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# Dietary phosphorus availability influences female cricket lifetime reproductive effort

LAKSANAVADEE VISANUVIMOL and SUSAN M. BERTRAM Department of Biology, Carleton University, Ottawa, Ontario, Canada

**Abstract.** 1. Recent ecological stoichiometric findings indicate that the relationships among key macronutrient elements [e.g. carbon (C), nitrogen (N), and phosphorus (P) of organisms and their resources] may underlie variation in life-history traits. The amount of phosphorus in an individual's body is often correlated with its rate of growth, and low-phosphorus diets are known to reduce growth in a number of insect and crustacean herbivores.

2. These findings suggest that the stoichiometric imbalance between organismal biomass requirements and the relative scarcity of nutrients in nature may also underlie variation in lifetime reproductive success.

3. This study investigated how dietary phosphorus availability during adulthood influenced lifetime reproductive effort, compensatory feeding, lifespan, condition, and stoichiometry of adult European House Cricket, *Acheta domesticus*.

4. Female crickets fed high amounts of phosphorus during adulthood laid significantly more eggs compared to those fed low amounts of phosphorus. Phosphorus availability did not directly influence lifespan, condition, or body stoichiometry, and crickets did not compensate for low phosphorus diets by eating more food.

5. A stoichiometric perspective may help understand the causes of variation in invertebrate fitness.

**Key words.** *Acheta*, behavioural stoichiometry, C:N, C:P, eggs, fitness, *Gryllus*, life history, N:P, nitrogen.

## Introduction

Organisms are likely to be more fit when their elemental compositions are in balance with their requirements (Boersma & Elser, 2006). Essential chemical elements cannot be synthesised by the organism; instead they must be obtained in sufficient quantities from the diet. Unfortunately, the diet is rarely in balance with the consumer's elemental needs (Sterner & Elser, 2002). Nitrogen and phosphorus, for example, are two of the most limiting and essential of the required chemical elements. Organisms require nitrogen to build proteins which act as enzymes catalysing vital biochemical reactions such as catabolism, DNA replication, DNA repair, and RNA synthesis (Sterner & Elser, 2002). Organisms require phosphorus to build ATP, RNA, DNA, and phospholipid molecules (Sterner & Elser, 2002). Unfortunately for plant eaters, the concentrations of nitrogen and phosphorus are typically 10–20 times lower in plants than in herbivores (Mattson, 1980; Elser *et al*., 2000). These stoichiometric mismatches between the consumer's needs and their diet have the potential to place severe constraints on the organisms' ability to meet their nutritional demands (Mattson, 1980; Strong *et al*., 1984; Elser *et al*., 2000).

Although nitrogen has traditionally been considered *the* essential element that limits production in many ecosystems (Schindler & Eby, 1997), phosphorus is also known to limit production in a number of ecosystems (Redfield, 1958; Hecky & Kilham, 1988; Vitousek *et al*., 1993; Verhoeven *et al*., 1996; Schindler & Eby, 1997; Karl, 1999). Body phosphorus content appears to correlate positively with growth in several invertebrate taxa (Quraishi *et al*., 1966; Elser *et al*., 2000, 2003; Eskelinen, 2002; Fagan *et al*., 2002; Schade *et al*., 2003; Perkins *et al*., 2004). Furthermore, experimental reduction of dietary phosphorus content appears to reduce invertebrate growth (Urabe & Sterner, 2001) while experimental supplementation appears to stimulate it (Elser *et al*., 2001; Perkins *et al*., 2004).

Less is known about how dietary phosphorus influences invertebrate fitness, but thus far the evidence is tantalising.

Correspondence: Susan M. Bertram, Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S 5B6, Canada. E-mail: Sue_Bertram@carleton.ca

For example, *Daphnia* females fed phosphorus-rich food produced larger yolkier eggs with higher developmental success than females that were fed phosphorus-deficient food (Urabe & Sterner, 2001). Further, female *Drosophila* use male donated phosphorus to synthesise nucleic acids necessary for egg production during oogenesis. Females are thought to delay oogenesis when they are fed reduced phosphorus diets (T. Markow, unpublished, cited in Markow *et al*., 2001). Additionally, Bertram *et al*. (2006) revealed a strong positive correlation between male cricket mate signalling effort and total body phosphorus content. Bertram *et al*.'s (2009) follow-up study revealed that dietary phosphorus availability strongly influences cricket signalling effort, an indicator of mating success (Cade & Cade, 1992; Crnokrak & Roff, 1995, 1998a,b; Hunt *et al*., 2004; Judge *et al*., 2008). Together, these findings suggest that dietary phosphorus availability influences invertebrate fitness.

In an attempt to quantify how dietary phosphorus availability influences insect reproductive effort, common European house crickets (*Acheta domesticus*) were reared on artificial diets that varied in phosphorus content. The following fitness components, behavioural characters, and life-history traits were then measured: (i) lifetime reproductive effort [female crickets were the focus of this study because Bertram *et al*. (2009) revealed that dietary phosphorus availability directly affects male lifetime signalling effort], (ii) lifespan, (iii) compensatory feeding [assessed because some species adapt to poor phosphorus diets by increasing their feeding activity and/or reducing their ingestion rates (e.g. Darchambeau, 2005; Huberty & Denno, 2006)], (iv) condition, and (v) stoichiometry.

## Materials and methods

European house crickets (*Acheta domesticus*) were purchased as fourth and fifth instar juveniles from Port Credit Pet Center in Port Credit, Ontario, Canada. They were raised communally in 36-litre rectangular plastic containers (36 l × 28 W × 23 H cm) in an insect-rearing facility in the Nesbitt Biology Building at Carleton University, Ottawa, Ontario, Canada. The rearing room had a temperature range of 26 ± 4 °C and a LD 12:12 h cycle. All juvenile crickets were provided with unlimited amounts of food (powdered Harlan Teklad Rodent no. 8604, 1% phosphorus content, manufactured by Harlan Teklad Inc., Madison, Wisconsin) until the day they matured into adults. They were also provided with unlimited access to water and cardboard cartons for shelter. Food and water were replenished and containers were cleaned on a regular basis. Crickets were examined daily to determine whether they had moulted to adulthood. Upon reaching adulthood, each individual was housed in a 500 ml plastic-coated paper container (7° × °11°cm, height° × °diameter) with shelter and unlimited access to water.

### *Dietary treatments*

Each cricket was assigned to one of five experimental diet treatments ranging from low (0.2%) to high (1.0%) phosphorus content. This phosphorus range was designed to mimic the range found in nature. Crickets typically feed on a variety of foods from plants to fungi to insects (including conspecific); these foods vary from an average of about 0.2% phosphorus in terrestrial plants to an average of about 0.8% phosphorus found in terrestrial insects (Sterner & Elser, 2002). Male and female crickets were provided with unlimited amounts of their assigned diet from the day they reached adulthood until the day they died a natural death.

The experimental diets (0.2%P, 0.4%P, 0.6%P, 0.8%P, 1.0%P) were designed and manufactured by Harlan Teklad (Harlan Teklad Inc.; Harlan Teklad identifying codes available upon request). Most of the phosphorus was delivered using calcium phosphate (1.0%P = 35.53 g $kg^{-1}$; 0.8%P = 26.75 g $kg^{-1}$; 0.6%P = 17.98 g $kg^{-1}$; 0.4%P = 9.21 g $kg^{-1}$; 0.2%P = 0.44 g $kg^{-1}$). However, 1.9 g $kg^{-1}$ of the phosphorus came from the casein used as a protein source. As calcium phosphate was used to deliver the phosphorus, the calcium levels were balanced across all diets with calcium carbonate such that each diet contained a total of 1% calcium (1.0%P = 0.0 g $kg^{-1}$; 0.8%P = 6.5 g $kg^{-1}$; 0.6%P = 12.75 g $kg^{-1}$; 0.4%P = 19.25 g $kg^{-1}$; 0.2%P = 25.75 g $kg^{-1}$). Each diet also contained 24% protein (236.6 g $kg^{-1}$ of protein; 272 g $kg^{-1}$ casein), fat (42.7 g $kg^{-1}$: 2.7 g $kg^{-1}$ from casein and 40 g $kg^{-1}$ from soybean oil), L-cystine (4 g $kg^{-1}$), corn starch (150 g $kg^{-1}$), maltodextrin (50 g $kg^{-1}$), cellulose (50 g $kg^{-1}$), minerals (13.4 g $kg^{-1}$; note no calcium or phosphorus included in the mineral mix), vitamins (10 g $kg^{-1}$), choline bitartrate (2.5 g $kg^{-1}$), and antioxidants (8.0 mg $kg^{-1}$).

### *Lifetime reproductive effort*

Fourteen days after females reached adulthood, they were mated with virgin males. Each mating pair was housed together for 48 h and allowed to mate freely. To control for any possible male mating effects, virgin males were raised communally and fed the original (non experimental) diet (Harlan Teklad Rodent diet no. 8604 that contained 1% phosphorus). A 29 ml container of moist sand was provided for each female to lay eggs in. The moisture levels in the sand containers were checked daily and the sand containers were replaced weekly. The number of eggs each female laid was quantified by sifting the egg container's sand through an 8 inch metal sieve with 300 μm holes. Eggs were then collected and counted. Ten eggs from each female were randomly selected and weighed together using the precision analytical balance (Denver Instruments Pinnacle Series model PI-114) to obtain an estimate of average egg weight. The number of eggs laid was recorded weekly throughout each female's lifespan. Eggs were dried and later quantified for carbon, nitrogen, and phosphorus content (details below).

### *Lifespan*

Each individual's lifespan was calculated using the difference between the day it reached adulthood and the day it died

a natural death. The few individuals that escaped prior to dying or did not have their death date recorded were excluded from analysis.

### *Compensatory feeding*

Consumption rate was monitored over a 3-day period starting at 10 days post final moult. A subset of individuals from each of the five diets were given a pre-weighed amount of food (more than they could consume). Twenty-four hours later all remaining food was reweighed and the quantity of food consumed was calculated. This process was repeated every 24 h to obtain three consumption measures per cricket. Average consumption rate was then calculated.

### *Condition*

Each cricket was weighed following its final moult to adulthood and was then added into the experiment. It was also weighed every week thereafter to ascertain how the availability of phosphorus in the diet influenced weight change. After dying a natural death, each cricket's head width, thorax width, thorax height, and thorax area were measured to the nearest 0.1 mm using a Zeiss dissection microscope (Discover 4. V 12). As these size measurements were significantly positively correlated with each other (correlations ranged from 0.6769 to 0.9130; all $P$-values $<0.0001$), a principal component analysis was used to minimise the number of size variables. The first principle component explained 99% of the variation in body size (eigenvalue = 15.1147; eigenvector loadings: thorax width = 0.14, thorax height = 0.11, thorax area = 0.98, head width = 0.07). This first principal component was used as an overall size measure (PC1 size). Each cricket's dry mass was then quantified using a Denver Instruments Precision Analytical Balance (model P-114) after the crickets were dried at 130 °C for at least 24 h in a Thermal Scientific (6520 series) drying oven. The residuals from the regression of body weight (dry weight) on body size were used as an overall measure of cricket condition at death. Condition was also quantified using the residuals from a regression of body weight at maturity on body size.

### *Stoichiometry*

Body phosphorus, nitrogen, and carbon content were quantified for a subset of crickets (10 males and 10 females per diet). Each dried cricket was pulverised to a fine uniform powder with a mortar and pestle. Cricket stoichiometry was assessed using 1–2 mg of powder per cricket. Phosphorus content was quantified using the persulphate oxidation technique followed by orthophosphate analysis using the acid molybdate technique (APHA, 1992). Carbon and nitrogen content were measured using Elementar's Vario Micro Cube CHN analyser (Elementar Americas Inc., Mount Laurel, New Jersey). Egg stoichiometry was also quantified for a subset of individuals following the same assays as described above, except that 10–20 eggs were ground together to provide a large enough sample.

### *Statistical analyses*

Statistical analyses were conducted using JMP 8.0.2 software (SAS Institute Inc., Cary, North Carolina). Shapiro–Wilk goodness-of-fit tests were used to ensure the data do not differ significantly from normality. Data that were not normally distributed (number of eggs, consumption, and lifespan) were transformed using log transformations to approximate normal distributions. A nominal logistic fit model was used to explore the factors influencing whether or not females laid eggs. Only females who lived long enough to mate and lay eggs were included in this analysis. Multiple regression models were used to quantify whether the availability of dietary phosphorus influenced the number of eggs laid, compensatory feeding, weight change, condition, and stoichiometry. A repeated measures ANOVA that modelled individual trajectories was used to quantify the factors influencing the age-dependent weight change effects. To quantify the factors influencing the number of eggs laid through time, the model assessed age-dependent means (week and the interaction between week and dietary phosphorus were included in the model). A repeated measure ANOVA using individual trajectories could not be used for the number of eggs laid model, because there were too many zeros in the fecundity data and zeros are not allowed in repeated measures when they dominate (48.5% of the egg cups had no eggs in them even though females were alive and laid eggs other weeks). A Cox proportional hazards survival model was used to determine what factors influenced cricket lifespan. Parameters and interactions included in each of these models are detailed in Table 1. Correlation analyses were used to examine the relationships between body and egg carbon, nitrogen, and phosphorus content.

## Results

### *Reproductive effort*

Most (82%) of the females laid eggs (Table 2). Lifespan, condition at death, and condition at maturity influenced the propensity to lay eggs (Table 3). Together these factors explained 52% of the variation in propensity to lay eggs. Females who lived a few weeks after mating were more likely to lay eggs than females who only lived only a few days after mating. Similarly, females in better condition at maturity were more likely to lay eggs than females in poor condition. Egg laying appeared costly, however, as females who laid eggs were in worse condition at death than females who did not lay eggs. There was also a near significant trend for phosphorus availability in the diet to influence egg laying propensity; females reared on high phosphorus diets tended to have a higher propensity to lay eggs compared to females reared on low phosphorus diets. Cricket body size did not influence the propensity to lay eggs. Likewise, the interaction effects between the aforementioned parameters and the availability of phosphorus in the diet were not significant (Table 3).

The number of eggs crickets laid was influenced by time since mating. Crickets laid more eggs in their first week following mating than they did in subsequent weeks (Tables 2

**Table 1.** Parameters included in the models used to analyse the factors contributing to life-history traits.

| Models | %P diet | Sex | Body size | Condition at maturity | No. eggs | Lifespan | Condition at death | Dry weight at death | %P diet interactions | Time | Time interactions |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Egg layer (Y/N)? | X | — | X | X | — | X | X | — | X | — | — |
| Eggs through time | X | — | — | — | — | — | — | — | X | X | X |
| Reproductive effort | X | — | X | X | — | X | X | — | X | — | — |
| Egg mass | X | — | X | X | X | X | X | — | X | — | — |
| Lifespan | X | X | X | X | — | — | X | — | X | — | — |
| Weight change | X | X | X | X | — | — | — | — | X | X | X |
| Condition at death | X | X | X | X | — | X | — | — | X | — | — |
| Compensatory feeding | X | X | X | X | — | — | — | — | X | — | — |
| %P in body | X | X | X | X | — | X | X | — | X | — | — |
| %N in body | X | X | X | X | — | X | X | — | X | — | — |
| %C in body | X | X | X | X | — | X | X | — | X | — | — |
| %P in eggs | X | — | X | X | X | X | — | — | X | — | — |
| %N in eggs | X | — | X | X | X | X | — | — | X | — | — |
| %C in eggs | X | — | X | X | X | X | — | — | X | — | — |

Factors (columns) with an X were included in the model (rows). Interaction effects between each parameter (X) and dietary phosphorus availability were also included in the models.

and 3). There was also a trend for phosphorus availability in the diet to influence the number of eggs laid per week. There was no interaction between time and phosphorus availability. Overall, this model explained 8% of the temporal variation in egg laying behaviour.

Females were wildly variable in the number of eggs they laid throughout their lives, ranging from 0 to 655 eggs in total, with an average lifetime number of eggs of 77 (all females) and 101 (only the females that laid eggs; Table 2). Lifetime reproductive effort was significantly influenced by the amount of phosphorus in the cricket's diet, condition at maturity, and condition at death (Table 3). Together these three factors explained 17% of the variation in female lifetime reproductive effort. Female crickets that were fed more phosphorus laid more eggs (Fig. 1). Likewise, female crickets that were in great condition at maturity laid more eggs. Cricket body size and lifespan did not influence lifetime reproductive effort. There were no interactions between the availability of phosphorus in the diet and all aforementioned parameters (Table 3). There was also no relationship between the number of eggs laid and egg mass (regression: $F_{1,136} = 1.0615$, $P = 0.3047$, $R^2_{adj} = 0.0004$).

Females who laid heavy eggs died more quickly than females who laid lighter eggs (Tables 2 and 3; Fig. 2). Egg mass was not influenced by maternal dietary phosphorus availability, body size, condition, lifespan, number of eggs, or the interaction between phosphorus availability and any of these aforementioned factors. Together almost 13% of the variation in egg mass was explained by this model.

### *Lifespan*

On average, crickets tended to live between 4 and 5 weeks following their moult to adulthood, however cricket lifespan was highly variable across individuals (Table 2). Lifespan was influenced by condition at death, body size, and sex (Table 3). Dietary phosphorus availability, condition at maturity, and the interaction between phosphorus availability and the aforementioned parameters did not influence cricket lifespan (Table 3).

### *Condition*

The repeated measures multivariate model explained significant variation among crickets, but not within crickets (Table 3). Crickets did not gain significant weight over time and there were no interactions between time and sex, size, condition at maturity, or the availability of phosphorus in the diet. Cricket weight was, however, dependent on cricket sex (females weighed more than males), size (larger crickets weighed more than smaller crickets), condition at maturity (by definition), and the availability of phosphorus in the diet (crickets fed diets with higher phosphorus content weighed more).

The key factors influencing condition at death was the cricket's condition at maturity and lifespan. Together these factors explained 28% of the variation in condition at death (Table 3). Crickets that were in better condition at maturity were also in better condition at death. Crickets that lived to a ripe old age were in worse condition at death than crickets that died young. Dietary phosphorus availability, body size, sex, and interactions between all the aforementioned parameters and phosphorus availability did not influence condition at death.

### *Compensatory feeding*

Crickets ate an average of 48 $\pm$ 32.8 mg of their diet per day (both sexes combined; Table 2). Crickets did not compensate for poor phosphorus availability by consuming more food. Similarly, foraging behaviour was not influenced by condition at maturity, body size, sex, or the interaction between phosphorus availability and the other aforementioned parameters (Table 3).

**Table 2.** Descriptive statistics for all variables measured in male and female *Acheta domesticus* bodies.

| Sex | Parameter | Mean ± *SE* | Min. | Max. | CV | *n* |
|---|---|---|---|---|---|---|
| Female | No. eggs (lifetime – all) | 76.61 ± 6.75 | 0.00 | 655.00 | 121.75 | 191 |
| Female | No. eggs (week 1 – all) | 49.19 ± 5.16 | 0.00 | 412.00 | 144.91 | 191 |
| Female | No. eggs (week 2 – all) | 28.13 ± 3.43 | 0.00 | 250.00 | 144.64 | 141 |
| Female | No. eggs (week 3 – all) | 10.95 ± 2.98 | 0.00 | 182.00 | 238.83 | 77 |
| Female | No. eggs (week 4 – all) | 4.67 ± 1.42 | 0.00 | 36.00 | 174.64 | 33 |
| Female | No. eggs (lifetime – layers) | 101.19 ± 8.33 | 1.00 | 655.00 | 96.74 | 156 |
| Female | No. eggs (week 1 – layers) | 64.57 ± 6.61 | 0.00 | 412.00 | 120.25 | 138 |
| Female | No. eggs (week 2 – layers) | 33.96 ± 3.97 | 0.00 | 250.00 | 125.04 | 114 |
| Female | No. eggs (week 3 – layers) | 13.70 ± 3.75 | 0.00 | 182.00 | 211.87 | 60 |
| Female | No. eggs (week 4 – layers) | 6.70 ± 1.89 | 0.00 | 36.00 | 135.57 | 23 |
| Female | Average weight of 10 eggs | 2.61 ± 0.05 | 1.00 | 4.50 | 24.27 | 152 |
| Female | Lifespan | 28.07 ± 1.40 | 1.00 | 130.00 | 94.99 | 365 |
| Male | Lifespan | 33.79 ± 1.88 | 1.00 | 138.00 | 99.36 | 318 |
| Female | Compensatory feeding (mg) | 54.41 ± 3.00 | 5.67 | 169.83 | 55.74 | 102 |
| Male | Compensatory feeding (mg) | 47.27 ± 3.71 | 3.63 | 167.50 | 80.48 | 105 |
| Female | Weight at start | 400.38 ± 5.00 | 135.50 | 705.80 | 25.69 | 423 |
| Male | Weight at start | 336.29 ± 4.32 | 143.00 | 624.70 | 23.71 | 340 |
| Female | Dry weight | 132.85 ± 3.02 | 35.40 | 285.80 | 37.51 | 272 |
| Male | Dry weight | 91.75 ± 1.81 | 36.40 | 189.70 | 32.73 | 275 |
| Female | Head width | 4.39 ± 0.03 | 3.06 | 6.03 | 12.20 | 248 |
| Male | Head width | 4.33 ± 0.03 | 3.06 | 5.46 | 10.35 | 243 |
| Female | Thorax width | 5.17 ± 0.03 | 3.72 | 6.65 | 10.21 | 254 |
| Male | Thorax width | 4.62 ± 0.03 | 3.36 | 6.08 | 10.01 | 248 |
| Female | Thorax height | 3.25 ± 0.03 | 2.09 | 4.26 | 12.39 | 254 |
| Male | Thorax height | 2.74 ± 0.02 | 1.87 | 3.94 | 11.94 | 248 |
| Female | Thorax area | 16.99 ± 0.23 | 8.17 | 28.32 | 21.71 | 254 |
| Male | Thorax area | 12.77 ± 0.16 | 6.32 | 21.58 | 20.09 | 248 |
| Female | %P eggs | 1.01 ± 0.06 | 0.50 | 1.82 | 32.16 | 30 |
| Female | %P body | 0.86 ± 0.02 | 0.66 | 1.17 | 13.96 | 54 |
| Male | %P body | 0.79 ± 0.01 | 0.58 | 1.02 | 13.52 | 52 |
| Female | %N eggs | 5.41 ± 0.31 | 1.70 | 8.28 | 32.16 | 32 |
| Female | %N body | 10.48 ± 0.09 | 8.80 | 11.77 | 6.44 | 55 |
| Male | %N body | 11.74 ± 0.17 | 8.79 | 14.32 | 10.23 | 51 |
| Female | %C eggs | 31.78 ± 1.66 | 10.70 | 43.48 | 29.54 | 32 |
| Female | %C body | 46.97 ± 0.32 | 43.26 | 53.21 | 5.12 | 55 |
| Male | %C body | 54.90 ± 0.74 | 47.10 | 65.63 | 9.52 | 51 |

All weight measures are in milligrams. All size measures are in millimetres. Min., minimum value recorded; Max., maximum value recorded; CV, coefficient of variation; *n*, sample size. The sample size for number of eggs laid is smaller because it does not include females that died prior before the mating session or individuals that escaped.

*Stoichiometry*

Cricket body stoichiometry was highly variable across individuals (Table 2). The key factor influencing cricket body phosphorus content was lifespan (Fig. 3; Table 3). Crickets that lived longer had less phosphorus in their bodies than crickets that died quickly. It is not possible to tease apart cause or effect with the available data, because phosphorus content was calculated following natural death. Dietary phosphorus availability, cricket condition at maturity, size, sex, condition at death, and interactions between these factors and dietary phosphorus did not influence body phosphorus content. Together these factors explained 11% of the variation in total body phosphorus.

The factors influencing cricket body nitrogen content were sex, condition at death, an interaction between lifespan and dietary phosphorus, an interaction between condition at death and dietary phosphorus, and body size. Together these factors explained 53% of the variation in nitrogen content. Males contained more nitrogen than females; crickets in good condition at death contained less nitrogen than crickets in poor condition at death. Nitrogen content was not directly influenced by dietary phosphorus availability, condition at maturity, or lifespan (Table 3).

The factors influencing cricket carbon content were sex, condition at death, an interaction between sex and dietary phosphorus content, condition at maturity, and an interaction between condition at maturity and dietary phosphorus content. Together these factors explained 53% of the variation in body carbon content. Females had significantly more carbon in their bodies than males. Crickets in good condition at adulthood had significantly less carbon in their bodies than crickets in poor condition. Crickets in good condition at death had significantly

**Table 3.** Factors influencing variation in life-history traits (factors with $P < 0.10$ included).

| Model | d.f. | $R^2_{adj}$/parameter | $F/X^2$ | $P$ |
|---|---|---|---|---|
| Female laid eggs? (Y/N) | 9,204 | Overall model; $R^2_{adj} = 0.5218$ | 151.6126 | <0.0001 |
| | | %P diet | 3.0245 | 0.0820 |
| | | Condition at maturity | 6.3545 | 0.0117 |
| | | Condition at death | 15.5039 | <0.0001 |
| | | Lifespan (log) | 102.7312 | <0.0001 |
| Eggs through time | 7,471 | Overall model; $R^2_{adj} = 0.0805$ | 6.9746 | <0.0001 |
| | | %P diet | 3.6764 | 0.0558 |
| | | Time (week) | 14.4088 | <0.0001 |
| Lifetime reproductive effort | 9,79 | Overall model; $R^2_{adj} = 0.1707$ | 3.0126 | 0.0038 |
| | | %P diet | 10.2323 | 0.0020 |
| | | Condition at maturity | 7.0294 | 0.0097 |
| | | Condition at death | 7.1140 | 0.0093 |
| Egg mass | 11,83 | Overall model; $R^2_{adj} = 0.1259$ | 2.2308 | 0.0200 |
| | | Lifespan | 20.4038 | <0.0001 |
| Lifespan | 9,422 | Overall model | 61.6829 | <0.0001 |
| | | Sex | 10.9469 | 0.0009 |
| | | Size | 12.2708 | 0.0005 |
| | | Condition at death | 34.1975 | <0.0001 |
| Weight change | 7,176 | Overall model (across) | 63.7651 | <0.0001 |
| | | %P diet | 4.6645 | 0.0321 |
| | | Sex | 140.7968 | <0.0001 |
| | | Size | 96.5389 | <0.0001 |
| | | Condition at maturity | 201.9500 | <0.0001 |
| | 21,500 | Overall model (within) | 0.7861 | 0.7381 |
| Condition at death (adults) | 9,412 | Overall model; $R^2_{ad} = 0.2830$ | 19.6430 | <0.0001 |
| | | Condition at maturity | 136.8199 | <0.0001 |
| | | Lifespan | 27.1204 | <0.0001 |
| Compensatory feeding | 7,127 | Overall model; $R^2_{adj} = 0.0109$ | 0.7934 | 0.5942 |
| %P body | 11,99 | Overall model; $R^2_{adj} = 0.1141$ | 2.1595 | 0.0238 |
| | | Lifespan | 12.2278 | 0.0007 |
| %N body (adults) | 11,85 | Overall model; $R^2_{adj} = 0.5325$ | 10.9413 | <0.0001 |
| | | Sex | 67.4726 | <0.0001 |
| | | Size | 5.0453 | 0.0273 |
| | | Lifespan (log) × %P diet | 8.0420 | 0.0057 |
| | | Condition at death | 27.1459 | <0.0001 |
| | | Condition at death × %P diet | 5.1519 | 0.0258 |
| %C body (adults) | 11,85 | Overall model; $R^2_{adj} = 0.5272$ | 11.1290 | <0.0001 |
| | | Sex | 82.8718 | <0.0001 |
| | | Sex × %P diet | 7.6220 | 0.0071 |
| | | Condition at maturity | 5.9457 | 0.0168 |
| | | Condition at maturity × %P diet | 5.3697 | 0.0229 |
| | | Condition at death | 8.0623 | 0.0057 |
| %P eggs | 9,18 | Overall model; $R^2_{adj} = 0.3688$ size | 2.7529 | 0.0322 |
| | | | 7.6783 | 0.0126 |
| %N eggs | 9,18 | Overall model; $R^2_{adj} = 0.0987$ | 1.3405 | 0.0987 |
| | | No. eggs × %P diet | 3.2167 | 0.0888 |
| %C eggs | 9,19 | Overall model; $R^2_{adj} = 0.2021$ | 1.7880 | 0.1370 |
| | | Condition at maturity | 5.3319 | 0.0323 |

Weight change was quantified using a repeated measures ANOVA; size, condition, lifetime reproductive effort, and stoichiometric measures were quantified using multiple regressions; The propensity to lay eggs was quantified using a nominal logistic fit model; lifespan was quantified using a Cox proportional hazards model; Factors included in each model are presented in Table 1.

 

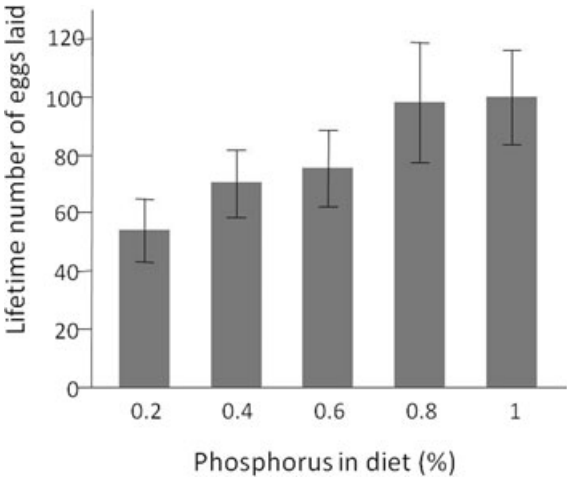


**Fig. 1.** Females that were fed diets with high phosphorus content laid significantly more eggs than those fed diets with low phosphorus content. The plot shows the mean number of eggs laid in the lifetime for all females capable of laying eggs in each dietary treatment; error bars indicate standard errors.

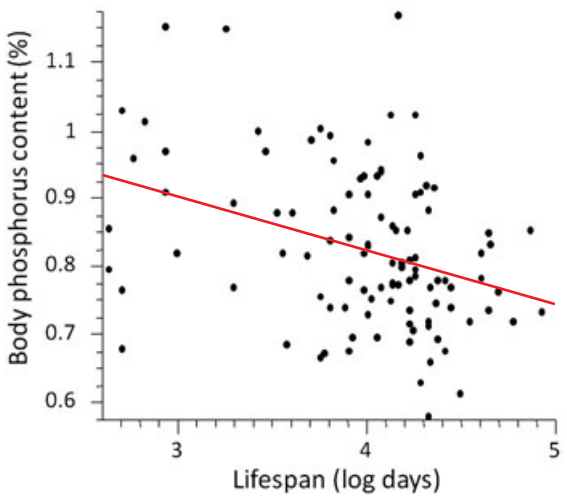


**Fig. 3.** Body phosphorus content decreased with increasing lifespan.

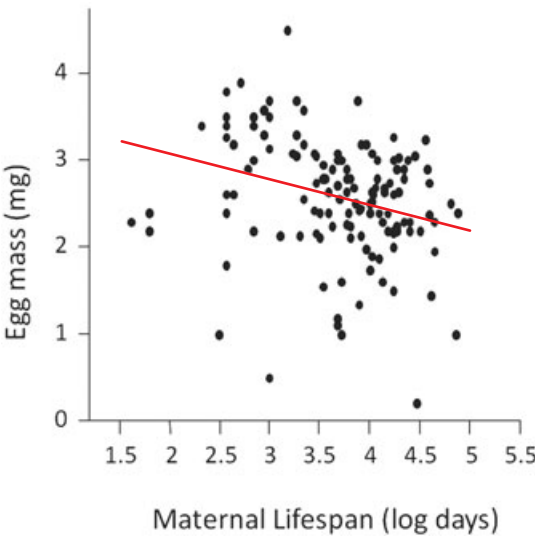


**Fig. 2.** Egg mass decreased with increasing maternal lifespan.

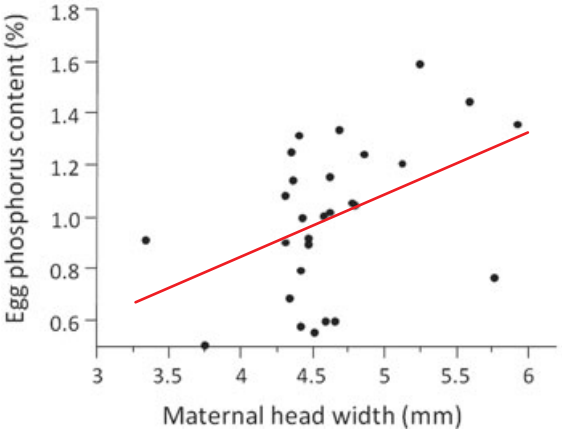


**Fig. 4.** Egg phosphorus content increased with maternal body size.

more carbon in their bodies than crickets in poor condition. Body carbon content was not influenced by dietary phosphorus content alone, size, or lifespan (Table 3).

There was a significant correlation between total body carbon and nitrogen content (correlation = 0.5237, $P < 0.0001$, $n = 104$). Total body nitrogen and phosphorus were not significantly correlated, neither were total body carbon and phosphorus.

Cricket eggs were also highly variable in their stoichiometry (Table 2). Egg stoichiometry was not correlated with maternal body stoichiometry. Eggs with lots of phosphorus contained lots of nitrogen and carbon; similarly, eggs with lots of nitrogen also contained lots of carbon (nitrogen and phosphorus correlation = 0.6908, $P < 0.0001$, $n = 27$; carbon and phosphorus correlation = 0.7274, $P < 0.0001$, $n = 27$; carbon and nitrogen correlation = 0.9402, $P < 0.0001$, $n = 30$). A key factor influencing egg phosphorus content was maternal body size. Larger females laid eggs with more phosphorus than smaller crickets (Fig. 4; Table 3). Maternal condition at maturity, lifespan, number of eggs laid, and availability of phosphorus in the diet did not influence egg phosphorus content. Likewise there was no interaction between maternal dietary phosphorus content and any of the aforementioned factors. Together these factors explained 37% of the variation in egg phosphorus content (Table 3). The models exploring the factors influencing egg carbon and nitrogen content were not significant (Table 3).

## Discussion

### *Reproductive effort*

Dietary phosphorous availability significantly influenced female lifetime reproductive effort. Crickets that were fed diets high in phosphorus laid significantly more eggs throughout their lives than crickets that were reared on low phosphorus diets. In fact, the availability of phosphorus in the diet contributed more to explaining variation in lifetime reproductive effort than any other factor examined including body size and condition at maturity. This finding suggests that dietary phosphorus availability has the potential to strongly impact invertebrate fitness. There was also a trend for dietary phosphorus to influence the propensity to lay eggs and the temporal variation in egg laying, although these parameters were not quite significant. Together, these findings support the handful of other studies showing that dietary phosphorus availability influences invertebrate reproductive success (e.g. Markow *et al*., 2001; Urabe & Sterner, 2001; Bertram *et al*., 2009). More researchers need to examine how dietary phosphorus contributes to invertebrate reproduction before we can understand how universal this finding is.

Females can enhance their reproductive success in two ways: they can produce more offspring (lay more eggs) or they can enhance the survival of their offspring (produce eggs with higher yolk content). Egg mass was not influenced by maternal access to dietary phosphorus. Instead, egg mass was directly linked to maternal lifespan: females that laid heavier eggs died young. This suggests that laying heavy eggs may be costly. Alternatively, crickets facing imminent death may invest as much as they can into their eggs, producing larger/heavier eggs.

### *Lifespan*

Dietary phosphorus availability did not influence cricket lifespan. This finding is also supported by previous work on adult male crickets (Bertram *et al*., 2009). Instead, variation in lifespan was partially explained by sex (males lived longer than females), size (larger crickets lived longer than smaller crickets), and condition at death (crickets that lived a long time tended to be in poorer condition at death). Given that the availability of phosphorus during development tends to influence invertebrate growth (Quraishi *et al*., 1966; Elser *et al*., 2000, 2001, 2003; Urabe & Sterner, 2001; Eskelinen, 2002; Fagan *et al*., 2002; Schade *et al*., 2003; Perkins *et al*., 2004), phosphorus availability has the potential to tangentially impact adult longevity. Because of the potential importance of dietary phosphorus to juvenile cricket growth, body size, condition at adulthood, reproduction, and adult longevity, we are conducting a follow-up experiment to test the idea that phosphorus availability during development influences cricket life history traits.

### *Condition*

Dietary phosphorus availability influenced body weight, but it did not explain any of the variation in cricket condition at death. Instead, variation in condition at death was explained by condition at maturity and lifespan. These findings suggest that the ability to gain weight as a juvenile is far more important a predictor of cricket condition at death.

### *Compensatory feeding*

Crickets did not compensate for low phosphorus availability by consuming more food. This finding does not preclude the possibility that *A. domesticus* compensates for poor quality food in a different way. For crickets to compensate for a missing essential element, they must be able to assess the current elemental state of their own bodies as well as the elemental state of their food, and then compare the two (Despland & Noseworthy, 2006). While it is unknown whether crickets do this, there is tangential evidence that suggests that they do. Crickets can become cannibalistic (Alexander & Otte, 1967; Kieruzel & Chmurzynski, 1987) when faced with nutrient limitations (Simpson *et al*., 2006). Given that herbivores contain approximately four times more phosphorus in their bodies than the typical plant (about 0.8% vs. 0.2%; Mattson, 1980; Sterner & Elser, 2002), cannibalism should greatly increase the availability of limited essential elements, resulting in enhanced lifetime reproductive success. Future research should examine whether cannibalistic crickets have higher fitness.

### *Stoichiometry*

Variation in body phosphorus content was influenced by cricket lifespan. Crickets that lived until they were old contained less phosphorus than crickets that died young. Variation in body phosphorus was not, however, dependent on the availability of phosphorus in the diet, body size, sex, and condition. Similarly, body phosphorus content was not correlated with body nitrogen or carbon content. These findings are somewhat surprising, given that the availability of phosphorus in the diet often impacts body phosphorus content (Schade *et al*., 2003); smaller crickets typically have more phosphorus in their bodies than larger crickets (Peters, 1983); females typically contain more phosphorus than males (Markow *et al*., 1999); and phosphorus tends to be positively correlated to nitrogen content and negatively correlated to carbon content (Bertram *et al*., 2008). One potential explanation for these 'classic' relationships to be lacking is that prior to the start of the experiment, the crickets were reared on diets that were plentiful in protein, lipids, carbohydrates, and phosphorus. When resources are plentiful throughout development, these classic relationships may not occur. If this is the case, they should be evident when juveniles are reared on nutrient-limited diets. A second potential explanation for these unusual findings is that the crickets were allowed to die a natural death before their body

stoichiometry was examined. Any classic relationship that existed could have been washed out by the extreme variation in lifespan, assuming older crickets utilise more of their nutrient stores thereby altering the proportion of nitrogen, phosphorus, and carbon in their bodies.

Egg phosphorus content was strongly influenced by maternal body size. Larger females laid eggs with more phosphorus in them than smaller females. This result has potentially important ramifications if egg phosphorus content influences early juvenile growth and development. If larger females can provide their offspring with extra phosphorus, their offspring may grow more quickly, reach larger sizes by adulthood, and produce more successful offspring. These ideas need to be tested.

*European house crickets*

The European house crickets (*Acheta domesticus*) used in this experiment were obtained from a commercial supplier. As such, they were bred in captivity for several generations, fed unlimited food, and exposed to a predator-free environment. They are thus likely to have experienced very different inbreeding and selection intensities from their wild cricket counterparts (Gray, 1997; Gray & Cade, 1999). Further, because these individuals were obtained from a commercial supplier, it is unknown what they were fed prior to arriving at Carleton University as third and fourth-instar juveniles. To control for this, the crickets were fed a control diet high in phosphorus (1%P) until they reached adulthood. Colony artefacts could, however, still remain. Care must therefore be taken when extending these results to other insect species.

## Conclusions

Diet is well known to affect cricket reproduction (Wagner & Hoback, 1999; Holzer *et al*., 2003; Scheuber *et al*., 2003; Mallard & Barnard, 2004; Zajitschek *et al*., 2009). However, until recently, the condition-dependent nature of these reproductive traits had not been hypothesised to relate to the demand for key macronutrient elements. The experiments presented here reveal that dietary phosphorus directly impacts female cricket lifetime reproductive effort. Crickets did not compensate for poor quality diets by eating more food. Cricket lifespan and condition were not directly impacted by dietary phosphorus availability. These findings, coupled with previous findings revealing that phosphorus influences invertebrate growth (Quraishi *et al*., 1966; Elser *et al*., 2000, 2001, 2003; Urabe & Sterner, 2001; Eskelinen, 2002; Fagan *et al*., 2002; Schade *et al*., 2003; Perkins *et al*., 2004) and sexually selected traits (Bertram *et al*., 2006, 2009) suggest a stoichiometric imbalance between the organism's nutrient requirements and the relative scarcity of nutrients in nature may underlie variation in fitness conferring traits. Given the limiting nature of phosphorus in many ecosystems (Redfield, 1958; Hecky & Kilham, 1988; Vitousek *et al*., 1993; Verhoeven *et al*., 1996; Schindler & Eby, 1997; Karl, 1999), variation in its availability may influence lifetime reproductive success in many invertebrate species. These findings suggest that the time is ripe to compare the importance of phosphorus availability during development to life-history traits.

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