulthood did not influence male time spent signalling in the spring field cricket (*Gryllus veletis*); instead, males simply needed to consume a lot of either diet.

Only a small subset of males were able to maximize all their fitness conferring traits simultaneously. For most others, they were unable to simultaneously maximize all their fitness conferring traits on either the protein- or carbohydrate-rich diets; instead, many experienced trade-offs between fitness proxies, cognitive ability, development time and/or body size. These trade-offs may have been less apparent had I also included a control diet that was rich in both proteins and carbohydrates. In fact, a study from our lab showed that when given a choice, crickets (*Gryllus veletis* and *Gryllus assimilis*) selected diets that maximized their lifespan as well as their reproductive performance (Harrison *et al.*, 2014). This supports the idea that the optimal diet for any individual is not that of extreme protein or carbohydrate intake, but varying levels of each macronutrient that may change with environmental pressures and life stage.

### *Future Directions*

In the wild, animals live in an ever-changing environment, and as such it is expected that their nutritional needs will change (Bazazi *et al.*, 2016). Depending on how these needs change, organisms may be forced to differentially allocate resources to maximize one, but not all, traits important for fitness. In the case of females, it could be that a diet effect was not detected in predicting fecundity and cognition trade-offs because female crickets may only produce eggs after their adult molt. If this is the case, adult diet is likely to be a more important factor in predicting egg count. However, as my research focused on the developmental stress hypothesis and therefore utilized diet manipulation during the juvenile life stage, I could not manipulate female adult diet to further test this idea. I had a working assumption that egg production begins before the female's adult molt, an assumption that should be tested.

Though longevity was not tested in this study, it would also be interesting to quantify whether females exhibit trade-offs between fecundity or cognitive abilities with lifespan, when resources are limited individuals are expected to trade-off the cost of one trait with another (Kerr *et al.*, 2010). As individuals were euthanized approximately two weeks post-adult molt, it would be interesting to observe how developmental diets influence the entire lifespan, as the diet manipulation in this case comprised about 50% of their lifespan. In my study, female resources were limited to either a reduced carbohydrate or reduced protein diet, and as such reduced longevity may have been observed if either cognition or fecundity was maximized. However, as females are known to invest heavily in reproduction, and females can produce more offspring if they live longer, it may be that this species exhibits a positive correlation between reproduction and longevity (Bateman, 194; De Loof, 2011; Rapkin *et al.*, 2018).

It is possible that the trade-offs that I observed in males were the result of the stressful diets, as individuals experienced either elevated carbohydrates or elevated protein during

development. Future work should aim to uncover whether males are more likely to exhibit trade-offs in fitness conferring traits under these stressful nutrient conditions compared when they are reared on a control diet that is rich in both carbohydrates and protein. Had I suspected I may have obtained a higher proportion of individuals who were able to simultaneously maximize their signalling effort or fecundity, their body size and their cognitive scores.

Looking into how males' trade-off cognitive ability and another fitness trait important to survival, such as spermatophore quality, would also be a fascinating way to observe whether males differentially allocate resources, or just have superior condition across the board. There is plenty of research showing that males typically favour a 'live fast, die young' strategy, as they prioritize reproduction over immunity and longevity (Trivers, 1972; Rolff 2002; Rapkin *et al.*, 2018). Finding support for this would also support my current findings and suggest that males allocate resources almost entirely to reproductive efforts, like signalling, and focus less energy towards cognitive ability, a trait that should also be important to survival.

## General Conclusions

Suboptimal nutrient intake is one of the most common experiences animals face during critical points in development (Bonaparte *et al.*, 2011), adding to my motive to investigate whether this nutrient availability during development influences cognitive ability (1<sup>st</sup> data chapter), and whether nutrient availability during development results in trade-offs between fitness proxies, cognition, and other life history traits (2<sup>nd</sup> data chapter). In my first experiment I found that individuals successfully learned the location of the food reward in a radial arm maze over the course of the four training trials. Counter to my expectations, however, neither sex nor developmental diet influenced cricket cognitive ability. Albeit puzzling, carbohydrates are important for fueling energetically demanding activities, such as cognitive exertion during training, so individuals fed carbohydrate-rich diets may have been able to designate a greater proportion of their carbohydrate rich nutrients to fueling cognitive brain power (Bertram *et al.*, 2009). Conversely, protein-biased individuals may have been more motivated to increase their carbohydrate intake with a carbohydrate-biased food reward, and as such initially showed faster learning within the RAM until a food cue was no longer present. If both of these scenarios occurred simultaneously, one might expect no significant effect of diet on learning during training.

Interestingly, and counter to my previous research, when the food reward was removed and no olfactory cues were present, crickets performed as poorly as they did on the first training trial. This may have occurred because both the protein rich and the carbohydrate rich diet I used during development were stressful to individuals. Both diets may have been developmentally stressful to individuals because excess protein or carbohydrates during development can negatively impact fitness (Raubenheimer and Simpson, 1997). This stress during development may have limited male and female ability to learn a cognitive task once the olfactory cues were

removed. Albeit perplexing that diet did not significantly influence cognition, I still consider myself to have found tangential support for the developmental stress hypothesis through my first experiment. Crickets learn, but it appears that developmental diet truly needs to be optimal in order to foster this learning into long-term memory. Future research should rear individuals on varying protein to carbohydrate-biased diets, as well as on a controlled standard diet, to carefully tease apart this hypothesis further.

My second experiment showed the complexity of studying trade-offs associated with fitness proxies. Female fecundity was influenced by body size and development time, with faster developers having more eggs, especially in larger females. If the developmental diets were stressful to the crickets, as suggested in my first data chapter, females who developed more quickly would have spent less time consuming the stressful diets. They would also have been switched back to a standard diet post-adult molt sooner than slowly developing females. This shift in nutrient intake, even over a short time as adults, could have reversed the adverse consequences of being on either extreme juvenile diet, as was found by a grasshopper study (*Ageneotettix deorum*; Joern and Behmer, 1997). Similar to what I observed in my study, other studies also provide evidence that growth and early fecundity can be beneficial to fitness (e.g., in the female eastern grey kangaroo *Macropus giganteus*; Quesnel *et al.*, 2018).

Developmental diet did not influence the likelihood that a male would adopt a caller strategy. Instead, I found that to have a high probability of adopting a caller strategy a male either had to be large, develop quickly, and perform well in the cognitive task, or exhibit trade-offs between these fitness conferring traits. For the males that adopted a caller strategy, their time spent calling was influenced by interactions between cognition and development time (for reward latency) and cognition and body size (for number of complete entries). Males that developed quickly and expressed strong cognitive behaviours also spent a large amount of time

signalling for mates. For all others, high signalling time only occurred if there was a trade-off with the other fitness conferring traits. Similar to females, it could have been that males who did not spend as much time on a 'stressful' diet somewhat reversed the effects of their developmental factors to adequately fuel signalling on a more balanced diet. On the other hand, diet may not have significantly influenced male signalling as another study found that male nutrient intake is not as important for signalling compared to the amount of food that is consumed (Harrison *et al.*, 2014). Kokko (1998) suggested that trade-offs may arise not only because individuals differ in how they allocate resources, such as dietary intake, but also in acquiring condition. Within this study I largely influenced the condition individuals would acquire by manipulating their juvenile diet. That being said, the good genes hypothesis further suggests that males in better condition, regardless of the assignment of a high-protein or high-carbohydrate diet, should be better able to handle the stress of those manipulations and prevent those trade-offs from occurring. My results suggest evidence for both the good genes hypothesis and trade-offs. Larger males who developed quickly and performed well on the cognition trials were more likely to develop a caller strategy, providing evidence that some males can simultaneously possess all the traits necessary for elevated fitness. Only a handful of individuals should, however, be able to afford these costs. For all others, if they displayed elaborate secondary sexual traits, they did not seem to be able to afford the cost of maximizing the other fitness conferring traits; they therefore exhibited trade-offs. Together these findings suggest that some males are in overall better condition, while others must trade-off fitness-related traits when under stressful conditions.

To my knowledge, my study is the first to show the full impact developmental diet can have on adult cricket fitness related traits and the trade-offs associated with cognition and how these traits are fostered. Future research should investigate whether these trade-offs occur when

individuals are reared on a standardized diet containing normal levels of protein and carbohydrates, to better compare the effects of dietary treatment on fitness trade-offs. When given a choice, animals will often regulate their relative intake of proteins and carbohydrates (Raubenheimer and Simpson, 1997; Boersma and Elser, 2006).

I have data on how female fecundity and male signalling were influenced by developmental diet. These individuals were not placed in the cognition experiment. By comparing the behaviour of individuals that were not cognitively tested to the cognitively tested individuals, I should be able to determine how fecundity, caller/satellite strategy, and time spent signalling for callers is impacted by cognitive training. In this way, I will be able to quantify whether diet has a more significant effect on fecundity and signalling when subjects do not have the additional stress of cognitive testing in radial arm mazes.

Ultimately, more research is required to delineate the factors driving variation in cricket cognition, other fitness conferring traits, and their trade-offs. Studies investigating how diet influences insect fitness and cognition are also important, so that researchers can understand how organisms are responding to everchanging human-influenced shifts in resource availability.

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## Supplementary Material



Supplementary Figure 1: Aerial view of a radial arm maze showing six radial arms extending from a central platform. Each arm had a unique colour and marking, and a metal food dish that sat flush with the floor of the maze.

Supplementary Table 1: Relationship between sex, diet, trial number, reward colour, latency to emerge, discovery motivation, chirps during test and cognition measures for male and female

Texas field crickets

Variable	Term	Estimate	Std Error	DFDen	t Ratio	Prob> t
Log(RewardLatency)	<b>Intercept</b>	<b>4.919</b>	<b>0.156</b>	<b>151</b>	<b>31.47</b>	<b>&lt;.0001</b>
	Trial[1]	0.173	0.108	499	1.60	0.1094
	Trial[2]	0.008	0.107	500	0.07	0.9420
	Trial[3]	-0.211	0.109	503	-1.93	0.0547
	<b>Trial[4]</b>	<b>-0.237</b>	<b>0.109</b>	<b>505</b>	<b>-2.16</b>	<b>0.0309</b>
	Diet[Carbohydrate]	0.029	0.065	130	0.44	0.6602
	Sex[Female]	-0.011	0.064	131	-0.18	0.8608
	Colour[Blue]	-0.076	0.124	130	-0.61	0.5399
	<b>Colour[Green]</b>	<b>-0.397</b>	<b>0.194</b>	<b>137</b>	<b>-2.05</b>	<b>0.0425</b>
	Colour[Orange]	-0.053	0.128	136	-0.41	0.6811
	Colour[Red]	0.218	0.143	128	1.53	0.1288
	Colour[White]	0.179	0.167	128	1.07	0.2855
	Emerge Latency	-3.19e-6	<0.001	558	-0.02	0.9608
	Discovery	0.005	0.017	130	0.31	0.7603
	Motivation					
	Log(#CompEntries)	Chirp During Test [No]	0.091	0.086	135	1.06
<b>Intercept</b>		0.947	0.070	153	13.58	<.0001
<b>Trial[1]</b>		<b>0.110</b>	<b>0.051</b>	<b>503</b>	<b>2.17</b>	<b>0.0304</b>
Trial[2]		-0.030	0.050	504	-0.59	0.5529
<b>Trial[3]</b>		<b>-0.164</b>	<b>0.051</b>	<b>506</b>	<b>-3.20</b>	<b>0.0015</b>
<b>Trial[4]</b>		<b>-0.122</b>	<b>0.051</b>	<b>509</b>	<b>-2.38</b>	<b>0.0177</b>
Diet[Carbohydrate]		-0.051	0.023	132	-1.77	0.0798
Sex[Female]		0.021	0.023	133	0.74	0.4604
Colour[Blue]		-0.084	0.055	131	-1.53	0.1292
Colour[Green]		-0.039	0.086	140	-0.45	0.6537
Colour[Orange]		-0.068	0.057	138	-1.20	0.2322
Colour[Red]		0.056	0.063	130	0.88	0.3780
<b>Colour[White]</b>		<b>0.152</b>	<b>0.074</b>	<b>130</b>	<b>2.05</b>	<b>0.0428</b>
<b>Emerge Latency</b>		<b>-0.0001</b>	<b>&lt;0.001</b>	<b>538</b>	<b>-2.28</b>	<b>0.0231</b>
Discovery		-0.010	0.008	131	-1.20	0.2310
Motivation						
Chirp During Test [No]	0.055	0.038	137	1.43	0.1548	

Supplementary Table 2. Relationship between sex, diet, trial number and all possible interactions, as well as four covariates, with reward latency and number of complete entries cognition measures.

<b>Variable</b>	<b>Source</b>	<b>PValue</b>	
Log(RewardLatency)	<b>Trial</b>	<b>0.0114</b>	
	Sex	0.8404	
	Diet	0.6688	
	Trial*Sex	0.9072	
	Trial*Diet	0.4450	
	Sex*Diet	0.7690	
	Trial*Sex*Diet	0.7531	
	Colour	0.2795	
	Chirp During Test	0.3249	
	Discovery Motivation	0.7807	
	Emerge Latency	0.8972	
	Log(#CompEntries)	Trial	<0.0001
		Sex	0.4977
Diet		0.0887	
Trial*Sex		0.6436	
Trial*Diet		0.3625	
Sex*Diet		0.6739	
Trial*Sex*Diet		0.8922	
Colour		0.1800	
Chirp During Test		0.1834	
Discovery Motivation		0.2508	
Emerge Latency		0.0266	



Supplementary Figure 2. ImageJ software used to count female eggs that were raised on either a protein or carbohydrate-rich diet for fecundity measure.



Supplementary Figure 3. Electronic Acoustic Recording System (EARS) used to record multiple parameters of male cricket signalling; also used to house males and females individually for duration of cognition experiment.



Supplementary Table 3. General linear model of relationship between female cricket egg count and diet, PC1 size, development time, reward latency as a cognition measure, and all possible interactions, along with reward colour and emerge latency.

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	6.605	0.757	8.73	<.0001
Diet [Carbohydrate]	-0.025	0.174	-0.15	0.8851
PC1Size	-0.198	0.122	-1.61	0.1148
Development Time	-0.078	0.029	-2.71	0.0101
Reward Latency	7.258e-5	0.001	0.11	0.9125
Diet[Carbohydrate]*(PC1Size-0.07534)	0.056	0.114	0.49	0.6265
Diet[Carbohydrate]*(Development Time-27.4)	-0.019	0.027	-0.68	0.4994
<b>(PC1Size-0.07534)*(Development Time-27.4)</b>	<b>-0.045</b>	<b>0.017</b>	<b>-2.62</b>	<b>0.0127</b>
Diet[Carbohydrate]*(PC1Size-0.07534)*(Development Time-27.4)	0.011	0.018	0.62	0.5398
Diet[Carbohydrate]*(Reward Latency-258.624)	-0.0002	0.001	-0.30	0.7639
(PC1Size-0.07534)*(Reward Latency-258.624)	-0.001	0.001	-1.37	0.1782
Diet[Carbohydrate]*(PC1Size-0.07534)*(Reward Latency-258.624)	-0.0004	0.001	-0.78	0.4412
(Development Time-27.4)*(Reward Latency-258.624)	-9.741e-5	0.0001	-0.82	0.4184
Diet[Carbohydrate]*(Development Time-27.4)*(Reward Latency-258.624)	-4.513e-5	0.0001	-0.35	0.7263
(PC1Size-0.07534)*(Development Time-27.4)*(Reward Latency-258.624)	0.00004	6.079e-5	0.74	0.4666
Diet[Carbohydrate]*(PC1Size-0.07534)*(Development Time-27.4)*(Reward Latency-258.624)	-0.0001	6.787e-5	-0.85	0.3986
<b>Colour[Blue]</b>	<b>0.675</b>	<b>0.294</b>	<b>2.29</b>	<b>0.0274</b>
<b>Colour[Green]</b>	<b>-0.900</b>	<b>0.424</b>	<b>-2.12</b>	<b>0.0404</b>
Colour[Orange]	-0.363	0.275	-1.32	0.1944
Colour[Red]	0.207	0.284	0.73	0.4717
Colour[White]	-0.003	0.437	-0.01	0.9940
Emerge Latency	-0.0003	0.0003	-0.96	0.3436

Supplementary Table 4. General linear model of relationship between female cricket egg count and diet, PC1 size, development time, number of complete entries as a cognition measure, and all possible interactions, along with reward colour and emerge latency.

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	5.707	0.754	7.57	<.0001
Diet [Carbohydrate]	-0.147	0.158	-0.93	0.3595
PC1Size	-0.129	0.117	-1.11	0.2753
<b>Development Time</b>	<b>-0.054</b>	<b>0.026</b>	<b>-2.09</b>	<b>0.0435</b>
Number Complete Entries	0.069	0.117	0.60	0.5553
Diet[Carbohydrate]*(PC1Size-0.07534)	0.017	0.116	0.15	0.8827
Diet[Carbohydrate]*(Development Time-27.4)	-0.025	0.025	-1.01	0.3209
<b>(PC1Size-0.07534)*(Development Time-27.4)</b>	<b>-0.060</b>	<b>0.016</b>	<b>-3.70</b>	<b>0.0007</b>
Diet[Carbohydrate]*(PC1Size-0.07534)*(Development Time-27.4)	-0.001	0.018	-0.06	0.9510
Diet[Carbohydrate]*(Number Complete Entries-2.5)	0.083	0.120	0.69	0.4939
(PC1Size-0.07534)*(Number Complete Entries-2.5)	0.076	0.097	0.79	0.4352
Diet[Carbohydrate]*(PC1Size-0.07534)*(Number Complete Entries-2.5)	0.118	0.097	1.22	0.2311
(Development Time-27.4)*(Number Complete Entries-2.5)	0.030	0.022	1.36	0.1822
Diet[Carbohydrate]*(Development Time-27.4)*(Number Complete Entries-2.5)	0.031	0.024	1.27	0.2102
(PC1Size-0.07534)*(Development Time-27.4)*(Number Complete Entries-2.5)	0.015	0.013	1.12	0.2694
Diet[Carbohydrate]*(PC1Size-0.07534)*(Development Time-27.4)*(Number Complete Entries-2.5)	0.001	0.014	0.06	0.9561
Colour[Blue]	0.581	0.288	2.02	0.0506
<b>Colour[Green]</b>	<b>-0.820</b>	<b>0.396</b>	<b>-2.07</b>	<b>0.0452</b>
Colour[Orange]	-0.269	0.258	-1.04	0.3047
Colour[Red]	0.025	0.266	0.09	0.9270
Colour[White]	0.364	0.480	0.76	0.4527
Emerge Latency	-0.0005	0.0003	-1.54	0.1325

Supplementary Table 5. Nominal logistic regression model of the relationship between male Texas field cricket's likelihood to call and their size, diet, development time, cognition (number of complete entries), and the interaction between size, development time and cognition influencing male field cricket signalling.

Cognition Variable	Source	ChiSquare	P
Number Complete Entries	PC1Size	0.7701	0.3802
	Diet	0.0035	0.9529
	Development Time	0.2843	0.5939
	Number Complete Entries	0.0006	0.9803
	PC1Size*Development Time	0.8772	0.3490
	PC1Size*Number Complete Entries	0.1314	0.7170
	PC1Size*Development Time*Number Complete Entries	0.8509	0.3563

\*Indicates an interaction between variables

Supplementary Table 6. General linear mixed model showing how male Texas field cricket time spent signalling is influenced by diet, body size, development time, reward latency, and their interactions.

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-4.781	4.985	-0.96	0.3518
Diet[Carbohydrate]	0.311	0.753	0.41	0.6846
PC1Size	1.913	1.415	1.35	0.1950
Diet[Carbohydrate]*(PC1Size+0.19633)	-2.418	1.415	-1.71	0.1067
Development Time	0.224	0.171	1.31	0.2074
Diet[Carbohydrate]*(Development Time-31.2188)	-0.231	0.171	-1.35	0.1950
(PC1Size+0.19633)* (Development Time-31.2188)	-0.012	0.192	-0.06	0.9513
Diet[Carbohydrate]*(PC1Size+0.19633)* (Development Time-31.2188)	0.011	0.192	0.06	0.9558
Reward Latency	-0.0004	0.002	-0.16	0.8763
Diet[Carbohydrate]*(Reward Latency-345.969)	0.001	0.002	0.40	0.6909
(PC1Size+0.19633)* (Reward Latency-345.969)	0.009	0.005	1.90	0.0763
Diet[Carbohydrate]*(PC1Size+0.19633)* (Reward Latency-345.969)	-0.009	0.005	-1.82	0.0874
(Development Time-31.2188)* (Reward Latency-345.969)	0.001	0.001	1.85	0.0822
Diet[Carbohydrate]*(Development Time-31.2188)* (Reward Latency-345.969)	-0.001	0.001	-1.61	0.1269
(PC1Size+0.19633)* (Development Time-31.2188)* (Reward Latency-345.969)	-0.0001	0.001	-0.20	0.8437
Diet[Carbohydrate]*(PC1Size+0.19633)* (Development Time-31.2188)* (Reward Latency-345.969)	0.0002	0.001	0.24	0.8122

Supplementary Table 7. General linear mixed model showing how male Texas field cricket time spent signalling is influenced by diet, body size, development time, number of complete entries, and their interactions.

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	5.486	3.440	1.59	0.1303
Diet[Carbohydrate]	0.211	0.781	0.27	0.7905
PC1Size	0.126	0.975	0.13	0.8988
Diet[Carbohydrate]*(PC1Size+0.19633)	-0.660	0.975	-0.68	0.5077
Development Time	-0.111	0.098	-1.13	0.2769
Diet[Carbohydrate]*(Development Time-31.2188)	0.066	0.098	0.67	0.5104
(PC1Size+0.19633)* (Development Time-31.2188)	0.116	0.103	1.13	0.2749
Diet[Carbohydrate]*(PC1Size+0.19633)* (Development Time-31.2188)	-0.126	0.103	-1.23	0.2383
Number Complete Entries	0.303	0.640	0.47	0.6422
Diet[Carbohydrate]*(Number Complete Entries-2.46875)	0.693	0.640	1.08	0.2945
(PC1Size+0.19633)* (Number Complete Entries-2.46875)	1.334	0.679	1.96	0.0670
Diet[Carbohydrate]*(PC1Size+0.19633)* (Number Complete Entries-2.46875)	-0.671	0.679	-0.99	0.3377
(Development Time-31.2188)* (Number Complete Entries-2.46875)	0.158	0.097	1.64	0.1213
Diet[Carbohydrate]*(Development Time-31.2188)* (Number Complete Entries-2.46875)	-0.083	0.097	-0.86	0.4016
(PC1Size+0.19633)* (Development Time-31.2188)* (Number Complete Entries-2.46875)	-0.002	0.092	-0.02	0.9829
Diet[Carbohydrate]*(PC1Size+0.19633)* (Development Time-31.2188)* (Number Complete Entries-2.46875)	0.064	0.092	0.70	0.4946