HOW DIFFERENCES IN DIETARY PHOSPHORUS CONTENT INFLUENCE INSECT FITNESS

By

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A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Master of Science

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Ottawa-Carleton Institute of Biology
Carleton University
Ottawa, Ontario
August 30, 2009

Your file Votre référence
ISBN: 978-0-494-60187-7
Our file Notre référence
ISBN: 978-0-494-60187-7

ABSTRACT

Nitrogen and phosphorus are thought to be essential limiting resources in many ecosystems. While much is known about how nitrogen and phosphorus influences aquatic invertebrates, little is known about how phosphorus influences terrestrial invertebrates. I investigated how dietary phosphorus influences growth, consumption rate, reproduction, survival, condition, and internal elemental composition in a terrestrial insect species. In one experiment, I fed newly moulted 8th instar adult *Acheta domesticus* crickets' diets that differed in phosphorus content. In a parallel experiment, I fed 5th instar juvenile *Acheta domesticus* crickets' diets that differed in phosphorus content and quantified their juvenile and adult life history traits and fitness. My results revealed that the availability of dietary phosphorus strongly influenced juvenile growth and adult female reproduction. Further, the amount of available phosphorus also influenced juvenile body elemental composition. These results suggest that dietary phosphorus availability influences life history traits and lifetime reproductive success.

ACKNOWLEDGEMENTS

The first and foremost person I want to sincerely thank is Dr. Susan Bertram, my supervisor, for allowing me to work under her guidance and for helping me embark on this rewarding journey. Your continuing support and inspiration are well appreciated and needed at times. Thank you for providing me with this wonderful opportunity and allowing me to learn what it is like to be a research scientist. Your guidance and patient have left me richer for the experience. I would like to thank the Bertram lab members, Emily Whattam, Crystal Vincent, Lauren Fitzsimmons and Vanessa Rook for their advice and constructive comments throughout my graduate career. Special thanks to Chris Seguin for his endless effort at collecting and entering data. Thanks to all the work-study students, Rita Xing and Brent Thompson for aiding me with the completion of the experiments. Special thanks to Nop Ariayaphanneekun, both for his help with cricket care and for his support.

Thank you to my thesis advisors, Dr. Naomi Cappuccino and Dr. Frances Pick, for their constructive comments and guidance. Thanks also to Dr. Susan Aitken for her generous advice and help on phosphorus extraction. Thanks to Dr. Jeff Dawson and Dr. John Vierula for the use of their equipments. Thanks to my mom for her comforting words and encouragement. Special thanks to my grandmother for her financial support throughout my graduate career. Thanks to all the staff at Carleton University Biology Department, you have made my stay at Carleton not only a pleasant one, but also a life-long memory.

This thesis was funded by Graduate Studies and Research at Carleton as well as the Petro–Canada Young Innovator awarded to Dr. Susan Bertram, and by Dr. Bertram's NSERC grant and Carleton's start-up funds.

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INTRODUCTION

Ecological stoichiometry is the relationship among chemical elements of organisms and their interactions in ecosystems (Sterner & Elser, 2002). Essential elements must be obtained in sufficient quantities from food because they cannot be synthesized. The two most limiting of the essential elements are nitrogen (N) and phosphorus (P). Nitrogen is essential for life; insects incorporate nitrogen into their biochemical metabolism. Because nitrogen is not a highly reactive element, it must be converted to the reduced state of ammonium (NH^{+}_{4}) or nitrogen oxide prior to being taken up (Suzuki and Grady, 2004). All organism require nitrogen to build proteins. Many proteins act as enzymes catalyzing biochemical reactions that are vital to insect metabolism including catabolism, DNA replication, repair and RNA synthesis (Sterner & Elser, 2002). Proteins can also have structural functions, and can be important in cell signalling, immune response, and the cell cycle (Cross *et al.*, 2003).

Phosphorus is a naturally occurring chemical element that comes in two main forms: organic and soluble. Organic phosphorus is found in all living organisms. Soluble or inorganic phosphorus, on the other hand, is located mainly in the soil and is usually taken up by plant tissues (Vance, 2001). Plants use this inorganic phosphorus to build complex organic phosphorus compounds such as nucleo-proteins and salts of phytic acid (Collison, 1912). Inorganic phosphorus is scarce in the environment; however it is essential in the synthesizing of proteins by ribosomes (Elser *et al.*, 2000). Phosphorus is used to build ATP, RNA, DNA, and phospholipid molecules (Sterner & Elser, 2002).

Both nitrogen and phosphorus are limiting resources in the environment. Nitrogen is thought to be *the* limiting nutrient in terrestrial and marine environment; phosphorus is thought to be *the* limiting nutrient in freshwater systems (Anderson *et al.*, 2004). Nitrogen is mismatched between terrestrial insects and their food source. In soil, nitrogen is found in only 5 parts per million; this low concentration of nitrogen can interfere with plant and insect nutrient interactions (Suzuki and Grady, 2004). Herbivorous insects face nutritional challenges because of the elemental imbalance between plant and animal tissues (White *et al.*, 1993). For example, in terrestrial systems the C:N ratio of foliage is 5-10 times higher than the C:N ratio of herbivorous insects (Elser et al., 2000). This means that, on average, the nitrogen content of autotrophs is 10 to 20 times lower than that of herbivores (Mattson, 1980). This stoichiometric mismatch has the potential to place severe constraints on the ability of insects to meet their nutritional demands (Mattson *et al.*, 1980).

Nitrogen has traditionally been considered *the* essential limiting resource in terrestrial invertebrates (Schindler and Eby, 1997). The amount of nitrogen in the herbivore body tends to correlate with the percentage of nitrogen in the diet it consumes (Cruz-Rivera, 2000). That said, some insect species seem to be able to compensate for low amounts of nitrogen in their diet. Slansky and Feeny (1977) examined the effect of nitrogen content on insect consumption rate and efficiency of food utilization in the cabbage butterfly (*Pieris rapae*). Larvae were reared on either their natural food source with various amounts of nitrogen added or food grown with nitrogen fertilizers. Cabbage butterfly larvae fed low nitrogen diets consumed food faster and used nitrogen more

efficiently than those fed high nitrogen diets (Slansky and Feeny, 1977). Larvae adjusted their feeding rate to maximize the rate of nitrogen that their body tissue could accumulate. This compensation did not seem to be completely sufficient, however, because herbivores that consumed nitrogen-poor diets also experienced slower growth and higher mortality rates (Joern & Spencer, 1998).

Although there have been extensive studies that have examined how nitrogen influences terrestrial invertebrate fitness, little is known about how dietary phosphorus influences insect growth, reproduction, and survival. In fact, phosphorus has rarely been thought of as a limiting resource in terrestrial ecosystems; most work on phosphorus has instead focused on freshwater aquatic ecosystems. However, like nitrogen, phosphorus is also mismatched between terrestrial insects and their food source (White *et al.*, 1993). The C:P ratio of foliage is 5-10 times higher than the C:P ratio of herbivorous insects (Elser *et al.*, 2000). This means that, on average, the phosphorus content of autotrophs is 10 to 20 times lower than that of herbivores (Mattson, 1980). This phosphorus mismatch has the potential to place severe constraints on the ability of insects to meet their nutritional demands (Mattson *et al.*, 1980). Below I review the published research that has examined the influence of phosphorus on invertebrate growth, body size, condition, consumption rate, reproduction, and survival. I then outline my research goals.

Phosphorus and Invertebrate Growth

In aquatic invertebrates, N:P ratios are correlated with organismal growth. Rapidly growing species (e.g., *Daphnia*) tend to have high concentrations of phosphorus

rich RNA, relatively high amounts of phosphorus in their bodies, and a relatively lower N:P ratios. *Daphnia*'s high growth rate requires greater ribosomal RNA content for protein synthesis. Reducing the availability of phosphorus in the diet results in a decrease in *Daphnia*'s growth (Sterner and Hessen, 1994). In a study by Elser et al. (2000), the C:P balance in *Daphnia* declined by up to 20% when the animals were fed on diets with greatly reduced phosphorus content. Further, almost one-half of the phosphorus incorporated into the *Daphnia's* body was lost as result of moulting during development (Verede *et al.*, 1999). Sterner *et al* (1993) observed that *Daphnia* that were fed on phosphorus deficient algae had problems with moulting, as the exoskeletons remained attached. These findings suggest that phosphorus is important for carapace formation and moulting, which in turn is essential for juvenile development (Verede *et al.*, 1999). Together, these results suggest that *Daphnia*'s growth, moulting, and body phosphorus content are all strongly affected by the ability to locate, consume and retain sufficient amounts of phosphorus (Elser *et al.*, 2000).

In comparison to fast growing species, slow growing species (e.g., freshwater copepods) tend to have a lower concentration of phosphorus rich RNA, relatively low amounts of phosphorus in their bodies, and a relatively higher N:P ratio. Elser *et al.* (1996) observed changes in invertebrate body phosphorus content that were correlated with increasing growth rate in organisms ranging from bacteria to large vertebrates. This relationship was partially correlated with an increase in ribosome content (Elser *et al.* 1996). These observations lead Elser *et al.* (1996) to propose the growth rate hypothesis. The growth rate hypothesis suggests that phosphorus contributes to organism growth rate

through RNA. RNA is the most phosphorus rich major component of the cell, containing almost 10% phosphorus. High levels of ribosomal RNA are needed to synthesize proteins during growth and development (Kita *et al.*, 1996). Organisms including bacteria, fish and copepods exhibit an increased amount of RNA during development (Elser *et al.*, 1996). The growth rate hypothesis suggests that variation in C:N:P stoichiometry may influence the amount of RNA available, and thus may drive variation in organismal growth rate (Elser *et al.*, 2000).

To date, only a handful of studies have investigated how phosphorus availability influences insect growth. Perkins *et al.* (2004) investigated the effect of dietary phosphorus on the growth, consumption, and body phosphorus content of the herbivorous insect, the tobacco hornworm, *Manduca sexta*. Perkins *et al.* (2004) reared *M. sexta* larvae on artificial and natural diets (*Datura wrightti* leaves) that differed in the level of phosphorus available. *Manduca sexta* that were reared on both artificial and natural diets with elevated phosphorus content showed an increased growth rate, body phosphorus content, and shortened time to final-instar moult (Perkins *et al.*, 2004). Caterpillars reared on medium and high phosphorus diets were also heavier than those reared on low phosphorus diets. Overall, Perkins *et al.*'s (2004) research suggests that limitation of phosphorus in both artificial and natural diets affects insect growth and body size.

Frost *et al.* (2002) examined how littoral mayfly growth was affected by phosphorus availability. Mayfly nymphs were given either high or low quantities of food that differed in phosphorus availability. Phosphorus content affected mayfly growth when the food was an abundant resource. Mayfly body phosphorus content also increased when

they were fed with artificial phosphorus enriched food (Frost *et al.*, 2002). Combined, these two studies suggest that dietary phosphorus availability can place constraints on insect growth.

Huberty and Denno (2006) examined the effect of nitrogen and phosphorus availability on two planthopper species *(Prokelisia dolus* and *Prokelisia marginata)* growth. Their result reveals that there were no growth effects, regardless of dietary phosphorus content. *Prokelisia dolus* and *P. marginata* growth remained relatively the same throughout the experiment. Phosphorus also did not influence body size of either planthopper species. When *P. marginata* were faced with low quality plants they did worse than *P. dolus.* The reason behind this appears to be that *P. dolus* can increase ingestion of poor quality food and therefore compensates for the lack of nutrients in the nutrient-deficit host plants (described in greater detail in the compensatory feeding section, below; Huberty and Denno, 2006).

Overall, the observed effects of dietary phosphorus availability on insect growth rate have not been consistent. Elevated dietary phosphorus availability increased growth in tobacco hornworms, *Manduca sexta* (Perkins *et al.*, 2004) and mayflies (Frost and Elser, 2002). However, growth was not influenced by dietary phosphorus in planthoppers, *P. dolus* and *P. marginata* (Huberty and Denno, 2006). Combined these studies indicate that the influence dietary phosphorus has on insect growth needs to be further investigated. I therefore examined how dietary phosphorus availability influences cricket growth and development.

Phosphorus and Invertebrate Consumption Rate

Foraging theory suggests that organisms must maximize their energy intake per unit time (Godfray 1994). In order to do that they must locate and consume food containing the appropriate amount of essential nutrients while expending the least amount of energy and time in the process. One of the factors that may influence whether growth, reproduction, and survival are influenced by dietary phosphorus availability is whether organisms are capable of distinguishing between food of different diet stoichiometry and preferentially consume the most appropriate diet. Some aquatic invertebrates preferentially feed on high nitrogen and phosphorus diets when given choice. Schatz & McCauley (2006) tested both juvenile and adult *Daphnia*'s ability to locate phosphorus rich food using a spatial gradient. *Daphnia* preferentially relocated to regions of high food quality (Schatz & McCauley, 2006).

A second way to compensate for poor quality food is to increase the consumption rate. Some aquatic invertebrates increase their foraging rate on low quality food when given no choice in what diet they consume. *Daphnia* can adapt to a poor phosphorus diet by altering their behavioural and physiological responses. *Daphnia* attempt to make up for the lower elemental concentrations in their food by increasing their feeding activity when they consume a poor phosphorus diet. Increased feeding rate is coupled with reduced ingestion rate, leading to higher residence time of food in the gut (Darchambeau, 2005). There are many other aquatic invertebrate consumers that regulate their gut enzymes depending on food concentration and nutrient-richness. Copepods, for example, control their digestive enzymatic activity based on food concentration in the gut

(Mayzaud & Poulet, 1978). These findings suggest that whenever possible, aquatic invertebrates attempt to compensate for poor quality diets by changing their behaviour and/or physiology.

To date only a handful of studies have examined how key chemical elements affect terrestrial invertebrate consumption rate. Huberty and Denno (2006) examined the effect of macronutrient limitation (both nitrogen and phosphorus) on two planthopper species *P. dolus* and *P. marginata. Prokelisia dolus* (the specialist species) compensated for low quality food by increasing its rate of ingestion and thereby enhancing its food intake on nutrient-deficient host plants (Huberty and Denno, 2006). On the other hand, when faced with low quality food, *P. marginata* (generalist species) migrated to plants of higher quality. When forced to stay on poor quality plants, *P. marginata* did not dramatically alter their foraging rates and instead suffered the consequences of poor quality food. Caterpillars (*M. sexta*) also did not engage in compensatory feeding on low quality phosphorus diets (Perkins *et al* 2004). Although caterpillars on high phosphorus diets ate less than caterpillars on low phosphorus diets in one experiment, this difference did not hold in other experiments. Overall, these studies suggest that some insects may be capable of compensating for low quality diets by consuming more food, while others may not. Because compensatory feeding behaviour could dramatically influence the effect diet quality has on insect growth and fitness, I also investigated whether cricket consumption rate was influenced by dietary phosphorus availability.

Phosphorus and Invertebrate Reproduction

Life-time reproductive success is the number of offspring that are produced by a female throughout its lifespan (Alcock, 2005). Because fast growing individuals typically require more phosphorus (Elser *et al.*, 2000), and most growth occurs during in juveniles (Elser *et al.*, 2000), juveniles tend to have a higher phosphorus body contents than adults (Hessen and Anderson 1991, DeMott 2003). This extensive demand for phosphorus during early development suggests that females may allocate excess phosphorus to reproductive tissues to enhance their reproductive success. Further, egg mass is thought to be an indicator of parental provisioning and high quality food sources are often associated with larger egg size (Berrigan, 1991). Conversely, the eggs produced by stressed organisms tend to be of poorer quality than those produced by non-stressed individuals (Helm *et al.*, 1973). Diet quality may, therefore, have the potential to influence egg size, egg stoichiometry, and overall reproductive success.

A handful of invertebrate studies suggest that phosphorus availability can influence reproductive success. Urabe and Sterner (2001) examined *Daphnia* reproduction and how it was influenced by food quality. When fed large amounts of phosphorus deficient food, *Daphnia* produced smaller eggs compared to individuals fed smaller amounts of phosphorus sufficient food. Further 15-32% of these smaller eggs did not develop (Urabe and Sterner, 2001). The larger eggs typically contained more yolk and the individuals from larger eggs were more likely to survive (Urabe and Sterner, 2001). Yolk is an indicator of maternal investment for egg development in *Daphnia*

(Goulden & Henry, 1987). These results suggest that phosphorus availability can dramatically influence invertebrate reproduction.

Less is known about insects, although there are hints that phosphorus may profoundly influence reproduction. Female *Drosophila* appear to have significantly higher body phosphorus content than male *Drosophila* (Markow *et al.*, 2001). Further, male *Drosophila* pass large quantities of ejaculatory proteins (rich in phosphorus) to females during mating (Markow *et al.*, 2001). Markow *et al.* (2001) tested whether female *Drosophila* use this phosphorus from donated male seminal fluids during oogenesis. Their results revealed that female *Drosophila* use male donated phosphorus to synthesize high levels of nucleic acids necessary for egg production. Markow *et al* (2001) also tested the effect of food source (that differed in phosphorus content) on *Drosophila* mating success. Oogenesis was delayed when females were fed reduced phosphorus diets. Phosphorus limitation has therefore been directly implicated in *Drosophila* reproductive success.

Two studies also suggest that phosphorus availability can influence male cricket reproductive success. Male crickets signal acoustically to attract mates. Several studies have revealed a positive relationship between signalling effort and male mating success (Droney, 1995; Gray, 1996; Wagner, 1999; Cade and Cade 1992). Using the Texas field cricket, *Gryllus texensis*, Bertram *et al* (2006) revealed a strong positive correlation between acoustic signalling effort and total body phosphorus content. Their study suggested that phosphorus availability may strongly impact male cricket calling behaviour and thus their lifetime reproductive success. Unfortunately, this initial study

was correlational in nature; it did not directly test whether phosphorus availability influenced signalling effort. As a follow-up study, we fed European house crickets (*Acheta domesticus*) diets that differed in phosphorus availability (Bertram *et al* 2009). We revealed that phosphorus availability strongly influenced male cricket mate signalling efforts. Crickets fed diets high in phosphorus called with significantly higher efforts than crickets fed diets with low phosphorus availability. These results reveal that phosphorus availability influences male cricket reproduction. Because the relationship between male calling effort and phosphorus availability has already been explored in male crickets, I focused my research efforts on examining how phosphorus availability influenced reproduction in female crickets. Specifically, I examined how female cricket reproduction was influenced by dietary phosphorus availability.

Phosphorus and Invertebrate Survival

Virtually nothing is known about how dietary phosphorus availability influences insect survival. There are, however, a few hints that it could be important. Urabe and Sterner (2001) showed that *Daphnia* that fed on low amounts of phosphorus not only grew more slowly but also produced a substantial number of eggs that ceased to develop prior to maturation. Egg survivorship was lowest in individuals fed on phosphorus deficient food (Urabe and Sterner, 2001). These results indicate that young *Daphnia* are vulnerable to phosphorus deficit food. Sufficient phosphorus therefore appears to be essential to juvenile survival in *Daphnia*. To my knowledge there has been no work that has looked at how phosphorus influences juvenile or adult survival in terrestrial insects.

Therefore I also investigated how dietary phosphorus availability influences insect survival both in the juvenile and adult stages.

Phosphorus and Invertebrate Condition

Insect body size and condition are important to lifetime reproductive success and survival. They can influence the cricket's ability to compete in aggressive interactions and therefore have the potential to influence dominance hierarchies (Harvery & Gange, 2006). Individuals with larger body size tend to have a higher resource holding potential because larger body size provides a physical edge for territory holders. Further, males with larger body size tend to be more attractive to females (Brown *et al.* 2006; Grey 1996). Thus, body size can directly impact lifetime reproductive success. To date, nothing is known about how phosphorus availability influences insect body size or condition. I therefore also investigated how phosphorus availability influences insect body condition. I used two measures of condition, residual mass (residuals from an allometric regression of body mass on body size; following Eiermann *et al.*, 2007) and the body content of carbon, hydrogen, nitrogen, and phosphorus (body stoichiometry).

I used body stoichiometry as one of my measures of condition so that I could explore the relationship between phosphorus availability in the diet, overall body size, and overall body stoichiometry. To date, there have only been a few studies that have examined the relationship between body size and phosphorus content among individuals. Bertram et al. (2008) explored the degree of intra-specific variation in body nitrogen and phosphorus content of a generalist field cricket (*Gryllus texensis*) and a specialist curculionidae weevil (*Sabinia setosa*). They found a four-fold difference in the

phosphorus content in the crickets, ranging from 0.32-1.27%; weevils exhibited a three-fold difference in the body phosphorus content (Bertram *et al.* 2008). Both the weevils and the crickets exhibited a significant inverse relationship between body size and body phosphorus content, suggesting that phosphorus availability may influence insect body size. However, the study was correlational in nature.

Research Goals and Study Organism

Because phosphorus may be important to insect fitness I reared the common European house cricket (*Acheta domesticus*) on artificial diets that varied in phosphorus content and then quantified how phosphorus affected cricket growth, consumption rate, lifetime reproductive success, survival, and condition. *Acheta domesticus* is a non-native species to North America but is presently widely distributed across the Eastern United States (with the exception of peninsular Florida) (Ghouri, 1961). *Acheta domesticus* serves as an ideal model organism for these experiments because the species varies considerably in its overall body size, suggesting the potential for strong dietary effects. Further, *A. domesticus* is a generalist species capable of consuming a variety of plant species which will likely have variable phosphorus contents. My experiment is designed to allow me to address following goals:

1. Does dietary phosphorus availability influence juvenile growth?
2. Do crickets compensate for poor quality diets by eating more?
3. Does dietary phosphorus affect female lifetime reproductive success?
4. Does dietary phosphorus affect juvenile and adult survival?
5. Does dietary phosphorus availability influence cricket condition?

METHODS

General Rearing Information

European house crickets (*Acheta domesticus*) were purchased as fourth and fifth instar juveniles (i.e four or five moults from adulthood) from Port Credit Center in Port Credit, Ontario. They were raised communally in 36-litre rectangular plastic containers (36 x 28 x 23 cm) in an insect rearing facility in the Nesbitt Biology Building at Carleton University, Ottawa, Ontario, Canada (Figure 1a). The rearing room was under controlled conditions with a temperature of 26±4° C and 12:12 hour light: dark cycle. All juvenile crickets were provided with an unlimited amounts of food (prior to the start of the experiment this was powdered Harlan Tekland Rodent no. 8604), water and cardboard cartons for shelter. Food and water were replenished daily and containers were cleaned on regular basis.

There were two parts to my experiment; the first part was to determine the effect phosphorus diet had on adult cricket life history traits and fitness (initiated with newly moulted 8^{th} instar adult crickets; Figure 2a). The second part was to determine the effect of phosphorus diet on juvenile fitness (initiated with newly moulted 5^{th} instar juvenile crickets; Figure 2b). I quantified cricket life history traits and fitness by measuring changes in body mass, as well as cricket consumption rate, egg production, longevity, body and egg elemental composition (Carbon/Hydrogen/Nitrogen/Phosphorus), body morphology, and body condition.

In the adult phase of the experiment, juveniles in communal containers were checked daily to identify individuals that had undergone their final moult and become an

adult within the last 24 hours. These adults were housed individually in a 500ml plastic-coated paper bowl (7 x 11 cm, height x diameter) with a shelter and unlimited water (Figure 1b). Each newly moulted adult was immediately and haphazardly assigned to one of five different diet treatments ranging from low to high phosphorus (0.2%, 0.4%, 0.6%, 0.8%, 1.0%; see below for details). Males and females were provided with unlimited amounts of their assigned diet from the day they reached adulthood until they died a natural death. All procedures that were conducted with 8th instar crickets (adults) were also carried out with 5th instar crickets (juveniles), with the exception that in the juvenile experiment, the crickets were placed in their own individual containers when they reached their 5th instar and then immediately placed on their experimental diet treatment.

Dietary Treatments

I fed males and females *Acheta domesticus* one of five custom powdered diets ranging from 0.2% - 1.0% phosphorus content. The phosphorus range was designed to mimic the natural range of food in the wild. Since crickets are ominivores they are capable of consuming a variety of foods, from plants to fungi to insects. These foods vary from an average of 0.2% phosphorus found in terrestrial plant matter to an average of 0.8% phosphorus found in insects (Sterner and Elser, 2002).

Each of the five diet treatments used in this experiment were designed, manufactured and purchased from Harland Teklad (Harlan Teklad Inc, P.O. Box 44220, Madison, WI, U.S.A.). The names in parentheses in the next sentence are Harlan Teklad

identifiers. The high diet (TD.07250) contained 1.0% phosphorus, the mid-high diet (TD.08058) contained 0.8% phosphorus, the medium diet (TD.07249) contained 0.6% phosphorus, the mid-low diet (TD.08057) contained 0.4% phosphorus, and the low diet (TD.07248) contained 0.2% phosphorus. All five of the research diets also contained 1% calcium and 24% protein. Most of the dietary phosphorus was delivered using calcium phosphate (high = 35.53 g/kg; mid-high = 26.75 g/kg; medium = 17.98 g/kg; mid-low = 9.21 g/kg; low = 0.44 g/kg), however, 1.9 g/kg of phosphorus came from the casein used as a protein source. Because we used calcium phosphate to deliver the phosphorus, we balanced the calcium levels across the diets with calcium carbonate (high = 0.0 g/kg; mid-high = 6.5 g/kg; medium = 12.75 g/kg; mid-low = 19.25 g/kg; low = 25.75 g/kg). Each diet also contained 236.6 g/kg of protein (272 g/kg casein), fat (42.7 g/kg: 2.7 g/kg from casein and 40 g/kg from soybean oil), L-cystine (4 g/kg), corn starch (150 g/kg), maltodextrin (50 g/kg), cellulose (50 g/kg), minerals (no calcium or phosphorus included in this mix; 13.4 g/kg), vitamins (10 g/kg), choline bitartrate (2.5 g/kg) and antioxidants (8.0 mg/kg). Table 1 shows the chemical components in each of the diets.

Weight Change

Initial cricket body mass was obtained by weighing the cricket on a precision analytical balance to 0.1g (model P-114) prior to initiating the diet treatment. Weekly change in body mass was quantified by weighing the cricket on the same balance each week until it died a natural death. Crickets were then placed in a drying oven (model 6520 series) at 130°C for 24 hours. The cricket's dry mass was measured using the same precision analytical balance (model P-114).

Consumption Rate

I monitored cricket consumption rate daily over a three day period starting at 10 days post final moult. A subset of males and females from each of the five diets were given a specific amount of food. Every 24 hours I carefully removed the food dish and weighed it using the precision analytical balance (model P-114), I then calculated how much food each individual had consumed in the last 24 hours. Table 2 shows the number of males and females that were monitored for consumption rate in each diet.

Reproduction

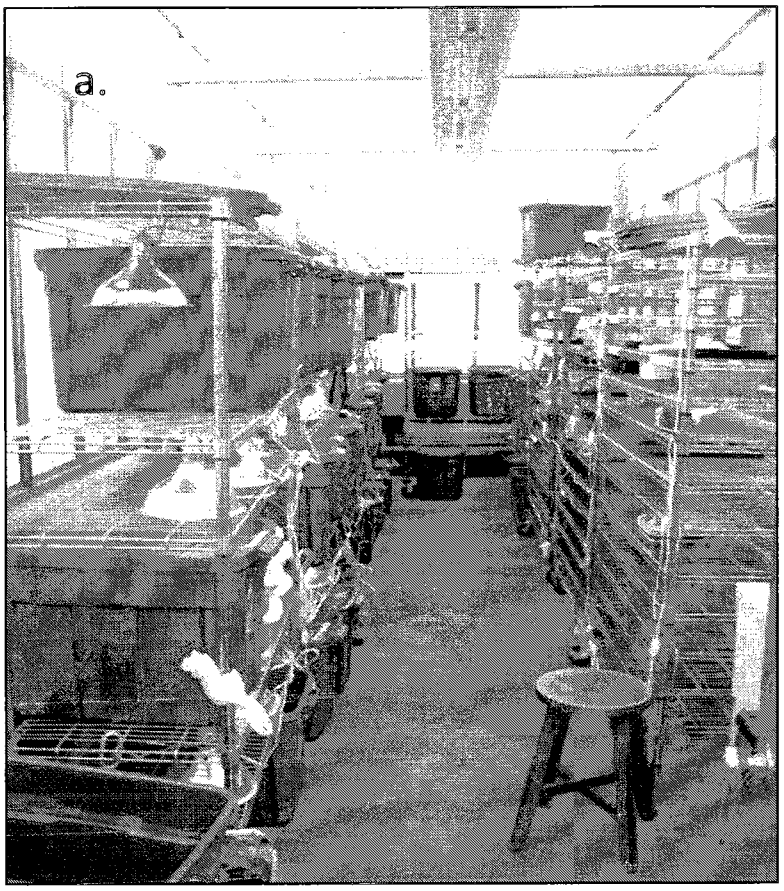
Fourteen days after females reached adulthood, they were placed with virgin males for 48 hours and allowed to mate freely. Virgin males that were used for mating were raised communally and fed the non experimental original diet (Harlan Teklad

Rodent diet no.8604). A 29 ml container of moist sand was provided for each female to lay eggs (Figure 3a). Prior to placing the sand in the container, the sand was sifted through an 8" diameter metal sieve with 300μm holes (Cole-Parmer Canada Inc; Figure 3b). After 48 hours, I removed each male cricket from the female's container leaving the sand container for a week in order for the female to lay eggs. On a weekly basis, I removed the sand container and replaced it with a new one.

I assessed the number of eggs laid by each female by sifting the female's egg laying sand through a 8" metal sieve with 300μm holes sieve. The total numbers of eggs laid were counted in the sieve. Ten eggs from each female were randomly selected and weighed together using the precision analytical balance (model P-114) to get an average egg weight. The total number of eggs laid and the average mass of eggs were recorded weekly throughout each female's lifespan.

Longevity

The date at which the cricket died a natural death was recorded to determine its longevity. Table 3 below shows the number of adult / juvenile males and females that were included in the longevity experiment (i.e., died a natural death versus escaping or dying an unnatural death [e.g. one cricket was accidentally run over by a cart following a boisterous weighing session]).



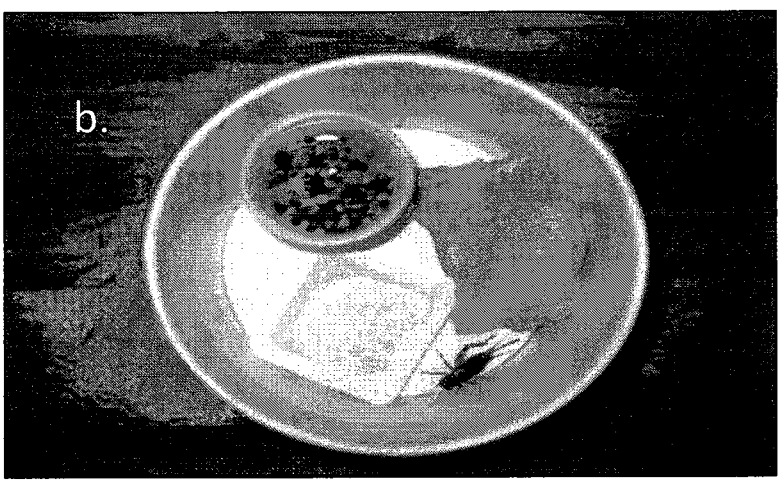


Figure 1. Photos of the rearing and monitoring protocol. a) Cricket rearing room in the greenhouse at Carleton University (large plastic bins were used for mass rearing, lights were on timers). b) Each cricket was placed in its own individual container with food (in the square bowl) water (in the bowl with gravel), and shelter (paper egg carton).

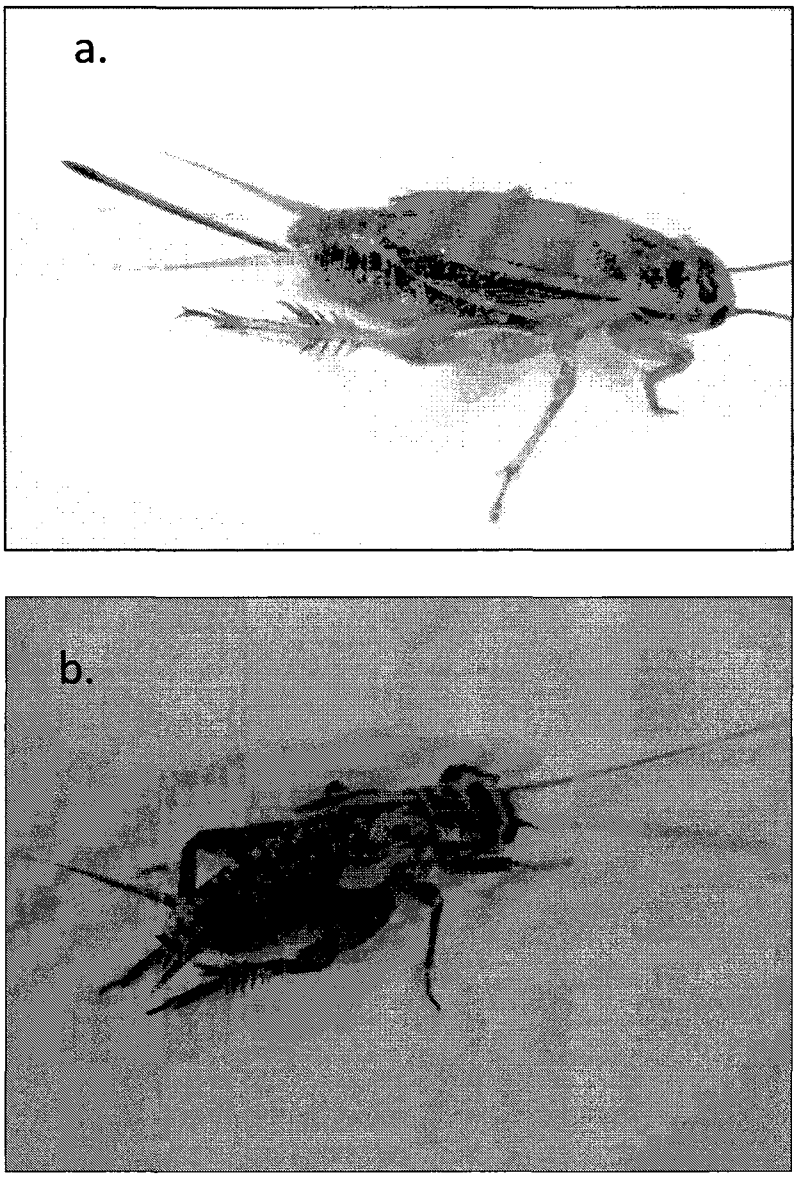


Figure 2. Photos of female *Acheta domesticus* a) newly moulted adult cricket 8th instar, notice the fully developed wings and ovipositor
b) 5th instar juvenile cricket, notice the development of the wing pads and the start of the ovipositor

Table 1. Chemical components in the custom research diets differing in phosphorus content (0.2% - 1.0% phosphorus)

Chemical Components (g/kg)	0.2% P	0.4% P	0.6% P	0.8% P	1.0% P
Casein	272.0	272.0	272.0	272.0	272.0
L-Cystine	4.0	4.0	4.0	4.0	4.0
Corn Starch	150.0	150.0	150.0	150.0	150.0
Maltodextrin	50.0	50.0	50.0	50.0	50.0
Sucrose	381.9	379.6	377.3	374.8	372.5
Soybean Oil	40.0	40.0	40.0	40.0	40.0
Cellulose	50.0	50.0	50.0	50.0	50.0
Mineral Mix, w/o Ca & P	13.4	13.4	13.4	13.4	13.4
Calcium Carbonate	25.7	19.2	12.7	6.5	0
Calcium Phosphate,	0.44	9.21	17.98	26.75	35.53
Vitamin Mix	10.0	10.0	10.0	10.0	10.0
Choline Bitartrate	2.5	2.5	2.5	2.5	2.5
TBHQ, antioxidant	0.008	0.008	0.008	0.008	0.008

Table 2: Number of individuals tested for consumption rate across the five phosphorus diet treatments.

Stage	Sex	0.2% P	0.4 % P	0.6 % P	0.8 % P	1% P
Adult	Female	23	20	20	20	19
Adult	Male	25	23	22	19	21
Juvenile	Female	22	23	23	24	23
Juvenile	Male	19	22	21	24	23

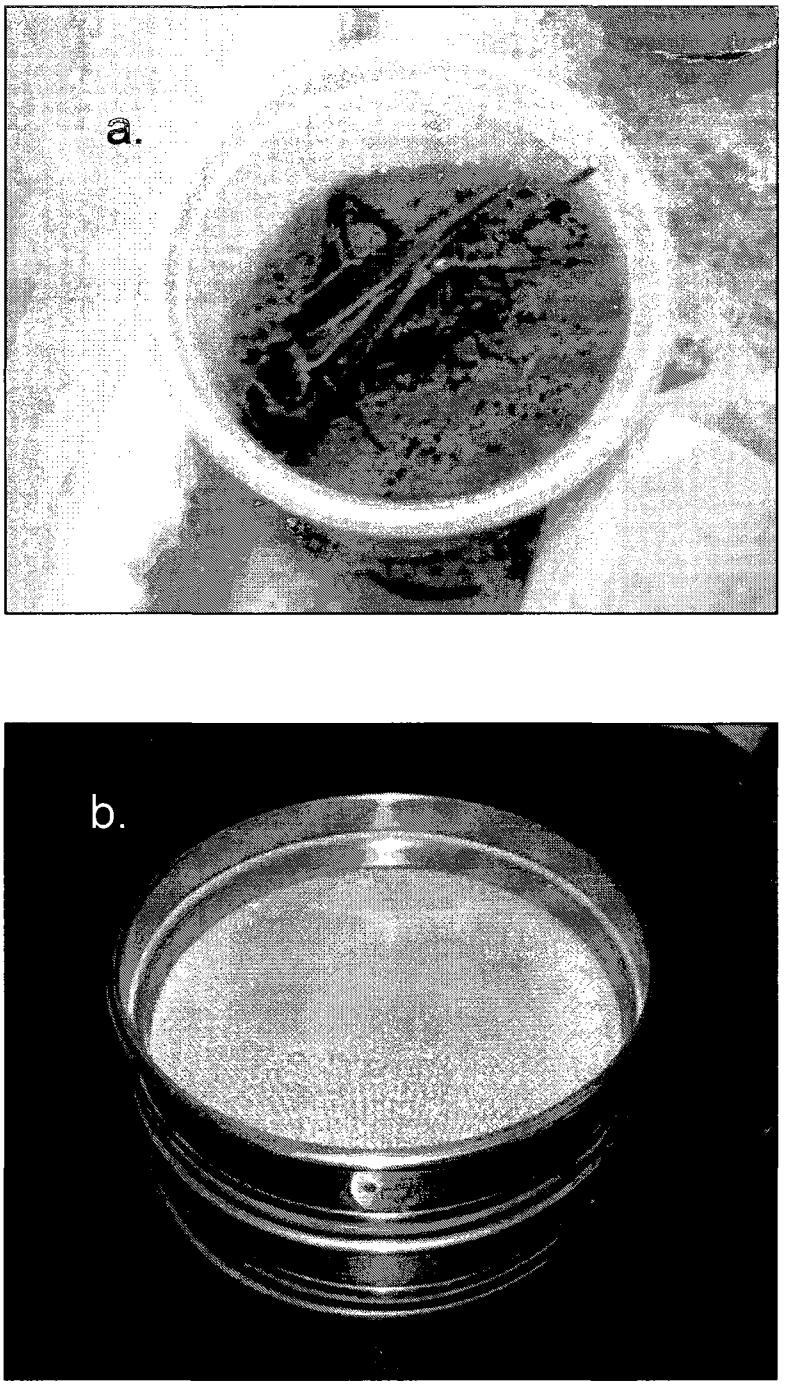


Figure 3. Photos of egg laying and sorting protocol. a) Female cricket laying eggs in the moist sand. b) 8" diameter metal sieve with 300μm holes used to sift cricket eggs

Table 3: Number of adult / juvenile males and females that were included in the longevity experiment (Note: the numbers are broken down by diet, sex, and stage)

Stage	Sex	0.2 % P	0.4 % P	0.6 % P	0.8% P	1 % P
Adult	Female	67	71	76	64	81
Adult	Male	66	62	61	56	60
Juvenile	Female	53	49	52	51	52
Juvenile	Male	56	46	53	47	60

Table 4: Number of adult / juvenile males and females that were measured for body morphology in each of the diets (Note: the numbers are broken down by diet, sex and stage)

Stage	Sex	0.2 % P	0.4% P	0.6 % P	0.8 % P	1 % P
Adult	Female	39	69	55	60	39
Adult	Male	59	32	54	51	55
Juvenile	Female	47	46	45	39	41
Juvenile	Male	42	26	35	21	38

Body Morphology

Once each cricket died a natural death, the cricket's body morphology was measured using Zeiss dissection microscope (Discover 4. V 12; Figure 4a). The cricket was placed under the microscope with a lamp shining upon its body. By using the Axio Vision software program and combined microscope, the head width, thorax width, thorax height, and thorax area were measured to the nearest 0.1 mm (Figure 4b). Table 4 below shows the number of adult / juvenile males and females that had their body morphology measured.

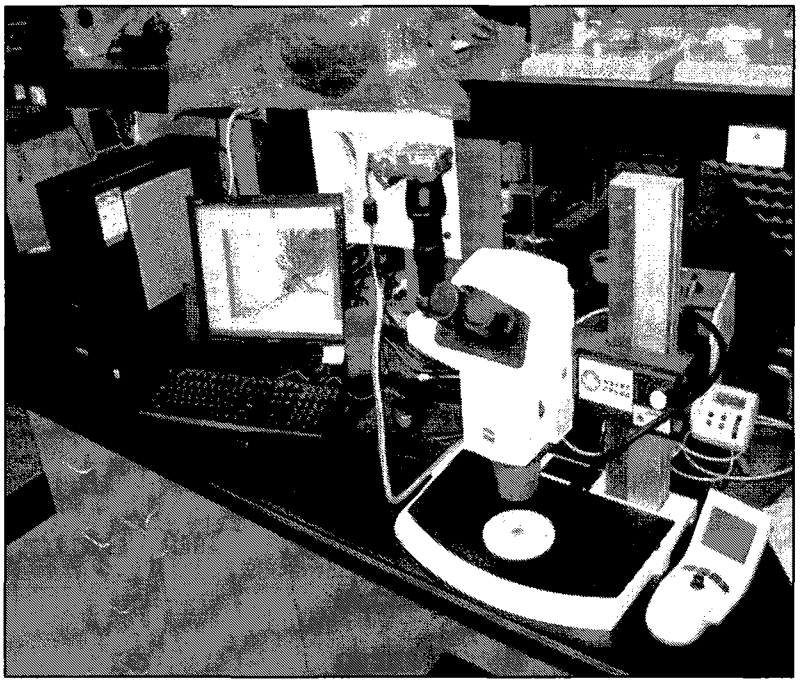
Elemental Stoichiometry

In order to quantify the percentage of carbon (C), hydrogen (H) and nitrogen (N) in cricket bodies and eggs I used a Vario Micro Cube CHN analyzer (figure 5). I individually pulverized each dried cricket to a fine powder using a mortar and pestle. I then used approximately 1 mg of powder from each individual to assess his or her body CHN. To run the analysis, I placed the weighed cricket powder in a small tin foil cup (the sample). Each sample was then placed in the Vario Micro Cube carousel and run through the combustion tube and reduction tube. This process provided a passage for gas (helium and oxygen) to flow through the sample allowing the sample to burn. Since each element requires a specific temperature to burn, each temperature set point gives a specific peak which can then be translated into the percentage of each element being examined (C,H, and N). I used the same process to analyze the amount of CHN in each female's eggs. In order to ensure accuracy of the elemental percentage given out by each sample, I set up a

lab standard which consisted of several hundred dried crickets that were ground thoroughly into a powder from the lab colony. This cricket lab standard was run prior to running the samples and re-run every ten samples to ensure consistency in the results.

I determined phosphorus content of the cricket's body by using approximately 1 mg of the ground up cricket powder. To quantify phosphorus content in the eggs, I used 10 dried eggs (approximately 1 mg). I used the persulfate oxidation technique followed by analysis of orthophosphate using acid molybdate technique (APHA 1992) to quantify phosphorus in these samples. Briefly, this method involves weighing out standard samples (stock phosphate solution from 50ul – 800 ul, apple leaves in powder form, and the colony cricket powder) and ~1mg of powdered cricket samples on a precision analytical balance (model P-114) and placing these in clean, dry, and pre-weighed test tubes. Dilute sulfuric acid and potassium solutions can then be added to each test tube, and the tubes can then be autoclaved for 90 minutes. After the test tubes cool, base NaOH is added until the solution turns faint pink. A drop of dilute sulfaric acid is added to the solution. Phosphate reagents are then added. The intensity of the color the solution turns shades of blue dependent on the phosphorus content in the solution. The test tube is then re-weighed to obtain the total extraction volume. The intensity of the blue colour (absorption) was measured using a spectrometer, Genesys 10vis. The absorption, extraction volume, and standards are used to calculate the amount of phosphorus in each sample. The number of individuals and eggs that were analyzed for their chemical composition in each diet can be found in Table 5.

a.



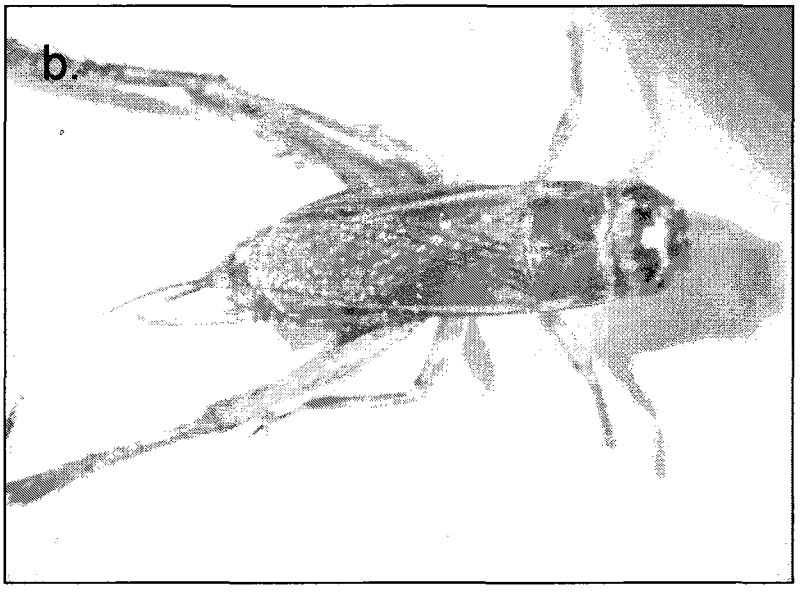


Figure 4. Photos of body morphology a) Zeiss dissection microscope Discover V.12. used to measure cricket body morphology b) Adult female cricket body morphology, red lines represent pronotum width (mm), height (mm) and overall area (mm^2)

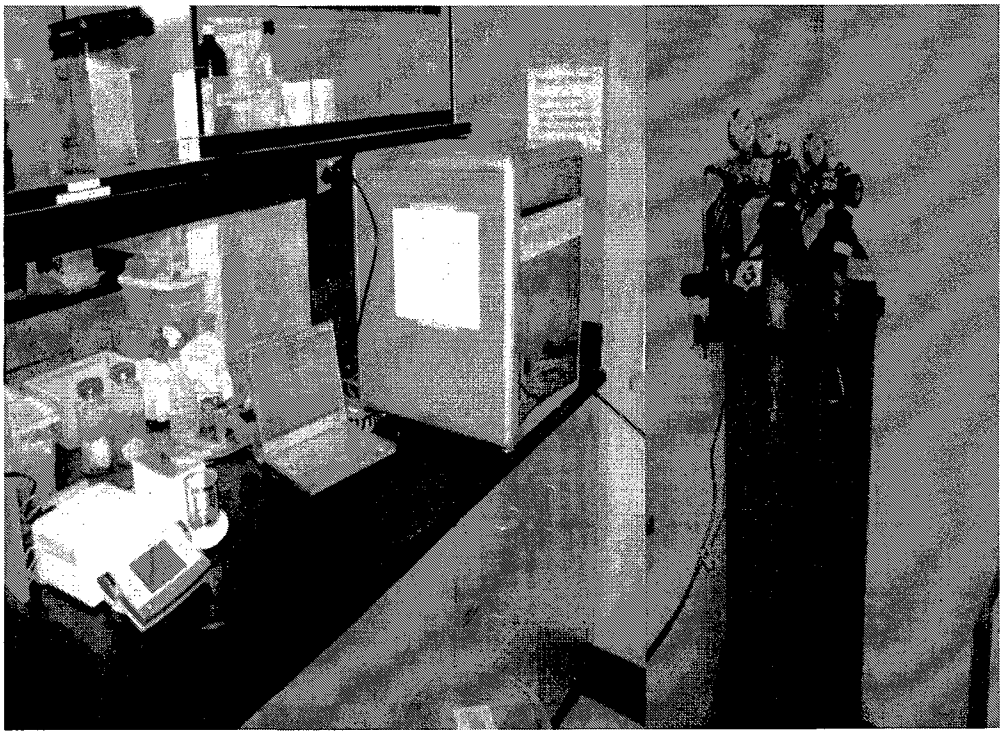


Figure 5. Photo of Vario Micro Cube CHN analyzer attached to the oxygen and helium tanks

Table 5. The number of individuals and eggs that were analyzed for their chemical composition (Note: numbers are broken down by diet, sex, stage and chemical element)

Eggs/ Body	CHN/ Phosphorus	Stage	Sex	0.2% P	0.4% P	0.6% P	0.8% P	1.0% P
Eggs	Phosphorus	Adult	Female	5	6	8	4	7
Eggs	Phosphorus	Juvenile	Female	3	4	7	6	10
Eggs	CHN	Adult	Female	6	7	8	5	7
Eggs	CHN	Juvenile	Female	4	4	8	8	12
Body	Phosphorus	Adult	Female	10	12	12	11	12
Body	Phosphorus	Adult	Male	10	10	11	10	11
Body	Phosphorus	Juvenile	Female	14	15	16	14	19
Body	Phosphorus	Juvenile	Male	14	17	12	11	12
Body	CHN	Adult	Female	10	12	12	11	12
Body	CHN	Adult	Male	11	10	12	10	11
Body	CHN	Juvenile	Female	14	15	16	14	19
Body	CHN	Juvenile	Male	14	17	12	12	12

Statistical Analyses

Statistical analyses were conducted using JMP 7.0.2 statistical software (SAS Institute Inc., Carleton University). I used a Shapiro-Wilk goodness-of-fit test to ensure the data did not differ significantly from normality. When the data were not normally distributed, I transformed them using a log transformation to approximate a normal distribution, thereby meeting assumptions for parametric statistics.

I used regression analyses to quantify whether diet influenced life history traits and fitness components (consumption rate, egg production, size, body stoichiometry, and egg stoichiometry). I used repeated measures ANOVA to determine whether weight change overtime was diet dependent. I used survival analyses to determine whether survival was influence by dietary phosphorus availability. I used a multiple regression model to explore the factors influencing longevity and reproduction. First principle component was used as an overall size measurement for body size.

I also examined the relationship among the variables I measured. I used a correlation analysis to examine the relationship between %C, %N, %P for juveniles and adults of both sexes. I also used regression analyses to determine the relationship between different fitness components (egg mass, egg number and longevity) and each dependent condition variable (size and consumption rate).

Lastly, I examined whether crickets reared on diets from the juvenile stage differed from crickets reared on diets from the adult stage in their life history and fitness measures. I also examined whether males differed from females. I used ANOVAs and survival analyses to assess these differences.

RESULTS

Growth

Prior to being placed on their respective phosphorus diets juvenile females had an average body mass of 0.204g ± 0.0879 (range = 0.043-0.552 g) and juvenile males had an average body mass of 0.200g ± 0.073 (range = 0.08-0.510 g). The sexes did not differ significantly in their masses (ANOVA: p=0.5370, df=1,572, F=0.3815, R^2adj=-0.00108). Further, individuals in each of the dietary phosphorus treatments did not differ significantly in their body mass at the beginning of the experiment (juvenile females: p=0.5510, df=1,286, t=0.4280, R^2adj=-0.0013; juvenile males: p=0.5510, df=1,286, t=0.60, R^2adj=0.0025). Juveniles gained weight over time (Figure 6a,b), with juvenile females gaining more weight than juvenile males (Table 6). This resulted in juvenile females obtaining higher body mass at the time of their death compared to juvenile males (figure 7a; juvenile female dry weight: 0.063 g ± 0.041, range = 0.03-0.218 g; juvenile male: 0.050 g ± 0.024, range = 0.02-0.136 g).

There was a significant diet effect on juvenile weight gain (Table 6). Juveniles reared on reduced phosphorus diets gained significantly less weight than those reared on phosphorus enhanced diets (statistics shown in Table 6). Diet did not significantly affect the time it took nymphs to reach their final instar (males: p=0.8578, df=1,37, R^2adj=-0.0261, t=-0.18; females: p=0.9425, df=1,90, R^2adj=-0.0111, t =-0.07). The average length of time it took juvenile females to reach adulthood was 22 ± 10 days (range = 1-52 days) while juvenile males took and average of 18 ± 9 days (range = 2-46 days).

Individuals from the different dietary treatments did not differ in their weights at the start of the experiment. Adult females on average had a body mass of 0.399 g ± 0.10 (range = 0.078–0.706 g), adult males had on average body mass of 0.336 g ± 0.079 (range = 0.143-0.635 g) at the start of the experiment. There was no effect of dietary phosphorus on changes in adult body mass (dry weight) of either sex (Table 6). Adult females weighed more at the time of their death compared to males (figure 7b; adult females: 0.134 ± 0.053 (range = 0.035-0.384 g dry mass), adult males: 0.101± 0.049 (range = 0.036-0.346 g dry mass).

Consumption Rate

Males and females did not differ in their phosphorus consumption but adults consumed almost twice as much food as juveniles regardless of their phosphorus diet (figure 8). Adult females consumed on average 0.049 g ± 0.027 of food per day (range = 0-0.119), whereas adult males consumed 0.047 g ± 0.037 of food per day (range = 0-0.165). Juvenile female consumed 0.027 g ± 0.022 of food per day (range = 0-0.111), while juvenile male consumed 0.027 g ± 0.024 of food per day (range = 0-0.132). Dietary Phosphorus availability did not influence consumption rate (figure 9; all $P>0.20$).

I also examined the relationship between consumption rate and body mass. With the exception of adult males, there was a positive relationship between body mass and consumption rate in adult and juvenile females (figure 10 a&c) as well as juvenile males (figure 10d). Adult/juvenile females and juvenile males that consumed more food had heavier bodies (dry mass). Conversely, there was a negative relationship between dry

weight and consumption rate in adult males (figure 10b). Adult males that consumed more food had lighter bodies (dry mass).

Reproduction

Most (82%) of adult females laid eggs, whereas only a few (17%) of the juvenile females that reached adulthood laid eggs. This difference in reproductive success is not significantly different between the two groups (Pearson=4.99, p=0.254) largely because of the extremely small number of juvenile females that reached adulthood. Adult females on average laid 75 eggs ± 92.82 (range = 0-655), whereas juveniles females that reached adulthood laid on average only 20 eggs ± 19.68 (range = 0-105).

Phosphorus availability significantly influenced lifetime reproductive success in female crickets. Females that were fed high phosphorus diets as adults laid significantly more eggs compared to those that were fed low phosphorus diets (figure 11a). Reproduction of females that were fed on their diet since the juvenile stages was not, however, significantly influenced by phosphorus availability (figure 11b). This latter result was likely due to a small sample size given that only 123 juvenile females reached adulthood before dying and only 45 of them laid eggs. Power analysis indicated that in order for me to accurately examine reproduction in juveniles, I would have to triple the sample size to 135 females who laid eggs.

Phosphorus availability did not affect average egg mass in adults (figure 11c). Likewise, the eggs of females fed different diets did not differ in percent phosphorus within the eggs (eggs from adult females: p=0.8701; df=1,28; R^2adj=0.0347; t-ratio=0.17;

eggs from juvenile females: p=0.9352; df=1,28; R^2adj=0.0355; t-ratio=-0.08; table 7). Similarly, the amount of nitrogen contained in the eggs was not dependent on phosphorus availability in the adult or juvenile mothers diet (Adult female: p=0.8701, df=1,28, t=0,17; Juvenile female: p=0.9352, df=1,28, t=-0.08).

Longevity

On average the mean lifespan for adult females was 27 ± 26.01 days (range: 1-128), adult males was 34 ± 33.46 days (range: 1-138), juvenile females was 39 ± 37.32 days (range: 1-157) and juvenile males was 22 ± 18.77 days (range: 1-124). Adult males lived longer than adult females (figure 12a). Juvenile females, on the other hand, lived longer than juvenile males (figure 12b).

Dietary phosphorus availability did not affect the survival of males or females, regardless of whether they started on their diets as juveniles or adults. Longevity remained relatively the same across all five diets (figure 13a-d).

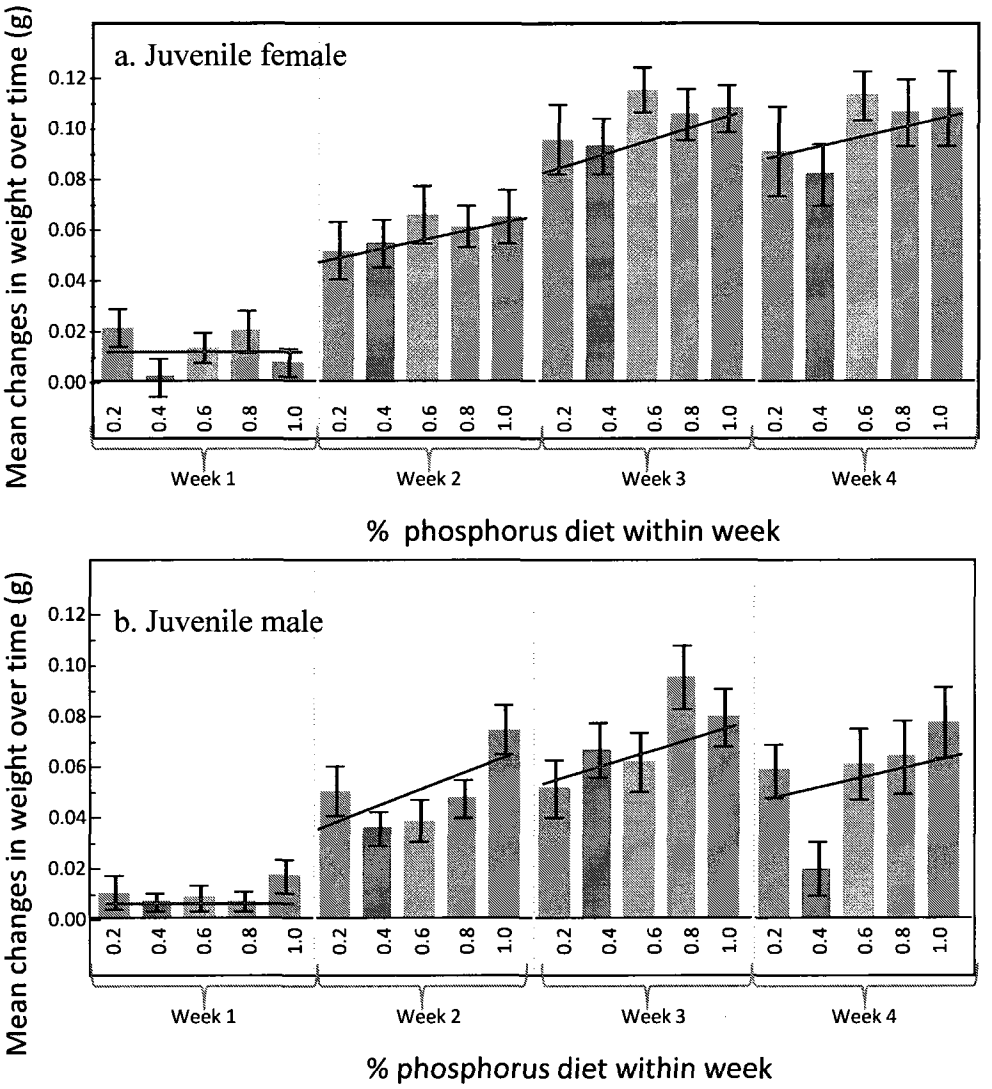


Figure 6 a & b. Relationship between % phosphorus diet and change in body mass of juvenile females and males (Statistics for these analyses can be found in table 6 a&b)

Table 6 : Results from the repeated measures ANOVA for how dietary phosphorus influences changes in body mass over time for a) adults b) juveniles

a.

Adult	Between / Within subject	Variable	F	Df	P
Adult	Between	Intercept	4.0444	1,274	0.0453
Adult	Between	Diet	1.6667	1,274	0.1978
Adult	Between	Male / Female	7.9206	1,274	0.0052*
Adult	Between	Male / Female * Diet	0.4447	1,274	0.5054
Adult	Within	Time	0.2287	2,273	0.7957
Adult	Within	Time* Diet	1.3203	2,273	0.2687
Adult	Within	Time * Male/Female	4.1935	2,273	0.0161*
Adult	Within	Time*Male/ Female *Diet	1.0831	2,273	0.3555

b.

Juvenile	Between	Intercept	39.8969	1,272	<0.0001*
Juvenile	Between	Diet	4.2417	1,272	0.0404*
Juvenile	Between	Male / Female	12.532	1,272	0.0005*
Juvenile	Between	Male / Female * Diet	1.2577	1,272	0.2631
Juvenile	Within	Time	34.2729	2,271	<0.0001*
Juvenile	Within	Time* Diet	3.9081	2,271	0.0212*
Juvenile	Within	Time * Male/Female	7.7862	2,271	0.0005*
Juvenile	Within	Time*Male/ Female *Diet	0.0331	2,271	0.9675

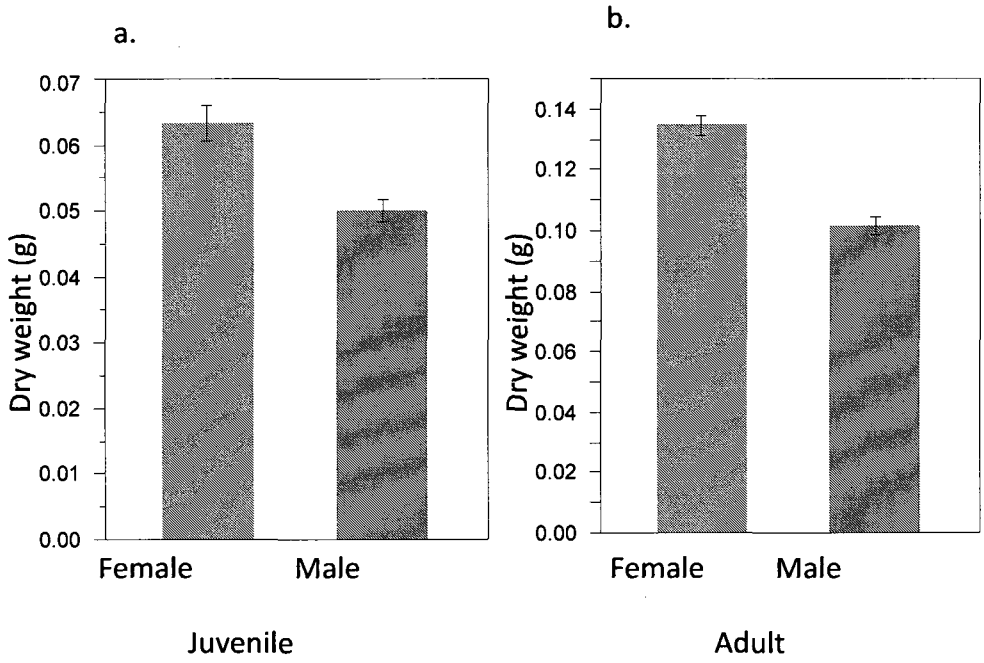


Figure 7. Differences in the dry body mass (g) between a) juvenile female and male (ANOVA: $F=18.4300$, $R^2adj=0.0340$, $df=1,491$, $p<0.0001$) b) adult female and male (ANOVA: $F=59.7900$, $R^2adj=0.0947$, $df=1,561$, $p<0.0001$) Note: error bars represent standard error

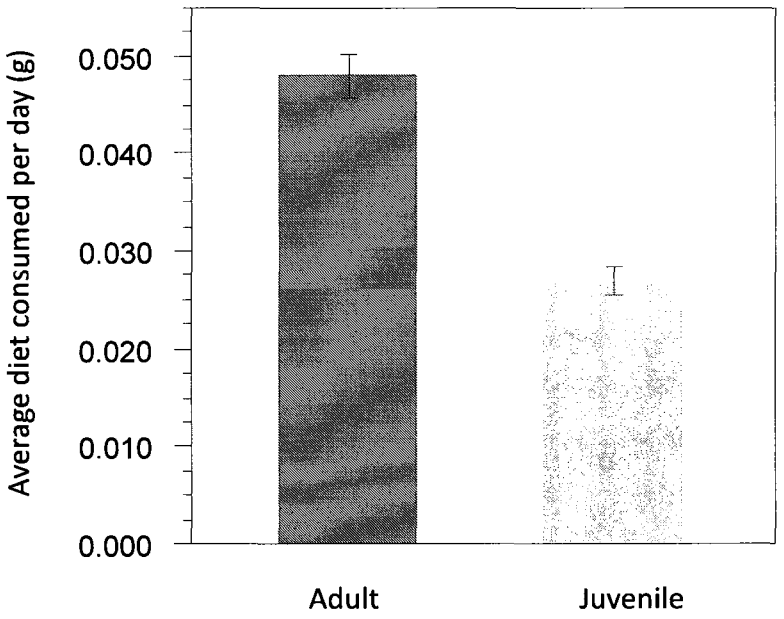


Figure 8. Differences in the rate of diet consumption (g) between adults and juveniles (ANOVA: $F=58.7900$, $R^2adj=0.1194$, $df=1,425$, $p<0.0001$)
Note: error bars represent standard error

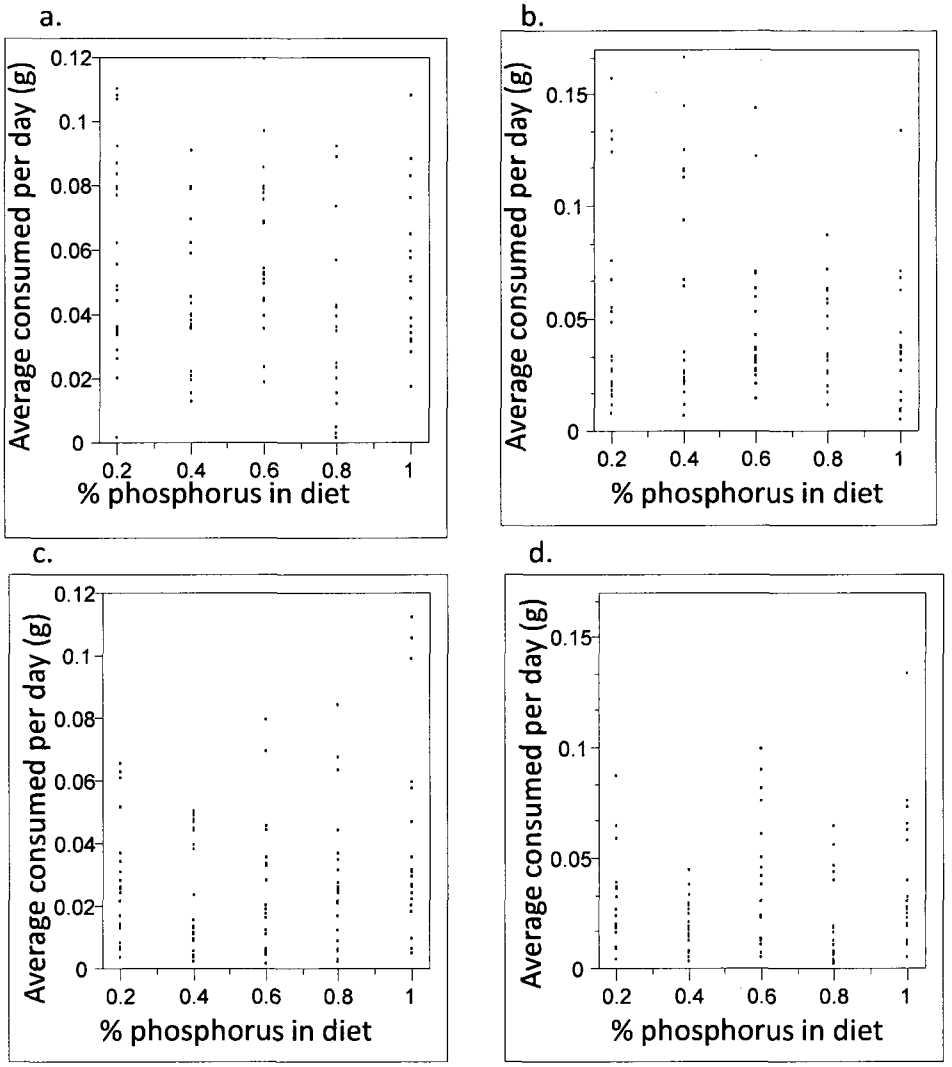


Figure 9. Relationship between phosphorus diet and average amount of diet consumed per day (g) in a) adult females ($P = 0.4222$, $df = 1,100$, $T = -0.81$; $R^2adj = 0.0030$); b) adult males ($P = 0.3387$, $df = 1,103$, $T = -0.96$; $R^2adj = 0.0007$); c) juvenile females ($P = 0.1951$, $df = 1,112$, $T = 1.30$; $R^2adj = 0.0060$); d) juvenile males ($P = 0.6143$, $df = 1,106$, $T = 0.51$; $R^2adj = 0.0070$).

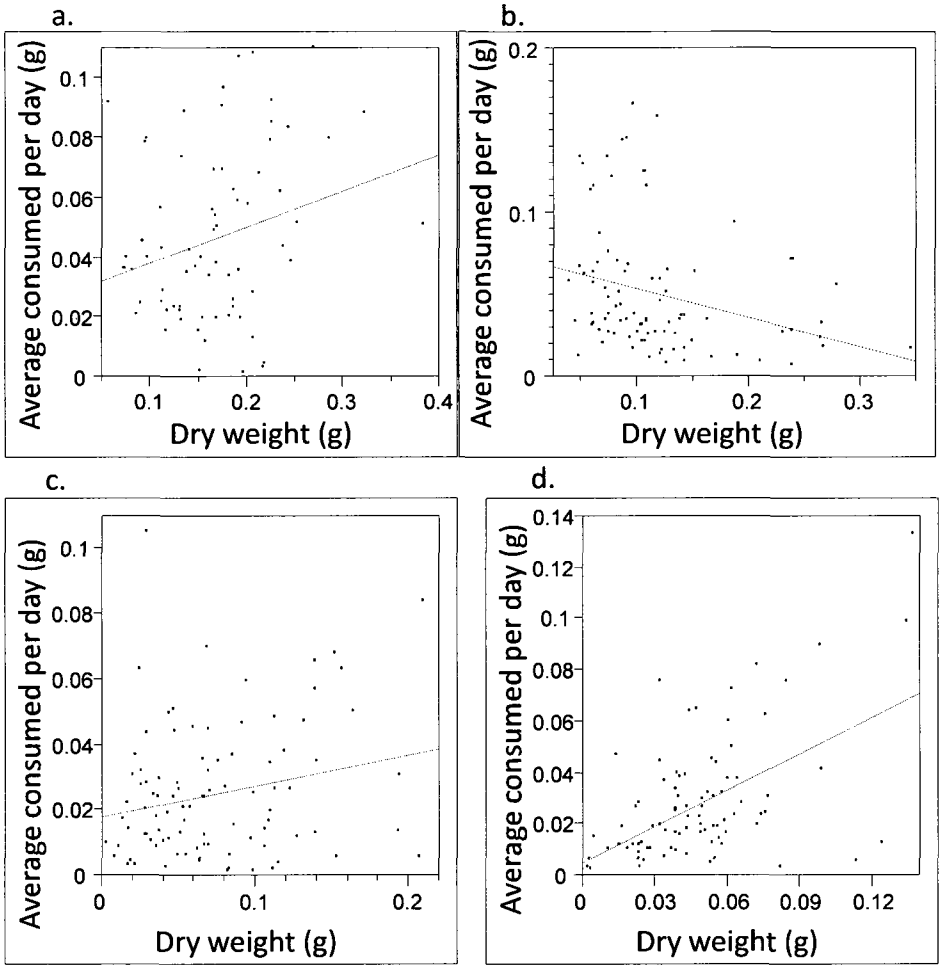


Figure 10. Relationship between consumption rate (g) and body mass (g) in a) adult females (P=0.0290*, df=1,69, t=2.23, R^2adj=0.0530; b) adult males (P=0.0068*, df=1,87, t=-2.77, R^2adj=0.0700); c) juvenile females (P=0.0290*, df=1,87,t=2.22, R^2adj=0.0400; d) juvenile males (P=<0.001*, df=1,232, t=4.29, R^2adj=0.2770).

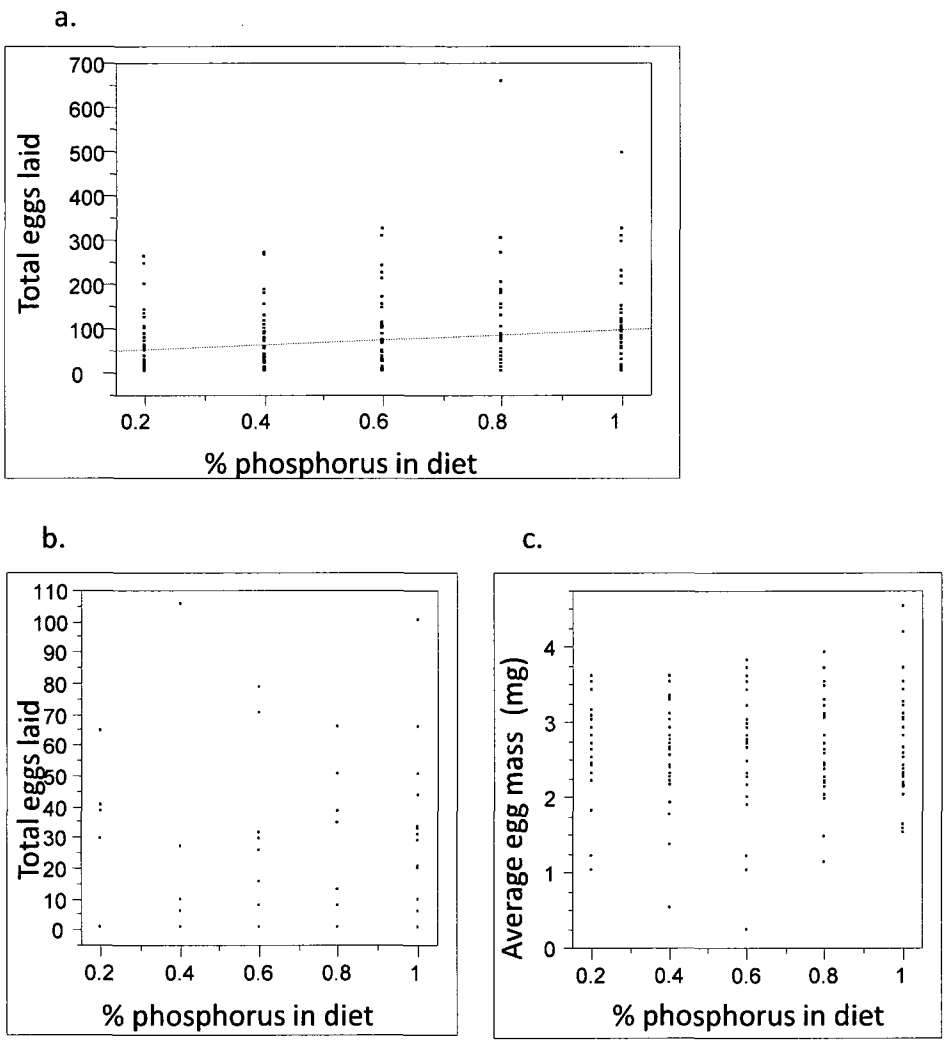


Figure 11. Relationship between phosphorus diet and reproduction in a) adult females egg number (P = 0.0221*; df = 1,160, T=2.31, R^2adj=0.0280) note: even with the two egg data remove, the statistic is still significant b) Females that were raised on phosphorus diet as juvenile stage (P = 0.8569; df = 1,33, T= -0.18, R^2adj=0.0292; c) adult females average egg mass (mg) (P = 0.5751; df =1,158, T = 0.56, R^2adj=0.0020).

Table 7. Variation in stoichiometric variables for eggs in adult and juvenile, female that were raised on phosphorus diet as a juvenile stage

Stage	Stoichiometry	Mean(%) ± SD	Min (%)	Max (%)
Adult	% P	0.98 ± 0.29	0.5	1.59
Adult	% C	32.54 ± 11.05	10.7	65.92
Adult	% N	5.35 ± 1.68	1.7	8.28
Juvenile	% P	2.24 ± 1.63	0.61	7.32
Juvenile	% C	40.67 ± 7.27	28.25	65.43
Juvenile	% N	7.67 ± 1.45	4.03	10.78

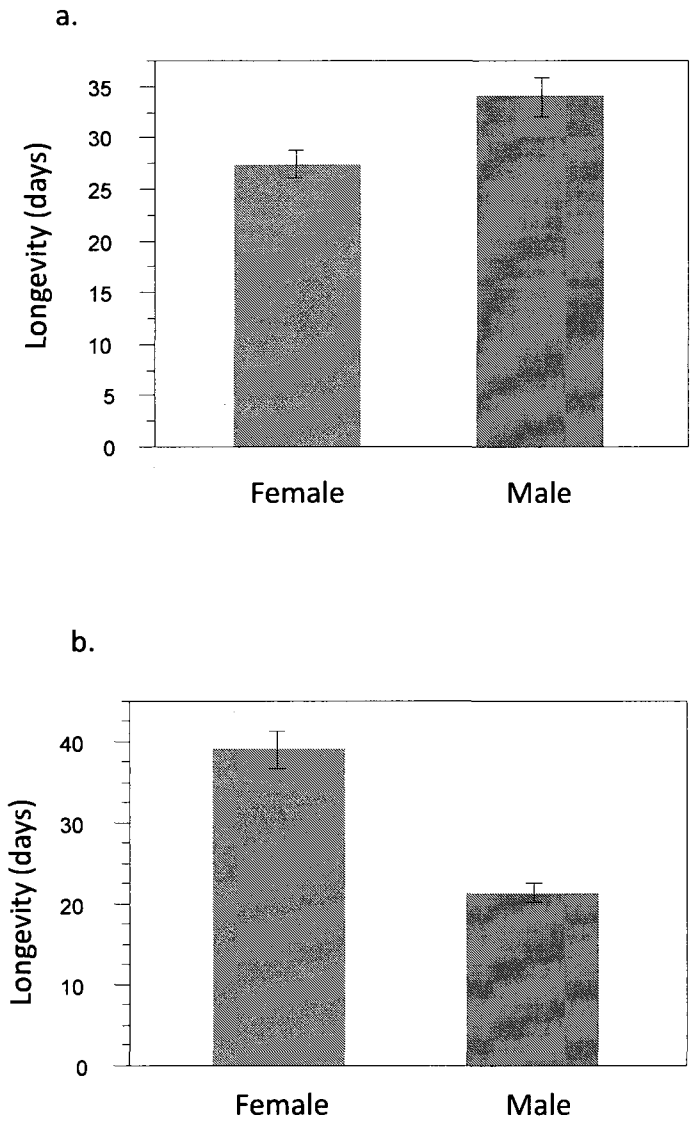


Figure 12. Differences between female and male longevity (days)
a) adults (ANOVA: P=0.0043, F=8.2260, df=1,669, R^2adj= 0.0106).
b) Juveniles (ANOVA: P<0.0001, F=46.32,df=1,523, R^2adj= 0.0796)
Note: error bars represent standard error

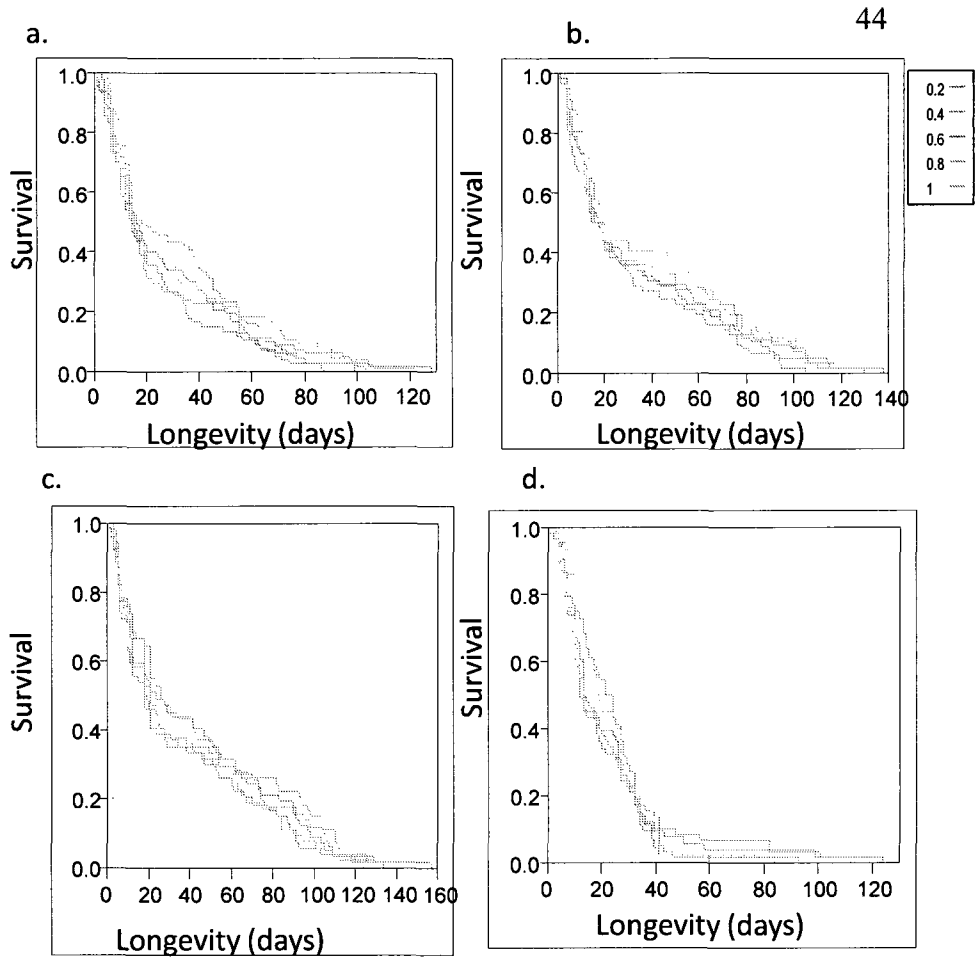


Figure 13. Relationship between phosphorus diets and longevity (days) in
a) adult females (Prob>chiSq. = 0.4923, Log Rank = 3.7320);
b) adult males (Prob>chiSq. = 0.6509, Log Rank = 2.3454);
c) juvenile females (Prob>chiSq. = 0.7434, Log Rank = 2.2738);
d) juvenile males (Prob>chiSq. = 0.6370, Log Rank = 0.3380)

Condition

Thorax width, thorax height, thorax area and head width were all significantly positively correlated (correlations ranged from 0.6769 to 0.9130; all p-value <0.0001; table 12). I therefore used a principal component analysis to reduce the number of size variables. The 1st principle component explained 85% of the variation in size (eigenvalue = 3.4171) and loaded each of the size components relatively equally (thorax width=0.5174, thorax height=0.465, thorax area=0.57839, headwidth=0.46729). Therefore, I used this one principle component as an overall size measure for all subsequent analyses.

I used residual mass (the residual between body size and body mass and dry weight) as one of my measures of condition. Dietary phosphorus did not influence body condition in the adults or juveniles of both sexes, all p-value >0.4367.

I also examined cricket body stoichiometry as a second measure of condition. There was extensive variation in cricket body stoichiometry (Table 8). There was an approximately two-fold difference in body carbon, nitrogen, and phosphorus content among individuals. Adult body carbon content ranged from 38-65%, body nitrogen content ranged from 8-17%, and body phosphorus content ranged from 0.57-1.16%. Lighter individuals contained relatively more nitrogen than heavier individuals (Table 9). Overall, there was no relationship between body mass and phosphorus content.

I determine the relationship between %C, %N and %P content within the cricket bodies. Both juvenile male and female body carbon content was positively correlated with nitrogen and phosphorus content (Table 9). The carbon content of adult female eggs

was also positively correlated with the nitrogen and phosphorus content of the eggs (Table 10).

Dietary phosphorus availability did not influence phosphorus, nitrogen or carbon body content in adult crickets (Table 11). Phosphorus availability in the diet did, however, influence phosphorus body content in juvenile crickets (figure 14a&b). Likewise, there were positive correlations between dietary phosphorus availability and the percentage of nitrogen and carbon content in juvenile bodies (Figures 14 c& d, Figure15).

Juvenile females that lived longer had higher percentage of phosphorus in their bodies compared to those with a shorter lifespan ($p=0.0267$, $df=1,71$, $R^2adj=0.0542$, $T=-2.26$). The same finding occurred for nitrogen content in juvenile females ($p=0.0209$, $df=1,70$, $R^2adj=0.0606$, $T=2.36$).

I found a strong inverse correlation between the percentage of nitrogen in the body and body mass (dry weight) in all crickets with the exception of juvenile females (figure 16). Heavier crickets contained less nitrogen in their bodies than lighter individuals. Overall, females that were fed on phosphorus diets during juvenile stage had higher phosphorus content in both body and eggs compared to those that were put on phosphorus diet as adults (Figure 17).

Factors Influencing Fitness

I used a multiple regression model to explore the factors influencing longevity and lifetime reproductive success. I included the following factors in the model for

longevity: diet, first principle component of body size, dry mass, residual mass, sex, and number of eggs laid. Longevity was most strongly influenced by the condition of the individual and the individual's sex. In fact, condition explained 44% of the variation in adult longevity; sex explained only 2% of this variation. Likewise, condition explained 58% of the variation in juvenile longevity, whereas sex explained only 2% of this variation. Neither reproductive effort nor dietary phosphorus availability played any role in explaining the variation in cricket longevity.

I included the following variables in the model for reproduction: diet, first principle component of body size, dry mass, residual mass, sex and longevity. Reproduction (number of eggs laid) was strongly dependent on phosphorus availability in the diet. Phosphorus diet explained 10% of the variation in egg laying for adult female. Body size, weight, condition, and longevity did not explain any of the variation in lifetime reproductive success. These models suggest that phosphorus availability strongly impacts lifetime reproductive success, while longevity is most strongly impacted by body condition (residual mass).

Table 8. Organismal stoichiometry descriptive statisitics of *Acheta domesticus*

Sex	Stage	Stoichiom-etry	Mean±SD	Minimum	Maximum
Female	Adult	% P	0.86 ± 0.12	0.67	1.16
Female	Adult	% C	47.00 ± 2.42	43.26	53.21
Female	Adult	% N	10.48 ± 0.68	8.80	11.77
Male	Adult	% P	0.79 ± 0.11	0.58	1.02
Male	Adult	% C	54.58 ± 5.66	38.49	65.63
Male	Adult	% N	11.85 ± 1.43	8.79	17.34
Female	Juvenile	% P	1.17 ± 0.25	0.71	1.69
Female	Juvenile	% C	46 ± 7.69	26.86	74.71
Female	Juvenile	% N	10.66 ± 1.79	6.26	18.66
Male	Juvenile	%P	0.97 ± 0.14	0.64	1.43
Male	Juvenile	% C	44.99 ± 6.48	36.49	57.60
Male	Juvenile	% N	11.12 ± 0.91	8.60	12.87

Table 9. Correlation between dry weight (g) and stoichiometry for adult/juvenile male and female crickets.

	Stages / Sex	Dry weight (g)	%C	%N	%P
%C	Adult female	0.2851	1.000	0.1576	0.0964
	Adult male	0.0755	1.000	0.2701	0.0964
	Juvenile female	-0.0569	1.000	0.7471	0.2108
	Juvenile male	0.2218	1.000	0.2301	0.5245
%N	Adult female	-0.4437	-0.1576	1.000	0.1508
	Adult male	-0.5330	0.2701	1.000	0.0605
	Juvenile female	-0.1705	0.7471	1.000	0.3773
	Juvenile male	-0.3214	0.2301	1.000	0.4206
%P	Adult female	0.0294	0.0964	0.1508	1.000
	Adult male	-0.1321	0.0964	0.0605	1.000
	Juvenile female	0.0599	0.2108	0.3773	1.000
	Juvenile male	-0.0821	0.5245	0.4206	1.000

Table 10. Correlation between stoichiometric balance for adult/juvenile female eggs

	Stages / Sex	%C	%N	%P
%C	Adult female	1.000	0.7816	0.7434
	Juvenile female	1.000	-0.1280	0.1072
%N	Adult female	0.7816	1.000	0.6895
	Juvenile female	0.7609	1.000	0.3687
%P	Adult female	0.7434	0.6895	1.000
	Juvenile female	0.1072	-0.2043	1.000

Table 11. Correlation between phosphorus diet and the adult male/female bodies elemental composition.

Stage	Sex	%C%H/%N	Yes / No	Prob > F	Df	R2 adj.	t
Adult	Female	% P	No	0.9856	1,52	0.01921	0.02
Adult	Female	% N	No	0.2852	1,53	0.003059	1.08
Adult	Female	% C	No	0.5259	1,53	0.01109	0.64
Adult	Male	% P	No	0.313	1,50	0.000759	1.02
Adult	Male	% N	No	0.2641	1,50	0.005377	1.13
Adult	Male	% C	No	0.1032	1,50	0.033264	-1.66

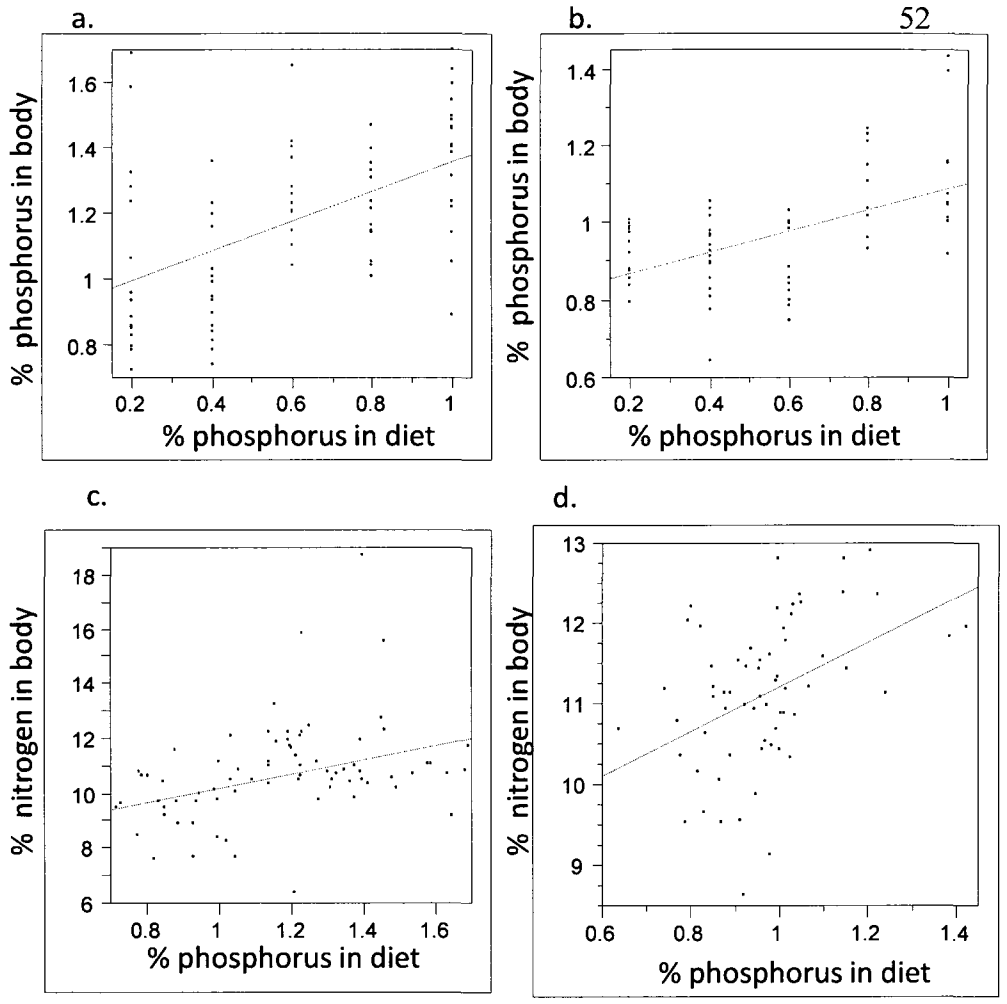


Figure 14. Relationship between phosphorus diet and %phosphorus in a) juvenile female bodies (P = <0.0001*;T= 5.41, df = 1,75; R^2adj = 0.2700); b) juvenile male bodies (P = <0.0001*;T-= 5.06, df = 1,63; R^2adj = 0.2770);. Relationship between % phosphorus diet and % nitrogen in c) juvenile female bodies (P = 0.0060*,df = 1,74, T= 2.83; R^2adj 0.0850); d) juvenile male bodies (P = 0.0019*,df = 1,64, T-= 3.24, R^2adj = 0.1270).

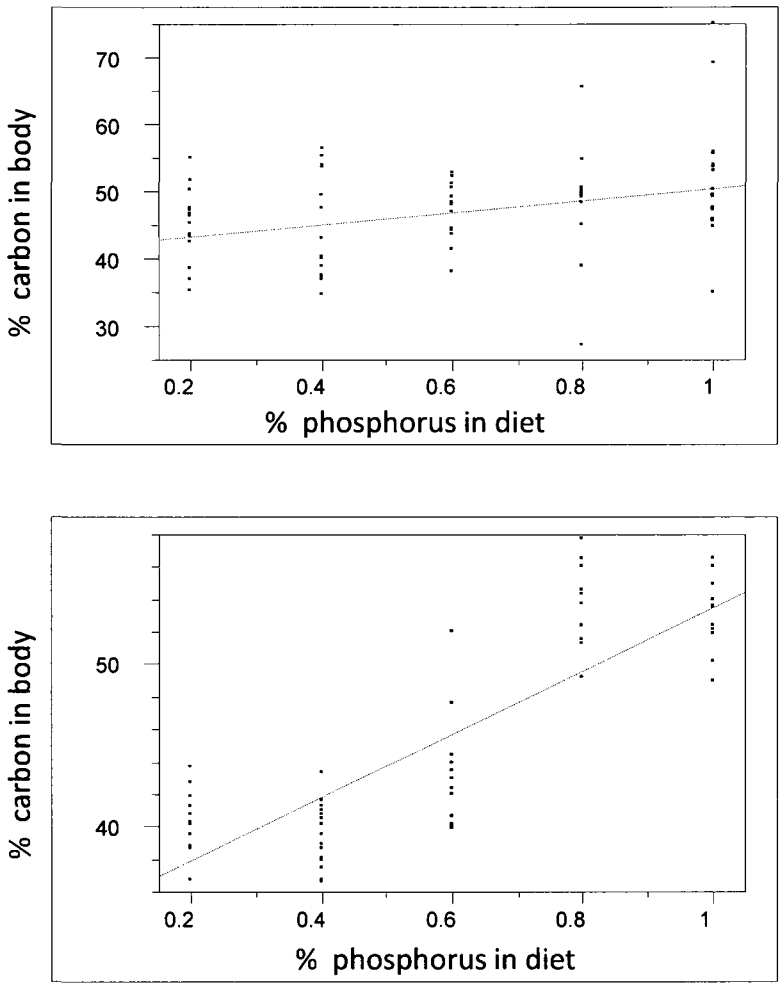


Figure 15. Relationship between phosphorus diet and %C in
a) juvenile female bodies (P = 0.0027*,df = 1,74, T-= 3.11, R^2adj= 0.1030);
b) juvenile male bodies (P = <0.0001*,df = 1,64, T= 2.85;R^2adj = 0.7160).

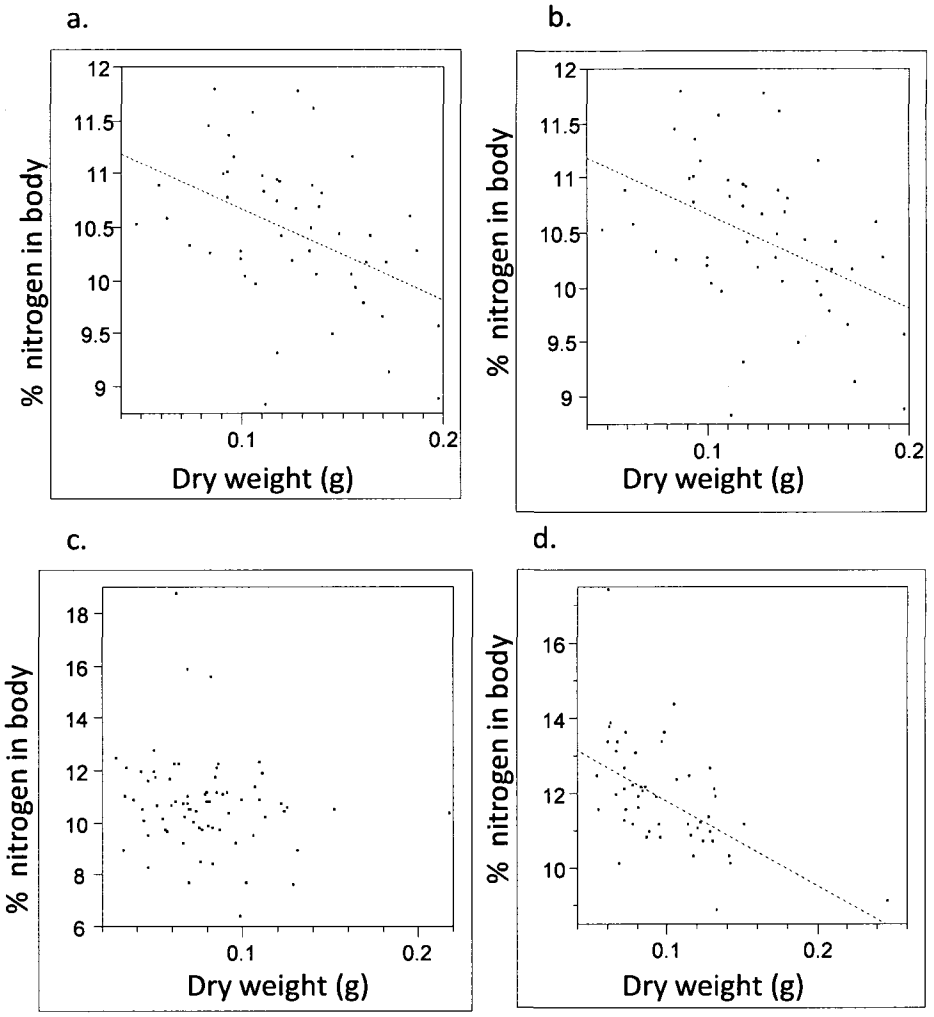


Figure 16. Relationship between body mass (g) and body nitrogen content in a. adult females (P=0.0010*; df=1,50; R^2adj = 0.1800; T= -3.50) b. adult males (P=<0.0001*; df=1,49; R^2adj = 0.2750, T= -4.48) c. juvenile females (P=0.1551; df=1,69; R^2adj = 0.0140, T= 1.44) d. juvenile males (P=0.0074*; df=1,63; R^2adj = 0.0940; T= -2.77).

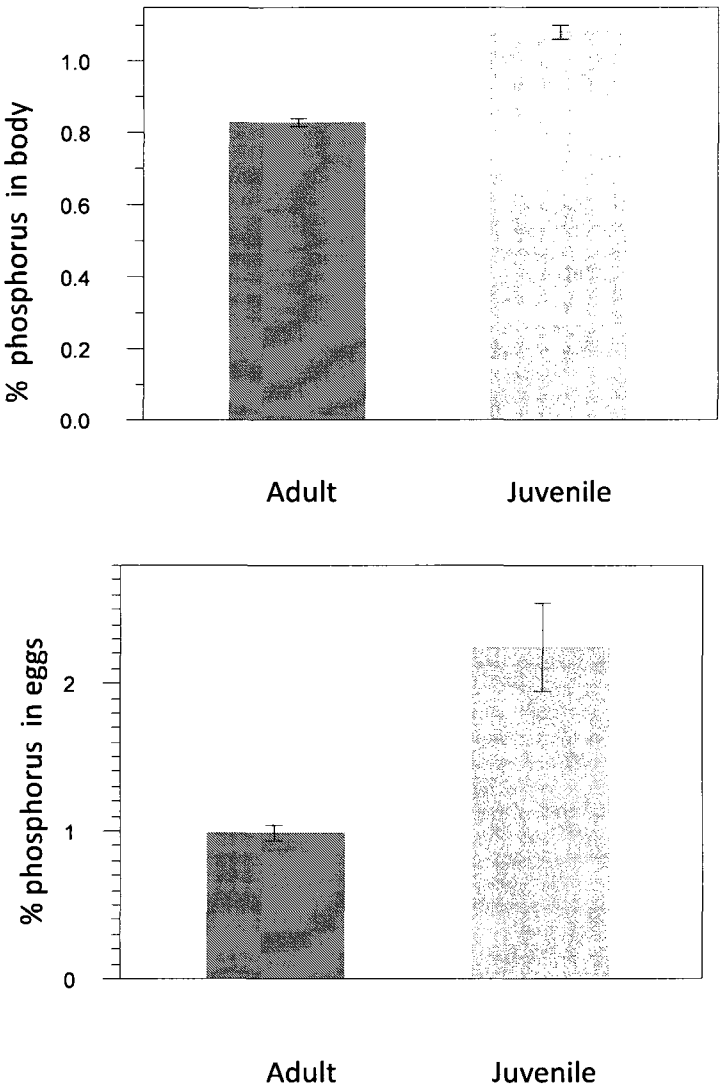


Figure 17. a. Differences in % phosphorus content of adult and juvenile bodies (Anova: F=108.46, R^2adj=0.3040, df=1,245, P= <0.0001*)
b. juvenile eggs, female that were raised on phosphorus diet during juvenile stage (Anova: F=16.36, R^2adj=0.2060, df=1,58, P= 0.0020)
Note: error bars represent standard error

Table 12. Relationship between dietary phosphorus availability and body morphology (thorax width and height, thorax area, head width)

	Sex	Body component	Prob > F	Df	R2 adj.	T
Adult	Female	Thorax width	0.5689	1,252	0.00267	-0.57
Adult	Female	Thorax height	0.1487	1,252	0.00432	-1.45
Adult	Female	Thorax Area	0.2196	1,253	0.002028	-1.23
Adult	Female	Head Width	0.5377	1,247	0.0025	-0.62
Adult	Female	Thorax Width	0.5689	1,252	0.00267	-0.57
Adult	Female	Thorax height	0.1487	1,252	1.45	-1.45
Adult	Female	Thorax Area	0.2174	1,245	0.002147	1.24
Adult	Female	Head Width	0.2183	1,244	0.002133	-1.23
Juvenile	Female	Thorax Width	0.336	1,209	0.0033	1.20
Juvenile	Female	Thorax Height	0.3069	1,208	0.000236	-1.02
Juvenile	Female	Thorax area	0.5312	1,208	0.00291	0.63
Juvenile	Female	Head Width	0.7841	1,209	0.00442	0.27
Juvenile	male	Thorax Width	0.6148	1,157	0.00474	0.5
Juvenile	male	Thorax Height	0.3704	1,157	0.00122	0.9
Juvenile	male	Thorax Area	0.2249	1,157	0.003055	1.22
Juvenile	male	Head Width	0.4365	1,157	0.00248	0.78

SUMMARY

Phosphorus availability influenced juvenile cricket growth and body elemental composition. Juvenile crickets that were reared on high phosphorus diets showed an increase in body mass compared to juveniles that were fed low phosphorus diets. Dietary phosphorus availability also influences juvenile male/female body elemental compositions (phosphorus/carbon/nitrogen). Dietary phosphorus availability did not influence juvenile cricket survival or body condition.

Adult female crickets that were fed high phosphorus diets laid significantly more eggs throughout their lives compared to those reared on low phosphorus diets. Dietary phosphorus availability did not influence average egg mass. As well, dietary phosphorus did not influence either adult or egg body elemental composition. It is important to note that crickets (adults and juveniles) that were fed on low phosphorus diets did not compensate by consuming more food. Overall, my findings suggest that dietary phosphorus availability impacts life history traits and explains 10% of the variation in adult female lifetime reproductive success.

DISCUSSION

Growth rate

Low phosphorus content in the food was associated with reduced growth in *Acheta domesticus*. Juvenile crickets reared on reduced phosphorus diets gained significantly less weight than those reared on phosphorus enhanced diets. My finding is consistent with previous studies of both aquatic and terrestrial invertebrates (Urabe & Watanabe 1992; DeMott *et al.* 1998; Sterner & Schulz 1998; Elser *et al.* 2001; Makino *et al.* 2002; Urabe *et al.* 2002). Tobacco hornworm larvae (Perkins, 2004) as well as mayfly nymphs (Frost & Elser, 2002) that were reared on elevated phosphorus diets showed an increase in growth. Combined, these results suggest that phosphorus is essential to juvenile development.

Although phosphorus availability did not significantly influence the time it took nymphs to reach their final instar in either sex, my observations suggest that the individuals reared on low phosphorus diets were more likely to die in the process of moulting, as they appeared to have a problem detaching their exoskeleton. This finding is similar to one described by Sterner *et al* (1993), *Daphnia* that feed on phosphorus deficient algae had problems with moulting, as their exoskeleton remained attached (Sterner *et al.*, 1993). Further more, phosphorus has been shown to be essential to juvenile aquatic moulting in *Daphnia*, as *Daphnia* looses approximately one-half of its phosphorus content from its body during moulting (Verede *et al.*, 1999). Combined, these results suggest that phosphorus is essential for carapace formation and moulting for both aquatic and terrestrial invertebrates. Phosphorus is important for the development of

juveniles; juvenile invertebrates not only require sufficient food to meet their energetic demands but they also require the essential element to maintain their somatic elemental demand (Elser *et al.*, 1996).

Phosphorus availability did not influence adult weight gain. Instead, cricket masses remained relatively constant regardless of phosphorus diet that the adult crickets were fed on. Huberty & Denno's (2006) study revealed a similar result as there was no effect of phosphorus diet availability on weight gain in the two planthopper species (*Prokelisia dolus* and *Prokelisia marginata*). A possible explanation for this could be that adult weight gain may be more dependent on different essential biochemical nutrients (e.g. fatty acids, amino acid, vitamins, etc.) than on phosphorus availability. An increase in phosphorus alone may not be sufficient to enhance consumer weight gain to its maximum potential (Gulati & DeMott 1997; Urabe *et al.* 1997). Another reason could be based on growth rate hypothesis which suggests that high growth rate organisms (e.g. *Daphnia*) require greater ribosomal RNA content (rich in phosphorus element) for protein synthesis. Main *et al.* (1997) indicated that organisms in their early life stages should have particular high phosphorus composition compared to adult stages. Therefore, juveniles should be more sensitive to phosphorus availability than adults.

Consumption Rate

Crickets that were put on the lower dietary phosphorus treatments did not compensate for the poor nutrient availability by consuming more food. Males and females of both stages consumed relatively the same amount of food per night regardless

of phosphorus diet they were fed on. In order for crickets to compensate for missing nutrients they must fulfill two criteria. First, they must be able to assess the current elemental state of their food; second they must be able to assess the current state of their own internal elemental component and compare the two (Despland *et al.*, 2006). Because *A. domesticus* are mobile, they have the ability to disperse to the other food sources when they are faced with limited nutrients. Because of this ability, *Acheta domesticus*, have likely never been selected to compensate for poor quality food by eating more. Instead, they probably just leave the area in search for better food. Our experimental design removed the option of cricket migration. With this option removed, we found that this cricket species appears incapable of modulating its response to its food state.

To our knowledge, there has been no research to address whether *A. domesticus* can assess its own internal elemental composition. In caterpillars, the measure of internal state is influenced by the concentration of trehalose, the main storage of sugar in insects, in the haemolymph (Thompson, 2003). The haemolymph trehalose concentration increases when caterpillars eat carbohydrates and decreases when they eat protein rich foods. This process in gustatory responses to nutrient stimuli causes the insect to switch between food types (Friedman *et al.*, 1991; Schiff *et al.*, 1989). Whether crickets exhibit the ability to assess their own internal element component remains unknown. Another reason *A. domesticus* may not compensate when consuming low quality food could be because they are incapable of reducing their ingestion rate. Copepods, for example, control their digestive enzymatic activity based on the food concentration in the gut (Mayzaud & Poulet, 1978). When copepods are faced with low quality food, they

increase their feeding rate and reduce their ingestion rate, leading to higher residence time of food in the gut (Darchambeau, 2005). *Acheta domesticus* may not be capable of regulating their gut enzymes based on food concentration and nutrient-richness, although this hypothesis remains to be tested.

Huberty and Denno's (2006) study revealed that *Prokelisia marginata* (the generalist species) dispersed to higher quality plant species when faced with low quality food. Fifth-instar locusts (*Locusta migratoria*) prefer to feed on high-protein food when given choices between high protein diet verses high carbohydrate diet (Behmer *et al.*, 2003). Likewise, invertebrate predators, when challenged by a nutritional imbalance, have the tendency to selectively forage for protein and lipid dietary resources to compensate for insufficient nutrition (Mayntz *et al.*, 2005). However, unlike these studies where the insects were given choices about their food quality, I did not provide the crickets with a choice of different food sources. Instead, *A. domesticus* was confined to a single food source. Generalist species are typically stronger at compensating for dietary imbalances than specialist species by either relocating or selecting different foods (Lee *et al.*, 2003; Lee *et al.*, 2005). Generalist locust species can tolerate, and compensate for more extreme dietary imbalances than specialist locust species (Raubenheimer & Simpson, 2003). It is, therefore, possible that if *A. domesticus* (a generalist species) were given choices between low phosphorus diets versus high phosphorus diets they could have differentiated between the two food resources. This hypothesis remains to be tested.

Adult crickets consumed twice as much food as juvenile crickets. Further, individuals that were larger in body mass generally consumed more food compared to

those smaller in body mass. This, however, did not hold true for adult males. Adult males that consumed greater amount of food had lighter body mass. A possible explanation for this difference with adult males could be that adult males utilize essential nutrients to expend energy for calling effort. Since calling effort requires energy, it could cause a reduction in body mass. In tangential support of this idea, Hunt *et al* (1994) revealed that male crickets reared on high protein diets lost a greater proportion of their body mass after each night's calling than males reared on low protein diets. Adult male crickets therefore may need to consume greater amounts of food to sustain calling and increase their mating success.

Reproduction

Phosphorous availability significantly influenced reproductive success. Females that were fed on high phosphorus diets as adults laid significantly more eggs compared to those reared on low phosphorus diets. My research appears to be the first look at how dietary phosphorus availability affects insect reproduction. The only other work to date on the subject is some by Markow *et al* (2001). Markow *et al.* (2001) found that oogenesis was delayed when *Drosophila* females were fed with reduced phosphorus diets. Phosphorus limitation has therefore been directly implicated in the manufacturing of eggs in *Drosophila.* My results suggest that phosphorus limitation also dramatically influences *A. domesticus* reproductive success, as females that were fed on reduced phosphorus content laid significantly fewer eggs.

Previous studies have also examined the effect of phosphorus diet on aquatic invertebrate reproduction. *Daphnia* that were fed on large amounts of phosphorus in insufficient food produced smaller eggs compared to individuals fed smaller amounts of phosphorus rich food. Further 15-32% of these smaller eggs did not develop (Urabe and Sterner, 2001). Boersma and Kreutzer (2002) found that female *Daphnia* that were fed on phosphorus sufficient algae gave birth to neonates with higher phosphorus content than those fed on phosphorus limited algae. This indicated that egg production under phosphorus limitation could potentially drain phosphorus resource from the female's body. These results suggest that phosphorus availability dramatically influences invertebrate reproduction. Future studies should examine the effects of dietary manipulations on *A. domesticus* lifetime fertility, the proportion of eggs fertilized, and the average number of hatchlings per female, to ascertain how lifetime egg laying translates into fitness.

As part of my experimental design, I chose to focus my study on the effect of phosphorus availability on female *A. domesticus* reproduction. The reason for focussing on only females was that our previous study revealed that phosphorus availability dramatically influenced male *A. domesticus* reproductive success. Male crickets that were fed on high phosphorus diets called with significantly higher effort than males were fed with on low phosphorus diets (Bertram *et al.*, 2009). This finding suggests that phosphorus availability influences lifetime reproductive success as males with higher signalling effort increase have greater chances of attracting more females (Bertram *et al.*,

2009). Together with my findings, these results indicate that phosphorus availability in *A. domesticus* strongly influences lifetime reproductive success.

Females can enhance their lifetime reproductive success in two ways. They can have more offspring, or they can increase the amount of energy they invest in their offspring to enhance the offspring's chances of survival. While phosphorus availability directly influenced the former, it did not affect the latter. The average weight per egg was consistent across all diet treatments, indicating that extra parental provisioning did not occur. There was no difference in the average weight of eggs from females who on average laid the fewest eggs compared to those who laid the most. This finding contradicts previous studies as most point to a trade-off strategy between number and size of offspring (e.g. Rotem *et al.*, 2003)

My reproductive results support other studies which found that phosphorus influenced reproduction in invertebrates (e.g. Urabe & Sterner, 2001; Markow *et al.*, 2001). Markow *et al* (2001) found that female *Drosophila* incorporate male ejaculate-donated phosphorus into the female ovaries during the mating process. *Drosophila* females used male donated phosphorus to synthesize high levels of nucleic acids necessary for making eggs. Copulatory transfer of phosphorus has also been documented in Lepidoptera (Lai-Fook, 1991) and mosquitoes (Quraishi, 1968). To date, there is an uncertainty to the form of phosphorus that is found in the male ejaculates (e.g., ATP, phospholipids or pyrophosphates in ejaculate) that is being incorporated by females. Further studies should examine whether excess phosphorus is passed to female crickets by males during mating in *A. domesticus*. Male *A. domesticus* that were fed on high

phosphorus diet could potentially sequester and transfer phosphorus to the female during copulation via their spermatophores. If this process occurs in *A. domesticus*, it would lessen any effects of phosphorus limitation in females. My results indicate that regardless of which phosphorus diet that females were fed on, the percentage of phosphorus within the eggs did not differ. Female *A. domesticus* could possibly partially depend on the phosphorus in male ejaculates for oogenesis.

Females regularly cannibalized males during the mating trials of this experiment, regardless of phosphorus dietary they were on. Cannibalistic females did not, however, lay significantly more eggs compared to non-cannibalistic females, suggesting that cannibalism does not influence reproduction. Maxwell (2000) examined the effect of cannibalism on female reproduction in *Iris oratoria* provided with unlimited food during the first 20-30 days then starved for 6-8 days prior to mating with the male. Similar to my study, cannibalism during mating did not affect female reproductive output as the number and mass of eggs remained relatively constant (Maxwell, 2000).

The finding that females reared on higher phosphorus diets laid more eggs than females reared on lower phosphorus diets was not really testable for juvenile females. This is due to a sample size issue as only 49% (123/250) of the juvenile females lived long enough to reach adulthood and only 18% (45/250) of these females laid eggs. With a sample size of 45 egg layers across five diets and extensive variation in egg numbers, there was insufficient data to properly ascertain whether diet influenced reproduction. That said, the egg laying data for cricket treated on phosphorus diet as juvenile (Figure 11b) certainly suggest that with a sufficient sample size there would be a significant

effect. Individuals in juvenile stages of development that live in a stressed environment (lack of essential nutrients) tend to be of poorer quality (Helm *et al.*, 1973). The manipulated diets may lack enough essential nutrients to allow juveniles to fulfil their development. Most of the dietary phosphorus was delivered using calcium phosphate. We therefore had to balance the calcium levels across the diets with calcium carbonate. High level of calcium carbonate may be harmful to insect developmental. Juvenile insects may be more susceptible to elevated level of calcium carbonate than adults. This hypothesis has not, to my knowledge, been tested.

Longevity

My experiment is one of the first to investigate the effect phosphorus has on terrestrial invertebrate survival. I found that dietary phosphorus did not influence cricket survival. Our previous work Bertram *et al* (2009) support my finding as it shows that dietary phosphorus availability does not influence adult male *Acheta domesticus* survival. Together, our finding indicates that longevity remains relatively the same across all phosphorus diets. To my knowledge, the work I present here and our earlier work (Bertram *et al.*, 2009) are the only studies to date that have investigated how phosphorus diet influence survival in terrestrial invertebrates.

Studies on aquatic invertebrates (mostly *Daphnia*) indicate that those fed on low amount of phosphorus produce a substantial number of eggs that ceased to develop prior to maturation (Urabe and Sterner, 2001). Boersma & Elser (2006) found that aquatic

invertebrate survival rate decreased when these invertebrate were provided with food containing a high-phosphorus content nutrient. Nutrient excess is thought to cause physiological damage to the invertebrate metabolism, resulting in a reduced ability to extract essential nutrients in the digestive tract (Boersma & Elser, 2006). Aquatic invertebrates therefore appear to regulate their nutrient intake to maintain fitness; when nutrients are very rich or very poor, survival is at stake.

Longevity was correlated with condition (residual mass). In fact, condition explained 44% of the variation in adult longevity and 58% of the variation in juvenile longevity. Individuals that lived longer were in better condition compared to those than survival for a shorter lifespan. Individuals with higher residual mass may have had more fat content or greater muscle mass which helped to sustain them. This hypothesis remains to be tested.

Elemental Stoichiometry

Acheta domesticus exhibited extensive variation in their elemental stoichiometry. The variation in nitrogen content was substantially larger than the previous published interspecific variation for the Texas field crickets, *Gryllus texensis*. Bertram *et al.*'s (2008) study showed that *G. texensis*'s nitrogen content ranged from 8-12%. My result revealed that *A. domesticus* nitrogen content ranges from 8-17%. Our results also differed for phosphorus content. *Acheta domesticus* exhibited substantially less variation in their body phosphorus content (0.57-1.16%, a two-fold difference) compared to field-captured *G. texensis* of 0.32-1.27%, a four-fold difference; Bertram *et al.*, 2006). Bertram *et al.*

(2006) found that crickets that were reared in the laboratory on a constant diet exhibited less variation in their stoichiometric variation compared to those captured in the field. A possible reason for this variation in body stoichiometry could be because field crickets consume a wide variety of food in nature, ranging from weeds to other insects.

Nitrogen body stoichiometry was strongly and inversely correlated with body size. There was a strong and inverse correlation between nitrogen body content and cricket body mass with the exception of juvenile females. This inverse relationship between body size and nitrogen body stoichiometry within a species is consistent to the findings that occur within other species and across insect taxa. Bertram *et al.* (2008) found a strong inverse correlation between nitrogen body content and insect body mass. Fagan *et al.*'s (2002) study also revealed that smaller species tend to have higher nitrogen content than larger species. Insects must allocate their resources to many tissues in order to maintain their physiological function. As body size increases, the overall distribution of N:P ratio becomes smaller, with respect to the total body mass (Fagan *et al.*, 2002).

Wood *et al.*'s (2004) study revealed a strong inverse correlation between insect body size and body phosphorus content. In contrast, I found no relationship between body mass and body phosphorous content in my adult experiment. Phosphorus availability in the diet did, however, influence phosphorus body content in juveniles. One factor that probably contributed to phosphorus allometry in juvenile stage could be that most growth occurred primarily in the nymphal stages (Woods *et al.*, 2004). The growth rate hypothesis suggests that growth rate is linked to phosphorus content of RNA; fast growing individuals should require more phosphorus content in their bodies. Commonly,

fastest growth is found in juveniles. Juvenile stages tend to have higher phosphorus body contents than adults (Heseen and Anderson 1991; DeMott 2003). Previous studies (Perkins 2001; Wood *et al.*, 2004) have examined the effect of phosphorus limitation on *Manduca sexta*, the weevil *Sabinia setose* (Schade *et al.*, 2003) and *Drosophila* (Markow, 2003). Their results indicated that smaller insects may be more likely to experience phosphorus limited growth than larger insects. My experiment supports the findings of these previous studies indicating dietary phosphorus contributes to juvenile insect body phosphorus content.

CONCLUSION

My comprehensive study is one of the first to investigate how phosphorus influences cricket growth, reproduction, survival, body condition and body elemental composition. My experiment revealed that dietary phosphorus directly impacts cricket growth and fitness. Juveniles that were fed diets with low phosphorus content gained significantly less weight compared to those that were fed diets with high phosphorus availability. Importantly, adult females that were fed poor phosphorus diets laid substantially fewer eggs compared to those reared on high phosphorus diets. In fact, phosphorus availability explained 10% of the variation in female lifetime reproductive success. My study, coupled with our previous study (Bertram *et al.*, 2009) suggests that phosphorus availability directly impacts cricket fitness. Future research should examine how phosphorus availability during development influences male aggressive behaviour

and the ability to obtain and retain a calling territory, as both of these factors will also influence reproductive success.

Crickets did not compensate for poor quality diets by eating more. Future work should examine whether crickets are capable of preferentially orienting to higher phosphorus diets when the diets they are on has insufficient phosphorus.

My study raised several interesting questions. Because phosphorus availability influences cricket reproduction, excess fertilizer (which is rich in phosphorus) could result in more insect crop damage in the agricultural sector. Fecundity increases with increasing phosphorus availability. Therefore the population density of pest insects should also increase with increasing phosphorus availability. Future work should probe into the effect of phosphorus on agricultural crops pests. An increase in level of phosphorus may result in better crop quality; however it may also increase the abundance of agriculture pests and subsequent crop damage.

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