

This is the peer reviewed version of the following article: Houslay, T. M., Houslay, K. F., Rapkin, J., Hunt, J. and Bussière, L. F. (2017), Mating opportunities and energetic constraints drive variation in age-dependent sexual signalling. *Funct Ecol*, 31: 728–741, which has been published in final form at <https://doi.org/10.1111/1365-2435.12766>. This article may be used for non-commercial purposes in accordance With Wiley Terms and Conditions for self-archiving.

Title: Mating opportunities and energetic constraints drive variation in age-dependent sexual signalling

Running headline: Drivers of variation in sexual signalling

Key words: Condition dependence, life history, phenotypic plasticity, sexual selection, trade offs.

Thomas M. Houslay^{*1,2}, Kirsty F. Houslay³, James Rapkin², John Hunt^{2,4}, Luc F. Bussière¹

¹ Biological and Environmental Sciences, University of Stirling, Stirling, United Kingdom.

² Centre of Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, Penryn Campus, Penryn, Cornwall, TR10 9FE, United Kingdom.

³ Department of Respiratory, Inflammation and Autoimmunity, MedImmune Ltd., Abingdon, United Kingdom.

⁴ School of Science and Health, Western Sydney University, Locked Bag 1797, Penrith, NSW, 2751, Australia

* Corresponding author: t.houslay@exeter.ac.uk

Other contact information:

KFH: houslayk@medimmune.com

JR: jr297@exeter.ac.uk

JH: J.Hunt@westernsydney.edu.au

LFB: luc.bussiere@stir.ac.uk

All data will be uploaded to Dryad upon acceptance of this manuscript.

Main text: 7415 words.

Figure count: 6.

Table count: 5.

Mating opportunities and energetic constraints drive variation in age-dependent sexual signalling

Thomas M. Houslay^{*1,2}, Kirsty F. Houslay³, James Rapkin², John Hunt², Luc F. Bussiere¹

¹ Biological and Environmental Sciences, University of Stirling, Stirling, United Kingdom.

² Centre of Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, Penryn Campus, Penryn, Cornwall, TR10 9FE, United Kingdom.

³ Department of Respiratory, Inflammation and Autoimmunity, MedImmune Ltd., Abingdon, United Kingdom.

* Corresponding author: t.houslay@exeter.ac.uk

Summary

1. When males repeatedly produce energetically expensive sexual signals, trade-offs between current and future investment can cause plasticity in age-dependent signalling. Such variation is often interpreted as alternate adaptive strategies: live fast and die young vs. slow and steady.
2. An alternative (yet rarely tested) explanation is that condition-dependent constraints on allocation cause variation in signalling with age ('late bloomers' do not have early investment options). Testing this hypothesis is challenging because resource acquisition and allocation are difficult to measure, and energetic reserves both affect and are affected by reproductive effort.

3. We simultaneously manipulated acquisition (through dietary nutrition) and access to potential mates (as a proxy for manipulating sexual trait allocation) in male decorated crickets (*Gryllodes sigillatus*), while measuring age- and signalling effort-mediated changes in energy storage components.
4. Increased diet quality caused increased signalling effort and energy storage, while access to females increased both the likelihood of and time spent signalling. Males with lower resource budgets signalled less, but still suffered energetic storage loss and viability costs.
5. Our results suggest that energetic constraints, rather than strategic resource accumulation, reduced signalling levels in males with lower resource acquisition ability. Our findings imply a non-adaptive explanation for age-dependent variation in sexual signalling, and an important role for energetic constraints in maintaining the honesty of costly behavioural displays.

KEY WORDS: Condition dependence, life history, phenotypic plasticity, sexual selection, trade offs.

64 The fitness of an individual depends on its investment in life-history traits (Stearns 1992). The
 65 expression of these traits is thought to be affected by trade-offs, caused when a fitness-improving
 66 increase in one life history trait is coupled to a fitness-reducing decrease in another (Stearns 1989).
 67 One trade-off universal to all organisms occurs because any individual has only a finite pool of
 68 resources, for which all of its traits are competing. Most studies on such trade-offs have focused on
 69 investment across traits at a single point in the life history, but allocation trade-offs can also occur
 70 between investments in the same trait at different ages. Although the concept of age-dependent
 71 changes in performance is prevalent in studies of senescence, it is often difficult to disentangle the
 72 effects of allocation from other potential causes of decreased performance (Monaghan *et al.* 2008;
 73 Nussey *et al.* 2008).

74 An individual's ability to acquire resources, known as its 'condition', is often strongly related to the
 75 expression of costly sexual traits in males (Rowe & Houle 1996). Condition is likely determined by a
 76 large number of loci because so many genes are involved in acquiring and metabolising resources. If
 77 the ability to pay fitness costs associated with increased investment in a sexual trait (e.g. reduced
 78 future reproductive effort or survival) is heritable, females can use a male's condition-dependent
 79 signals to assess the quality of his genes (in terms of the probable condition of offspring; Nur and
 80 Hasson 1984; Andersson 1986; Grafen 1990; Iwasa and Pomiankowski 1994).

81 This relationship between genetic quality and condition depends on trade-offs, with genetic
 82 correlations among fitness-related traits affected by relative levels of genetic variation in both the
 83 acquisition and allocation of resources (Van Noordwijk & De Jong 1986; Houle 1991; Hunt *et al.*
 84 2004b). While theory suggests males should benefit by sacrificing investment in longevity in favour
 85 of increased early reproductive success (Vinogradov 1998; Bonduriansky *et al.* 2008), studies often
 86 show the opposite pattern, such that males that signal most intensely are also those that survive the
 87 longest (Jennions, Møller & Petrie 2001). One possible explanation for this positive covariance is that
 88 high-condition individuals have more resources to allocate to all aspects of their life history, masking

real life history trade-offs between expensive traits (Van Noordwijk & De Jong 1986; De Jong & Van Noordwijk 1992; Reznick, Nunney & Tessier 2000; Hunt *et al.* 2004b).

Assessing how life history trade-offs affect investment in sexual trait expression is a challenging task.

The resource pool itself cannot be measured directly via phenotypic traits: many such traits may covary with an individual's resource budget, but must themselves have been constructed using resources and therefore will necessarily be traded against other life history traits (Hunt *et al.* 2004b; Houslay & Bussière 2012). Residuals of body mass over a fixed measure of body size are commonly used as a proxy for condition (e.g., Peig and Green 2009), yet are unlikely to be consistently adequate (Wilder, Raubenheimer & Simpson 2015; Barnett *et al.* 2015): for example, energetically expensive trait expression (such as sexual display) is fuelled by sugars that are supplied by fat stores (Tomkins *et al.* 2004). Consequently, condition indices can only be used to reliably compare individuals of different sizes if we know how fat content scales with size (Kotiaho 1999). Ideally, direct physiological analyses (rather than genetic or phenotypic approaches) are needed to study the mechanisms mediating energy storage and use, and their consequences for variation in sexual trait expression (Zera & Harshman 2001).

The problems inherent in assessing resource trade-offs are compounded when an individual expresses a sexual trait repeatedly over its lifetime. Such individuals face trade-offs not only between the focal trait and other components of life history, but also between current and future expression of the sexual trait: a male that invests many resources when young has fewer resources to spend when old, and vice versa (Badyaev & Qvarnström 2002). To save resources for critical periods, males may elect not to signal at certain ages, or to delay maturation until later in life (Kokko 1997). These trade-offs create variation between individuals in patterns of age-dependent expression of sexual traits, and impede females from using signal expression as an index of a male's overall acquisition ability. In other words, investment in sexual signalling at any given age is less

likely to be an honest indicator of quality than if this trait remained constant across his lifetime (Kokko 1997).

The honesty of sexual traits has long been of interest to students of sexual selection (Zahavi 1975; Andersson 1982; Nur & Hasson 1984; Grafen 1990; Johnstone 1995; Kokko 1997). Strict honesty in sexual trait expression may not always be fulfilled in a population (Candolin 1999; Hunt *et al.* 2004a), but the ‘on-average’ honesty enforced by life history trade-offs maintains stability in systems where male advertisement is subject to within-individual variation over lifetimes (Kokko 1997). Identifying costs associated with sexual trait expression is therefore crucial to determining variation in age-dependent signalling, and pinpointing mechanisms enforcing honesty in such systems.

A primary model for studying investment in age-dependent sexual expression is acoustic signalling (Andersson 1994). In crickets, males produce an advertisement call through stridulation of their forewings, and the amount of time a male spends calling (‘calling effort’) is a strong predictor of mating success in nature (Hunt *et al.* 2004a; Bentsen *et al.* 2006; Rodriguez-Munoz *et al.* 2010). Calling is highly energetically expensive (e.g., Kavanagh 1987; Hunt *et al.* 2004a; Ophir *et al.* 2010; Mowles 2014), and may increase mortality risk from both intrinsic (calling diverts energy from other functions, such as somatic maintenance, Hunt *et al.* 2004a) and extrinsic sources (male calls attract predators, Walker 1979, or parasitoids, Cade 1975). Among crickets, calling is likely fuelled by aerobic metabolism (Prestwich & Walker 1981; Bailey *et al.* 1993; Prestwich 1994; Hoback & Wagner 1997), with variation in calling effort linked to the ability to acquire and mobilise combinations of glycogen, carbohydrates and lipids (*Acheta domesticus*, Bertram *et al.* 2011; *Gryllus assimilis*, *Gryllus texensis*, Thomson, Darveau & Bertram 2014). Studies that manipulated resource acquisition have shown that male condition strongly affects the timing and magnitude of calling effort in several different cricket species (*Gryllus pennsylvanicus*, Judge, Ting & Gwynne 2008; *A. domesticus*, Bertram *et al.* 2009; *Teleogryllus commodus*, Maklakov *et al.* 2009; Zajitschek *et al.* 2009, 2012; *Gryllodes sigillatus*, Housley *et al.* 2015), and also that high-condition males sometimes invest so heavily in

sexual displays that they suffer heightened mortality compared to lower-condition individuals (*T. commodus*, Hunt et al. 2004a).

However, manipulating acquisition alone may not necessarily reveal differential costs of sexual trait expression, as males in good condition have more resources to allocate to all traits (Kotiaho 2001). Testing for differential costs requires manipulating both acquisition and trait investment, then studying their effects on other life history traits (e.g., future reproductive effort or survival). Moreover, behavioural signals are highly phenotypically plastic, which may allow males to react quickly to changes in the local environment so as to maximise fitness (Komers 1997; Bretman, Gage & Chapman 2011). We do not know the importance of resource trade-offs relative to other factors in causing distinct age-specific investment strategies (live fast and die young vs. slow and steady). Male crickets respond plastically to the social environment: juveniles reared in environments indicating high competition for females invest more in reproductive tissues (*T. oceanicus*, Bailey et al. 2010) and age-specific calling effort (*T. commodus*, Kasumovic et al. 2012a). During adulthood, rival male presence can cause plastic shifts towards increased calling effort (*T. commodus*, Callander et al. 2013), while the introduction of females can affect call frequency (*Gryllus bimaculatus*, Simmons 1986) and timing (*G. sigillatus*, *Gryllus veletis*, Burpee & Sakaluk 1993). The simultaneous manipulation of diet and the socio-sexual environment can therefore be used to evaluate both the energetic and life history consequences of trait investment, and reveal whether plasticity in the male signal is due to the strategic saving of resources or resource constraints.

In this study, we measure changes in allocation to sexual signalling and resource storage as a function of both acquisition and the demands of prior signalling in male decorated crickets (*G. sigillatus*). We measure calling effort, and use biochemical assays to estimate the energetic resource budgets of separate subsets of males before and after manipulating resource acquisition and access to potential mates. Furthermore, we assess the consequences of our experimental manipulations on longevity. Our measurements of male daily calling behaviour reflect two processes: a male's decision

as to whether or not he calls, and – given a positive decision – his calling effort. The presence of females should present universal incentive in the decision to call, enabling us to separate this choice from the more resource-dependent component (the non-zero amount of calling effort). By isolating components of male investment decisions in this way, we can disentangle variation due to constraints from variation due to adaptive planning. If future planning is the primary force shaping an individual's response to social conditions, low levels of calling early in life among males with reduced resource acquisition ability should also be associated with increased energy reserves and greater calling later in life (Kokko 1997; Hunt *et al.* 2004a). If, however, there is no evidence of greater energy reserves for allocation at a later point, it is likely that low calling levels are due simply to a lack of energy for current allocation to reproductive effort. In the latter case, we would also expect all males to respond to the availability of potential mates by investing their resources more heavily in calling effort, rather than saving for the future.

Materials and Methods

CRICKET HUSBANDRY

We used *G. sigillatus* (Figure 1) descended from a 2001 collection of 500 adult crickets in Las Cruces, New Mexico, which had been used to initiate a laboratory culture maintained at a population size of approximately 5000 crickets and allowed to breed panmictically (Ivy & Sakaluk 2005). Crickets were housed in 15-litre plastic containers in an environmental chamber maintained at $32\pm 1^{\circ}\text{C}$ on a reversed 14:10 hours light/dark cycle. We provided juvenile crickets with egg cartons for shelter, *ad libitum* water in cotton wool-plugged test tubes, and a standard diet of ground cat food (Friskies Go-Cat Senior®, Purina, London, UK). We provided moistened cotton wool pads to adults as an oviposition substrate. We maintained crickets at a density of approximately 300 individuals per container.

EXPERIMENTAL PROTOCOL

We manipulated male resource acquisition through diet treatment, and also manipulated access to females (as a proxy for manipulating resource allocation). We investigated variation in allocation to four different energy storage components (carbohydrates, glycogen, lipids and proteins; details given below) by sacrificing subsets of the experimental population (by placing individuals in a -80°C freezer) at three different ages: immediately at eclosion (baseline variation); one week post-eclosion (effect of resource acquisition manipulation); and two weeks post-eclosion (effects of both resource acquisition and female-access manipulations). A fourth group of males were allowed to die naturally, to estimate how acquisition and prior signalling investment affected lifespan.

We separated 630 male cricket nymphs from laboratory stock cultures during the final instar. Upon eclosion, we photographed individuals (Motic SMZ-168 with Moticam 2000) and measured pronotum length using NIH ImageJ v1.48 (Schneider, Rasband & Eliceiri 2012). We measured body weight using a high-precision electronic balance (Denver Instrument, model PI-225DA) at eclosion and weekly intervals thereafter. At eclosion, we assigned males randomly to one of four groups: those to be sacrificed immediately ($n = 58$), after one week ($n = 56$), after two weeks ($n = 256$), or allowed natural lifespan ($n = 260$). We transferred male crickets that were not immediately sacrificed to individual clear plastic containers (5×5×5cm), each containing plastic mesh as substrate, a cotton wool-plugged water bottle, and the food treatment. We cleaned containers and provided fresh food and water weekly.

We quantified allocation to the male sexual trait as ‘calling effort’, the duration (in seconds) that each individual spent broadcasting his sexual advertisement call. We measured each male overnight from 2pm-9am (dusk until afternoon of the following day on the reversed light cycle; lights off 2.30pm-12.30am, with 30 min rampdown) every day from the day after eclosion up to a maximum of 35 days. We mounted a microphone (C1163, Dick Smith Electronics) in the lid of each individual container, placing that container into a hollowed-out cube of soundproofing foam (15×15×15cm) to

minimise outside disturbance and prevent crosstalk between containers during recording. We removed a corner of the foam lid to maintain crickets on the set light cycle. An electronic acoustic recording system (Bertram and Johnson 1998) sampled from each microphone of each 10 times per second to determine whether or not a male was calling (see Hunt et al. 2004a).

CREATION OF ARTIFICIAL DIETS

We manipulated resource acquisition by creating seven artificial, dry, granular diets that varied in the amount of total nutritional content (see Appendix S1). All diets had a protein:carbohydrate ratio of 1:8, based on the effect of a high-carbohydrate diet in maximising calling effort and longevity in this species (J. Hunt, unpublished data). Diets ranged from 12-84% (in 12% increments) in total protein and carbohydrate content (referred to throughout as nutritional content).

FEMALE-ACCESS TREATMENT

During the second week post-eclosion, we allowed male crickets access to a single different adult female cricket during the recording period each night for between 0 and 7 consecutive nights (always beginning on the first day of the second week post-eclosion; i.e., eight distinct treatment groups). As logistical constraints prevented us from providing virgin females of similar age each day, we instead controlled female ‘experience’ to be similar to the male with which she would be placed: on the first day virgin males had access to virgin females, on the second day a male would gain access to a female that had had a single night’s access to males, and so forth. When not housed with an experimental male in his container, females were maintained in single-sex groups according to experience, and provided with water, shelter, and stock diet. Female experience was manipulated to the necessary level by housing them overnight with random stock males. Before being placed with an experimental male, females were also given at least a day without access to a male (considered sufficient as female *G. sigillatus* have a high remating rate, averaging 22 times over a 20-day period; Sakaluk 1987). During the week that access to females was manipulated, the food was removed

from each male's container during the call recording period (regardless of female presence). Males were given access to food whenever calling effort was not being recorded (9am – 2pm).

During the female-access treatment period, we allowed paired crickets to mate freely. In many cricket species, males stop calling after mating until they have generated a new spermatophore and are ready to mate again (Huber 1962; Loher 1974; Cade & Wyatt 1984; Wagner 2005); this is also the case in *G. sigillatus* (T. Houslay, personal observation). Males transfer a 2-part spermatophore, comprising the sperm ampulla and a spermatophylax 'nuptial gift' (Sakaluk 1984). While these take an average of 3.25 hours to compose (Sakaluk 1985), they consist of 80-85% water and have little or no nutritional value (Will & Sakaluk 1994; Warwick *et al.* 2009), and as such the metabolic costs of their generation are uncertain. We anticipate that one major effect of mating will be a reduction in calling, with the concomitant saving in stored resources that might otherwise be spent on calling activity. Our large sample size and use of continuous (rather than categorical) treatments enable us to capture diet- and calling-mediated changes in energy storage despite any additional effects of mating on males.

MEASURING ENERGY STORAGE

We used a modified version of Foray *et al.* (2012) to measure the major components of energy storage in each cricket: carbohydrates, glycogen, lipids and proteins (see Appendix S2). These can be assumed to comprise an individual's entire current energy budget. Cricket flight is fuelled by lipid breakdown (Zhao & Zera 2002), and the nature of the underlying pathways makes it likely that lipids are the major energy store for stridulatory calling effort. Their presence is likely to be greatest in males that have access to high quality resources. Glycogen is the storage form of glucose, and can be broken down in quick response to the need for energy in high-intensity activity (Campbell & Farrell 2003). High circulating glycogen levels may indicate males that are prepared to engage in sustained calling bouts, while carbohydrate content shows the existence of free sugars that can be used to fuel energetically expensive signalling. Protein contains the same energy content per gram as

carbohydrates, but takes longer to break down and is not typically used as a short-term energy source. The relative value of these energy storage forms for sexual signalling is currently unclear; lipids provide more energy than sugars on a gram-for-gram basis (Campbell & Farrell 2003), yet mobilisation of shorter-term energy forms is probably important for plastic responses to reproductive opportunities. If males were future planners, such that low-acquisition males restrict early signalling in favour of storage for use later in life, we would expect these males to show greater storage of lipids relative to glycogen and carbohydrate.

STATISTICAL ANALYSIS

We performed all statistical analyses using R 3.1.1 (R Core Team 2014). Independence between linear and quadratic forms of numeric predictors (e.g., diet and diet²) was achieved by centring the input variable before squaring (Gelman & Hill 2007). Diet treatments were centred and scaled to single-unit deviations, such that model coefficients show the effect on the response variable equivalent to one unit of change in diet treatment. In analyses of daily calling effort (discussed further below), male age post-eclosion was also centred but not scaled (coefficients show effect equivalent to one day's change). Continuous covariates (e.g., measurements of energy storage when used as predictors) were standardised by mean-centring and scaling (dividing by 1 standard deviation), putting them on a common scale and aiding interpretation of main effects (Gelman & Hill 2007; Schielzeth 2010). Unless otherwise stated, we performed model simplification by dropping non-significant higher-order terms (interactions and quadratic effects) from the full model sequentially, and using likelihood ratio tests to compare nested models. We retained more complex models whenever simplification resulted in a significant increase in model deviance. We observed no cases in which non-significant main effects became significant during model simplification steps that did not involve the deletion of an interaction term including that particular main effect.

Daily calling decisions and effort

Daily calling effort data were over-dispersed and zero-inflated; for analyses of these data, we used the R package MCMCglmm (Hadfield 2010) to fit a zero-altered Poisson (ZAP) model: a two-part model that includes a logistic regression for the zeroes in the data and a Poisson regression for the zero-truncated counts (see Appendix S3). We accounted for repeated observations on individuals by specifying a random effect of cricket ID. The ZAP model enabled us to ask two distinct questions within a single statistical structure (Atkins *et al.* 2013): what factors influenced whether a male called or not (*i.e.*, non-zero or zero), and, if he did call, what factors influenced the magnitude of calling (Houslay *et al.* 2015)?

For the first week post-eclosion, the full model included predictor variables of linear and quadratic terms for both diet treatment and days post-eclosion (corresponding to male age, modelled as a continuous variable), and all interactions (except those between the linear and quadratic forms of the same predictor, e.g. $\text{day} \times \text{day}^2$, as such interactions are not interpretable).

For analysis of daily calling effort across the second week post-eclosion, female-access treatment could not be entered in the model as a single predictor because the differences among treatment groups only become apparent as the week progresses. Instead, we use both a binary predictor of 'female presence' (indicating whether a male had access to a female on the current day of observation) and a continuous 'prior female access' variable (the number of days prior to the current one in which a male had access to females). The full model for daily calling over the second week post-eclosion therefore included predictor variables of female presence (binary variable), linear and quadratic terms for both diet treatment and male age (in days post-eclosion), prior female access, and up to three-way interactions between these (except those featuring linear and quadratic forms of the same predictor).

Changes in body mass

We used multiple regression to assess the linear and quadratic effects of diet treatment on changes in individual body mass (using the scaled mass index, SMI; Peig and Green 2009) over the first week post-eclosion. SMI is a better index of the relative size of energy reserves (Peig & Green 2010), and a better correlate of standardised mass in crickets (*A. domesticus*, *G. texensis*; Kelly, Tawes & Worthington 2014), than other commonly-used proxies.

To estimate the effects of both acquisition and allocation on the change in SMI (Δ SMI) over week 2, our predictors were diet treatment and log-transformed total calling effort. We used a separate model to investigate the effects on Δ SMI of diet treatment, female access treatment, and their interaction. Given that our individuals had already been subject to their diet treatments for the first week post-eclosion, we corrected Δ SMI over week 2 for the effect of regression to the mean, using the method outlined by Kelly and Price (2005).

Changes in energy storage

For those individuals sacrificed at eclosion, we used linear regression models to test whether carbohydrate, glycogen, lipid or protein content predicted an individual's scaled body mass (SMI). We log-transformed SMI so that model residuals conformed to a normal distribution. We excluded one individual because its SMI was over 4 standard deviations from the mean (all others were within ± 2.2 standard deviations).

We also used linear regression models to investigate the causes of variation in energy storage components at the end of the first week post-eclosion. For each response variable (protein, lipid, glycogen and carbohydrate), we estimated approximate changes over this period by subtracting the mean concentration of each storage form at eclosion from every individual's measurements. We then tested for the effect of diet treatment on these changes. The values representing changes in carbohydrates, glycogen and lipids were log-transformed prior to analysis. We also tested whether the changes in each form of energy storage predicted changes in SMI.

We estimated approximate changes in energy storage components over the second week by subtracting the diet-specific mean content of each storage form at the end of week 1 from each individual's measurements. We then tested whether these estimated changes in energy storage were associated with individual changes in SMI. We also tested whether individual energy storage measurements at the end of week 2 predicted SMI at the same stage.

Results

As expected (because treatments began after males reached eclosion), there were no significant differences between diet or female-access treatment groups in pronotum length, raw body mass, or scaled body mass (scaled mass index, SMI) at eclosion (all $P > 0.37$). Of the four energy storage forms measured, only lipids were a significant predictor of SMI at eclosion (lipid coefficient = 0.065 ± 0.030 , $t_{1,50} = 2.19$, $P = 0.033$).

MANIPULATION OF RESOURCE ACQUISITION IN EARLY ADULTHOOD

The likelihood of calling and calling effort both increase with diet quality and age

The simplified ZAP model for days 2-7 post-eclosion included predictor variables of diet, linear and quadratic terms for the day of calling (i.e., the age of an individual post-eclosion), and an interaction between diet and the linear day term. The likelihood of calling increased significantly along with both nutritional diet content and age over the first week (Table 1, Fig. 2(a)). The age-related increase in the likelihood of calling was less pronounced towards the end of the week. When males did call, there was a significant two-way interaction between day and diet on the amount of calling effort: effort increased over the course of the week, especially when males were fed higher quality diets (Table 1, Fig. 2(b)). The main effects of age and diet were to increase calling effort during this period, age acted in a non-linear manner. This non-linear effect of age can be seen in the change in the

intercept (centred at 48% diet) across panels in Fig. 2(b): the age-related change in the intercept does not increase at a constant rate, with the rate of increase dropping at higher values of male age.

Greater resource acquisition leads to increases in body mass and energy storage

The change in SMI among individuals increased with improving dietary nutrition, although this increase levelled off at higher diets (negative quadratic effect of diet; Table 2(a)). We estimated changes in energy storage content by comparing individual measurements of each storage component to averages taken from the subset of crickets sacrificed at eclosion, enabling us to investigate the underlying physiological response to our dietary manipulation. Improving dietary nutrition significantly increased carbohydrate, glycogen and lipid reserves, but not protein (Table 2(b)). Separate models also showed that the estimated changes in lipid and glycogen were significant predictors of changes in SMI over the first week post-eclosion (carbohydrate = 0.015 ± 0.004 , $t_{1,43} = 1.70$, $P = 0.096$; glycogen = 0.008 ± 0.003 , $t_{1,47} = 2.30$, $P = 0.026$; lipid = 0.008 ± 0.003 , $t_{1,46} = 2.34$, $P = 0.024$; protein = -0.001 ± 0.003 , $t_{1,48} = -0.01$, $P = 0.992$).

Relationship between calling and change in body mass is dependent on resource acquisition

The significant interaction term (Table 3) indicated that the relationship between total calling effort and change in SMI over week 1 was strongly dependent upon diet, switching from a negative to a positive relationship as diet improved (Figure 3): increased dietary nutrition meant males that called more were also better able to increase body mass.

MANIPULATION OF RESOURCE ACQUISITION AND ACCESS TO POTENTIAL MATES

In the first part of our study, males on lower quality diets called less often and less intensely than those on higher-quality diets (Fig. 2). While total calling effort and storage appeared to covary negatively among those low-acquisition males, those males did not invest more in energy reserves relative to higher-acquisition individuals (Fig. 3). To demonstrate ‘future planning’, low-acquisition

males should remain less likely to call even in the presence of females, instead conserving energy reserves for greater calling later in life.

Males are more likely to call when females are present

The simplified ZAP model for daily calling effort during the female-access treatment period included predictor variables of diet, female presence on the current day, the age of an individual at the time of measurement (in days post-eclosion), and prior female access (the number of days the male had access to a female prior to the current measurement). Multiple interactions were retained in the model, including a three-way interaction between prior female access, diet, and day of measurement.

Female presence significantly increased the likelihood of calling (Table 4, Fig. 4(a)). Males were less likely to call as the week progressed, but this decrease was not observable when females were present ('Female presence \times day' interaction in Table 4; Fig. 4(a)). Finally, call likelihood was significantly reduced in males that had greater access to females prior to the current measurement ('Prior female access', Table 4). Diet had no effect on the likelihood of calling: low-acquisition males were just as likely to call as high-acquisition males.

The positive effect of diet on calling effort is reduced by greater prior access to females

Calling effort increased significantly when a female was present, but the effect decreased over the week ('Female presence \times day' interaction term in Table 4; Fig. 4(b)). Dietary nutrition also significantly increased calling effort (Fig. 5), but this effect was reduced in males with greater access to females as the week progressed ('Prior female access \times diet \times day' interaction in Table 4; Fig. 5). These effects are visualised in Fig. 5, which shows the relationship between diet treatment and calling effort for the first (left panels) and final (right panels) days of the female-access manipulation, for those given no female access (lower panels) or daily female access (upper panels).

Diet-related increase in body mass is reduced by access to females

Having excluded males that did not call or that cannibalised females during the female-access treatment period, we found that increased total calling effort during week 2 was associated with decreased SMI ($\log \text{total calling} = -0.0018 \pm 0.0007$, $t_{1,307} = -2.55$, $P = 0.011$). Diet treatment increased individual SMI, but this effect was reduced at higher diets (diet = 0.0021 ± 0.0006 , $t_{1,307} = 3.51$, $P < 0.001$; diet² = -0.0018 ± 0.0003 , $t_{1,307} = -3.76$, $P < 0.001$). Separately, we found no effect of diet on the frequency with which males cannibalised females during the female access treatment period ($\chi^2_6 = 1.10$, $P = 0.98$).

Table 5 shows the minimum adequate model for a separate analysis of the effects of our experimental manipulations on the change in SMI over week 2 (again, excluding males that cannibalised females). The effect of diet depended on female-access treatment; in the absence of females, males on high-nutrition diets tended to gain more body mass during this period than those on low diets; however, high-nutrition males with consistent access to potential mates lost more mass ('Diet \times Female-access' interaction term in Table 5; Fig. 6).

Changes in body mass are associated with changes in carbohydrate and glycogen reserves

We found that the estimated change in carbohydrate and glycogen reserves were significant predictors of changes in individual SMI over the second week post-eclosion (carbohydrate = 0.0045 ± 0.0008 , $t_{1,143} = 5.52$, $P < 0.001$; glycogen = 0.0039 ± 0.0009 , $t_{1,145} = 4.56$, $P < 0.001$; lipid = -0.0006 ± 0.0009 , $t_{1,142} = -0.72$, $P = 0.470$; protein = 0.0007 ± 0.0009 , $t_{1,139} = 0.70$, $P = 0.486$).

We also tested whether any of our energy storage measurements correlated with individual SMI at the end of week 2; only lipid content significantly predicted SMI (lipid = 0.014 ± 0.005 , $t_{1,142} = 2.96$, $P = 0.004$).

EFFECTS OF RESOURCE ACQUISITION AND ACCESS TO POTENTIAL MATES ON SURVIVAL

Due to the high proportions of deaths in the two lowest diet treatments (100% and 60.5% respectively), we excluded individuals in those treatments from our analysis of the likelihood of

dying during the female-access period. Greater female access significantly increased the risk of death during this period, while diet significantly reduced it (diet = -0.225 ± 0.113 , $t_{1,377} = -1.99$, $P = 0.047$; female-access = 0.226 ± 0.074 , $t_{1,377} = 3.04$, $P = 0.002$; Fig. S2). A model retaining the excluded diet treatments gave qualitatively similar results, which are not presented here.

Our female-access treatment had a significant effect on lifespan after the conclusion of the female access treatment period (i.e., after the end of the second week post-eclosion); males with greater access to females during week 2 had shorter lifespans (female-access = -3.913 ± 0.937 days, $t_{1,145} = -4.18$, $P = 0.001$; Fig. S3(a)). Lifespan was significantly increased by greater dietary nutrition, although this effect disappeared at higher quality diets (diet = 11.823 ± 1.734 days, $t_{1,145} = 6.82$, $P < 0.001$; diet² = -2.491 ± 0.869 , $t_{1,145} = -2.87$, $P = 0.005$; Fig. S3(b)).

Discussion

Male sexual signals are often condition-dependent, with trait expression closely associated with the size of the resource budget (e.g. Andersson 1994; Johnstone 1995). Few studies have simultaneously manipulated resource acquisition and access to reproductive opportunities while also examining the resultant patterns of energy storage and use (Kotiaho 2000). Recently, it has become clear that traits used to measure condition are themselves condition-dependent (and are built using the resources that make up condition), meaning that traits reflecting condition must inevitably trade-off with one another (Tomkins *et al.* 2004; Houslay & Bussière 2012). This fact prescribes careful interpretation of covariances between proxies for condition and other life history traits, and highlights the need for more direct measurements of energy reserves (Morehouse 2014; Wilder *et al.* 2015). Despite the inherent challenges, identifying relationships between trait investment and stored energy reserves can illuminate mechanisms leading to changes in resource storage and use, and therefore help

clarify the adaptive significance of variation in resource allocation among males as well as the consequences of energetic trade-offs for honesty in sexual trait expression.

By manipulating dietary nutrition in male crickets, we showed that greater resource acquisition led to an increase in both the likelihood and amount of signalling in early adulthood (Table 1, Fig. 2), as well as greater investment in energy stores (lipid, glycogen and carbohydrate) during this period (Table 2). The relationship between allocation to scaled body mass (as scaled mass index, SMI) and calling effort depended on diet quality, switching from negative covariance when diet quality was low to positive covariance when high (Table 3, Fig. 3). While low-acquisition males called less often, and less intensely, during this period than did higher-acquisition males, we found no evidence that such a suppression of signalling effort led to increased energy reserves. If low calling effort among low-acquisition males was due to energy constraints rather than future planning, we predicted that these males would respond to the presence of potential mates by investing more in calling instead of saving energy for the future.

Sexual trait expenditure was highly plastic depending on the availability of a potential mate: males responded strongly to the presence of a female by increasing both the likelihood of calling and time spent calling (Table 4, Fig. 4). During the female-access treatment period, diet did not affect the probability of signalling on any given day. It did, however, have a strong positive effect on the amount of time spent calling (Fig. 5), mediated by female-access: the positive effect of diet on calling effort decreased with greater exposure to females over the course of the week (Fig. 5). The change in male scaled body mass (SMI) as a function of diet during this period was non-linear and also depended on access to females (Table 5, Fig. 6). In the absence of females, males fed higher nutrition diets tended to increase or maintain body mass more than those on lower-nutrition diets. However, greater female-access effectively reversed this relationship: males on high-nutrition diets tended to lose more SMI than those on lower-nutrition diets when given daily access to females over the treatment period. Diminishing calling effort by the end of week 2, even by males on high-quality

diets in the presence of females, suggests an exhaustion of available resources over this period (Fig. 5).

Indeed, the change in male body mass over week 2 covaried negatively with total calling effort, and the likelihood of calling decreased as previous exposure to females increased (Fig. 4(a), Table 4) – both results irrespective of diet. Greater exposure to potential mates also increased the risk of dying during week 2, although this effect was mitigated by diet quality (Fig. S2). There were lasting consequences of our experimental manipulations: longevity following week 2 was increased by dietary nutritional quality, and decreased by greater prior exposure to females (Fig. S3). However, this reduced viability cannot be considered a true cost unless it is accompanied by a net decrease in fitness, because any decrease in lifespan may be offset in fitness terms by increased early reproductive success (Kotiaho 2001; Hunt et al. 2004a,b).

FUTURE PLANNING OR BUDGET CONSTRAINTS?

Crickets are ideal for testing hypotheses concerning age-related reproductive effort because we can precisely quantify sexual advertisement effort by males. Several studies have manipulated resource acquisition to investigate its effects on life history traits, with largely consistent results: males fed higher-quality diets typically increase their calling effort with age, and longer-lived males call more (Judge *et al.* 2008; Maklakov *et al.* 2008; Zajitschek *et al.* 2009, 2012; Houslay *et al.* 2015). One exception is the study of Hunt et al. (Hunt *et al.* 2004a), in which males fed a high-protein diet called earlier and far more intensely than those fed lower protein diets; high-protein diet males also suffered survival costs such that they died earlier than low-protein diet males. This pattern of age-related allocation may reflect status-dependent plasticity in allocation to reproduction and survival in some cases (Kokko 1997, 1998; Höglund & Sheldon 1998; Vinogradov 1998). In particular, low-condition males can benefit from suppressing signalling at early ages and investing in longevity and sexual advertisement later in life (Kokko 1997). If this adaptive plasticity in investment applied to *G.*

sigillatus, males restricted to lower-nutrient diets should have allocated resources preferentially to energy storage instead of early signalling.

Males fed lower nutrition diets in our study called less, and the correlation between allocation to storage (individual change in SMI) and calling effort was negative during week 1 among males on lower-quality diets (Table 2, Fig. 3). However, while diet-restricted males were less likely to call over the first week (Table 1, Fig. 2(a)), this effect was not evident during week 2 (Table 4). If males on low-quality diets had continued to show a depressed propensity to call, and had increased body mass over this period, that would be more consistent with crickets suppressing advertisement in favour of increased future investment. Instead, it appears more likely that variation in calling effort was due simply to budget constraints: i.e., having less energy to allocate to signalling. The non-adaptive explanation for this variation among males is given further credence by dietary nutrition being a strong predictor of survival, with males fed lower-nutrient diets suffering reduced longevity. The fact that a male's current signalling effort is primarily governed by his acquisition ability and previous expenditure also supports previous assertions that energetic trade-offs provide a mechanism for maintaining honesty on average in male behavioural display traits (Kokko 1997).

VARIATION IN USAGE AND IMPORTANCE OF ENERGY STORAGE FORMS

Increased resource acquisition from higher-nutrient diets enabled male crickets to allocate more resources to storage after eclosion in the forms of greater lipid, glycogen, and carbohydrate content (Table 2(b)). These appear to represent the major forms of energy stores; protein levels did not vary over the three time points that these stores were measured, indicating that males seem to maintain protein content rather than increase it (although the nutrient ratio in our diet treatment was heavily in favour of carbohydrate rather than protein, the variation in total nutrient content would enable individuals to differ in allocation if required). We hypothesised that 'future planning' would result in low-acquisition males prioritising storage in the form of lipids; the positive effect of diet on this form indicates that this was not the case. Our results further corroborate findings in other cricket species,

in which calling effort covaried with the ability to acquire, metabolise and mobilise these three storage forms (Bertram *et al.* 2011; Thomson *et al.* 2014).

Males tended to lose scaled body mass (SMI) over week 2 (Table 5), and individual SMI change in this period was associated with estimated changes in glycogen and carbohydrate reserves – both of which are quick-release forms of energy. Absolute SMI at the end of that period was associated with current lipid reserves. Together, these results suggest that glycogen and carbohydrate are more freely expendable forms of energy, while lipids are more carefully conserved. Such variation in the conservation of storage forms indicates that lipids differ from carbohydrate and glycogen in usage and importance, and highlights the importance of estimating multiple metabolic reserves when examining life-history trade offs. The patterns of change in individual body mass and energy reserves over weeks one and two suggest that resource acquisition early in adulthood tended to be mobilised for fuelling calling effort as soon as the social circumstances favour it.

ACQUISITION CAN AFFECT COVARIANCES BETWEEN EXPENSIVE LIFE HISTORY TRAITS

Variation in resource acquisition ability among individuals can lead to positive correlations between life history traits, as predicted by Van Noordwijk and De Jong (1986) and often found in nature (Jennions *et al.* 2001). Fig. 3 demonstrates clearly how such a correlation can arise when some males have acquired a greater amount of resources and can then allocate to two competing traits. Increased nutrient availability can mask any negative correlation between two traits because individuals have more resources to allocate to both, as seen in the increasingly positive relationship between calling effort and body mass when dietary nutrition increases. We found that the mean change in body mass also increased with diet and with total calling effort, indicating that individuals allocate more resources to both storage and current reproductive effort as acquisition increases. These results have implications for how males are likely to sample their social environment through calling effort: males probably call irregularly early in life, and the extent to which this activity affects the accumulation of resources depends on nutrient intake. The accumulation of body mass with

increases in both diet and total calling effort may be due partly to those individuals on higher quality diets having a greater resource intake, and also because of the smaller marginal costs of increased advertisement to higher condition males, as predicted by models of sexual selection under condition-dependence (Grafen 1990; Rowe & Houle 1996). These findings mirror interspecific (Judge *et al.* 2008) and intraspecific (Hunt *et al.* 2004a) differences in the covariance between expensive traits under contrasting conditions, although it is not clear the extent to which our results are related to selection in the lab rather than in the wild. We hope to see more studies in more taxa that directly assess the storage and use of multiple energy forms over time – particularly in the face of varying resource acquisition regimes, and with varying reproductive opportunities.

SOCIALLY MEDIATED PLASTICITY IN REPRODUCTIVE EFFORT

Male decorated crickets responded strongly to female presence, with significant increases in both the likelihood and the time spent calling. To date, most work on male responses to the social environment in crickets have focused on adaptive plasticity for anticipating future conditions (Kasumovic & Brooks 2011): manipulations of juveniles' perceived density and future competition can affect adult life history traits (*T. oceanicus*, Bailey *et al.* 2010; *T. commodus*, Kasumovic *et al.* 2012a,b). Manipulations of the social environment in adult crickets have thus far been less common. Callander *et al.* (2013) measured the calling effort of male Australian black field crickets (*T. commodus*) that were either kept isolated or housed with a rival male after reaching maturity. However, all males in that study were isolated for the measurement period itself, which might explain the lack of behavioural plasticity across treatments (Callander *et al.* 2013). The extreme phenotypic flexibility of behavioural traits means individuals can adjust trait expression rapidly in response to social cues (Ghalambor, Angeloni & Carroll 2010; Bretman *et al.* 2011); indeed, female presence has previously been shown to affect the frequency and timing of cricket calling (Simmons 1986; Burpee & Sakaluk 1993; although see Bertram, Orozco & Bellani 2004). Storage of resources in 'quick-release' form as sugars (carbohydrate and glycogen) may provide male crickets the flexibility

to signal more effectively in the presence of females, explaining the increased calling effort by males on higher-quality diets during our female-access treatment period.

HOW STRONG IS THE EVIDENCE FOR ADAPTIVE PLASTICITY IN AGE-SPECIFIC REPRODUCTIVE EFFORT?

An unresolved question from our study relates to the decrease in calling effort over the second week due to greater previous exposure to females (Fig. 5). Early theoretical models concluded that reproductive investment should increase with age (Williams 1966), but recent studies using game theory and life history theory paint a more complex picture (Kokko 1997, 1998; Lindström *et al.* 2009). Kokko (1998) illustrated three phenomena that can prevent positive correlations between life history traits: 1) trade-offs acting over lifetimes, such that fitness benefits accrued early in life outweigh late life fitness (Hansen & Price 1995); 2) increasing marginal gains in fecundity could favour decreased survival and intense sexual advertisement for high-quality males; and/or, 3) suboptimal allocation. The last point is of particular interest: if individuals can plastically vary trait expression in heterogeneous environments, and trait expression is under selection, shouldn't we generally expect adaptive plasticity for continuous traits (Scheiner & Holt 2012)? One explanation for suboptimal allocation is that the genetic and regulatory machinery required for controlling allocation across traits and over time is expensive to maintain, and may not confer a sufficiently high selection advantage over a simpler set of rules: spend resources if you have them, and if not, focus on acquiring more resources instead. The differences we find in individuals that signal at high and low levels early in life may be primarily determined by budget constraints, as opposed to adaptive perception of future opportunities.

AUTHOR CONTRIBUTIONS

TMH, LFB and JH conceived the ideas and designed the experiment; JR advised on design and created the artificial diets; TMH performed the experiment and collected the data; KFH performed the energy storage estimation assays; TMH analysed the data; TMH, LFB and JH led the writing of the manuscript. All authors contributed critically to manuscript drafts and gave final approval for publication.

ACKNOWLEDGMENTS

TMH and LFB were supported by the University of Stirling. JH was funded by a University Royal Society Fellowship. JR was funded by a NERC studentship (awarded to JH). Thanks to E. Herridge, R. Murray, E. Petrova, and particularly P. Monteith and J. Weir for technical support and assistance with cricket husbandry; also to T. Ivy and S. Sakaluk for initiating the population from which the animals in this study were descended. We are grateful to Matt Hall, Joseph Tobias, Christine Miller and three anonymous reviewers for comments that greatly improved the manuscript.

DATA ARCHIVING

All data will be uploaded to Dryad upon acceptance of this manuscript.

613 **LITERATURE CITED**

- 614 Andersson, M. (1982) Female choice selects for extreme tail length in a widowbird. *Nature*, **299**,
615 818–820.
- 616 Andersson, M. (1986) Evolution of Condition-Dependent Sex Ornaments and Mating Preferences:
617 Sexual Selection Based on Viability Differences. *Evolution*, **40**, 804–816.
- 618 Andersson, M. (1994) *Sexual Selection*. Princeton University Press, Princeton, N.J. (USA).
- 619 Atkins, D.C., Baldwin, S.A., Zheng, C., Gallop, R.J. & Neighbors, C. (2013) A tutorial on count
620 regression and zero-altered count models for longitudinal substance use data. *Psychology of*
621 *addictive behaviors : journal of the Society of Psychologists in Addictive Behaviors*, **27**, 166–77.
- 622 Badyaev, A. V. & Qvarnström, A. (2002) Putting sexual traits into the context of an organism: A life-
623 history perspective in studies of sexual selection. *The Auk*, **119**, 301–310.
- 624 Bailey, N.W., Gray, B. & Zuk, M. (2010) Acoustic experience shapes alternative mating tactics and
625 reproductive investment in male field crickets. *Current biology : CB*, **20**, 845–9.
- 626 Bailey, W.J., Withers, P.C., Endersby, M. & Gaull, K. (1993) The energetic costs of calling in the
627 bushcricket *Requena verticalis* (Orthoptera: Tettigoniidae: Listroselidinae). *Journal of*
628 *Experimental Biology*, **178**, 21–37.
- 629 Barnett, C.A., Suzuki, T.N., Sakaluk, S.K. & Thompson, C.F. (2015) Mass-based condition measures
630 and their relationship with fitness: in what condition is condition? *Journal of Zoology*, **296**, 1–5.
- 631 Bentsen, C.L., Hunt, J., Jennions, M.D. & Brooks, R. (2006) Complex Multivariate Sexual Selection on
632 Male Acoustic Signaling in a Wild Population of *Teleogryllus commodus*. *The American*
633 *Naturalist*, **167**, 102–116.
- 634 Bertram, S.M. & Johnson, L. (1998) An electronic technique for monitoring the temporal aspects of
635 acoustic signals of captive organisms. *Bioacoustics*, **9**, 107–118.
- 636 Bertram, S.M., Orozco, S.X. & Bellani, R. (2004) Temporal shifts in conspicuousness: Mate attraction
637 displays of the Texas field cricket, *Gryllus texensis*. *Ethology*, **110**, 963–975.
- 638 Bertram, S.M., Thomson, I.R., Auguste, B., Dawson, J.W. & Darveau, C.-A. (2011) Variation in cricket
639 acoustic mate attraction signalling explained by body morphology and metabolic differences.
640 *Animal Behaviour*, **82**, 1255–1261.
- 641 Bertram, S.M., Whattam, E.M., Visanuvimol, L., Bennett, R. & Lauzon, C. (2009) Phosphorus
642 availability influences cricket mate attraction displays. *Animal Behaviour*, **77**, 525–530.
- 643 Bonduriansky, R., Maklakov, A.A., Zajitschek, F. & Brooks, R.C. (2008) Sexual selection, sexual conflict
644 and the evolution of ageing and life span. *Functional Ecology*, **22**, 443–453.
- 645 Bretman, A., Gage, M.J.G. & Chapman, T. (2011) Quick-change artists: male plastic behavioural
646 responses to rivals. *Trends in ecology & evolution*, **26**, 467–73.
- 647 Burpee, D.M. & Sakaluk, S.K. (1993) The effect of pair formation on diel calling patterns in two
648 cricket species, *Gryllus veletis* and *Gryllodes sigillatus* (Orthoptera: Gryllidae). *Journal of Insect*
649 *Behavior*, **6**, 431–440.
- 650 Cade, W. (1975) Acoustically Orienting Parasitoids: Fly Phonotaxis to Cricket Song. *Science*, **190**,
651 1312–1313.
- 652 Cade, W.H. & Wyatt, D.R. (1984) Factors Affecting Calling Behaviour in Field Crickets, *Teleogryllus*
653 and *Gryllus* (Age, Weight, Density, and Parasites. *Behaviour*, **88**, 61–75.
- 654 Callander, S., Kahn, A.T., Hunt, J., Backwell, P.R.Y. & Jennions, M.D. (2013) The effect of competitors
655 on calling effort and life span in male field crickets. *Behavioral Ecology*, **24**, 1251–1259.

- 656 Campbell, M.K. & Farrell, S.O. (2003) *Biochemistry*, 4th ed. Thomson/Brooks/Cole, Belmont, CA.
- 657 Candolin, U. (1999) The relationship between signal quality and physical condition: is sexual
658 signalling honest in the three-spined stickleback? *Animal behaviour*, **58**, 1261–1267.
- 659 Foray, V., Pelisson, P.-F., Bel-Venner, M.-C., Desouhant, E., Venner, S., Menu, F., Giron, D. & Rey, B.
660 (2012) A handbook for uncovering the complete energetic budget in insects: the van Handel's
661 method (1985) revisited. *Physiological Entomology*, **37**, 295–302.
- 662 Gelman, A. & Hill, J. (2007) *Data Analysis Using Regression and Multilevel/hierarchical Models*.
663 Cambridge University Press, New York.
- 664 Ghalambor, C.K., Angeloni, L. & Carroll, S.P. (2010) Behavior as phenotypic plasticity. *Evolutionary*
665 *Behavioral Ecology* (eds C.W. Fox & D.F. Westneat), pp. 90–107. Oxford University Press, New
666 York.
- 667 Grafen, A. (1990) Biological signals as handicaps. *Journal of theoretical biology*, **144**, 517–546.
- 668 Hadfield, J.D. (2010) MCMC Methods for Multi-Response Generalized Mixed Models: The
669 MCMCglmm R Package. *Journal of Statistical Software*, **33**, 1–25.
- 670 Hansen, T.F. & Price, D.K. (1995) Good genes and old age: Do old mates provide superior genes?
671 *Journal of Evolutionary Biology*, **8**, 759–778.
- 672 Hoback, W.W. & Wagner, W.E. (1997) The energetic cost of calling in the variable field cricket,
673 *Gryllus lineaticeps*. *Physiological Entomology*, **22**, 286–290.
- 674 Höglund, J. & Sheldon, B.C. (1998) The Cost of Reproduction and Sexual Selection. *Oikos*, **83**, 478–
675 483.
- 676 Houle, D. (1991) Genetic covariance of fitness correlates: what genetic correlations are made of and
677 why it matters. *Evolution*, **45**, 630–648.
- 678 Houslay, T.M. & Bussière, L.F. (2012) Sexual Selection and Life History Allocation. *Encyclopedia of Life*
679 *Sciences*, 1–8.
- 680 Houslay, T.M., Hunt, J., Tinsley, M.C. & Bussière, L.F. (2015) Sex differences in the effects of juvenile
681 and adult diet on age-dependent reproductive effort. *Journal of Evolutionary Biology*, **28**,
682 1067–1079.
- 683 Huber, F. (1962) Central Nervous Control of Sound Production in Crickets and Some Speculations on
684 Its Evolution. *Evolution*, **16**, 429–442.
- 685 Hunt, J., Brooks, R.C., Jennions, M.D., Smith, M.J., Bentsen, C.L. & Bussière, L.F. (2004a) High-quality
686 male field crickets invest heavily in sexual display but die young. *Nature*, **432**, 1024–7.
- 687 Hunt, J., Bussière, L.F., Jennions, M.D. & Brooks, R.C. (2004b) What is genetic quality? *Trends in*
688 *ecology & evolution*, **19**, 329–33.
- 689 Ivy, T.M. & Sakaluk, S.K. (2005) Polyandry promotes enhanced offspring survival in decorated
690 crickets. *Evolution*, **59**, 152–9.
- 691 Iwasa, Y. & Pomiankowski, A. (1994) The evolution of mate preferences for multiple sexual
692 ornaments. *Evolution*, **48**, 853–867.
- 693 Jennions, M.D., Møller, A.P. & Petrie, M. (2001) Sexually selected traits and adult survival: a meta-
694 analysis. *The Quarterly Review of Biology*, **76**, 3–36.
- 695 Johnstone, R.A. (1995) Sexual selection, honest advertisement and the handicap principle: reviewing
696 the evidence. *Biological Reviews*, **70**, 1–65.
- 697 De Jong, G. & Van Noordwijk, A.J. (1992) Acquisition and Allocation of Resources: Genetic (CO)
698 Variances, Selection, and Life Histories. *The American naturalist*, **139**, 749–770.
- 699 Judge, K.A., Ting, J.J. & Gwynne, D.T. (2008) Condition dependence of male life span and calling

700 effort in a field cricket. *Evolution*, **62**, 868–78.

701 Kasumovic, M.M. & Brooks, R.C. (2011) It's All Who You Know: The Evolution Of Socially Cued
702 Anticipatory Plasticity As A Mating Strategy. *The Quarterly Review of Biology*, **86**, 181–197.

703 Kasumovic, M.M., Hall, M.D. & Brooks, R.C. (2012) The juvenile social environment introduces
704 variation in the choice and expression of sexually selected traits. *Ecology and Evolution*, **2**,
705 1036–1047.

706 Kasumovic, M.M., Hall, M.D., Try, H. & Brooks, R.C. (2013) Socially cued developmental plasticity
707 affects condition-dependent trait expression. *Behavioral Ecology*, **24**, 429–434.

708 Kavanagh, M.W. (1987) The efficiency of sound production in two cricket species, *Gryllotalpa*
709 *australis* and *Teleogryllus commodus* (Orthoptera: Grylloidea). *Journal of Experimental Biology*,
710 107–119.

711 Kelly, C. & Price, T.D. (2005) Correcting for regression to the mean in behavior and ecology. *The*
712 *American naturalist*, **166**, 700–707.

713 Kelly, C.D., Tawes, B.R. & Worthington, A.M. (2014) Evaluating indices of body condition in two
714 cricket species. *Ecology and Evolution*, **4**, 4476–4487.

715 Kokko, H. (1997) Evolutionarily stable strategies of age-dependent sexual advertisement. *Behavioral*
716 *Ecology and Sociobiology*, **41**, 99–107.

717 Kokko, H. (1998) Good genes, old age and life-history trade-offs. *Evolutionary Ecology*, **12**, 739–750.

718 Komers, P.E. (1997) Behavioural plasticity in variable environments. *Canadian Journal of Zoology*, **75**,
719 161–169.

720 Kotiaho, J.S. (1999) Estimating fitness: comparison of body condition indices revisited. *Oikos*, **87**,
721 399–400.

722 Kotiaho, J.S. (2000) Testing the assumptions of conditional handicap theory: costs and condition
723 dependence of a sexually selected trait. *Behavioral Ecology and Sociobiology*, **48**, 188–194.

724 Kotiaho, J.S. (2001) Costs of sexual traits: a mismatch between theoretical considerations and
725 empirical evidence. *Biological reviews of the Cambridge Philosophical Society*, **76**, 365–76.

726 Lindström, J., Pike, T.W., Blount, J.D. & Metcalfe, N.B. (2009) Optimization of resource allocation can
727 explain the temporal dynamics and honesty of sexual signals. *The American naturalist*, **174**,
728 515–25.

729 Loher, W. (1974) Circadian Control of Spermatophore Formation in the Cricket *Teleogryllus*
730 *commodus* (Walker). *Journal of Insect Physiology*, **20**, 1155–1172.

731 Maklakov, A.A., Hall, M.D., Simpson, S.J., Dessmann, J., Clissold, F.J., Zajitschek, F., Lailvaux, S.P.,
732 Raubenheimer, D., Bonduriansky, R. & Brooks, R.C. (2009) Sex differences in nutrient-
733 dependent reproductive ageing. *Aging cell*, **8**, 324–30.

734 Maklakov, A.A., Simpson, S.J., Zajitschek, F., Hall, M.D., Dessmann, J., Clissold, F.J., Raubenheimer,
735 D., Bonduriansky, R. & Brooks, R.C. (2008) Sex-specific fitness effects of nutrient intake on
736 reproduction and lifespan. *Current biology : CB*, **18**, 1062–6.

737 Monaghan, P., Charmantier, A., Nussey, D.H. & Ricklefs, R.E. (2008) The evolutionary ecology of
738 senescence. *Functional Ecology*, **22**, 371–378.

739 Morehouse, N.I. (2014) Condition-Dependent Ornaments, Life Histories, and the Evolving
740 Architecture of Resource-Use. *Integrative and comparative biology*, **54**, 1–10.

741 Mowles, S.L. (2014) The physiological cost of courtship: field cricket song results in anaerobic
742 metabolism. *Animal Behaviour*, **89**, 39–43.

743 Van Noordwijk, A.J. & De Jong, G. (1986) Acquisition and allocation of resources: their influence on

744 variation in life history tactics. *American naturalist*, **128**, 137–142.

745 Nur, N. & Hasson, O. (1984) Phenotypic Plasticity and the Handicap Principle. *Journal of theoretical*
746 *biology*, **110**, 275–297.

747 Nussey, D.H., Coulson, T., Festa-Bianchet, M. & Gaillard, J.-M. (2008) Measuring senescence in wild
748 animal populations: towards a longitudinal approach. *Functional Ecology*, **22**, 393–406.

749 Ophir, A.G., Schrader, S.B. & Gillooly, J.F. (2010) Energetic cost of calling: general constraints and
750 species-specific differences. *Journal of evolutionary biology*, **23**, 1564–9.

751 Peig, J. & Green, A.J. (2009) New perspectives for estimating body condition from mass/length data:
752 the scaled mass index as an alternative method. *Oikos*, **118**, 1883–1891.

753 Peig, J. & Green, A.J. (2010) The paradigm of body condition: a critical reappraisal of current
754 methods based on mass and length. *Functional Ecology*, **24**, 1323–1332.

755 Prestwich, K.N. (1994) The energetics of acoustic signaling in anurans and insects. *Integrative and*
756 *Comparative Biology*, **34**, 625–643.

757 Prestwich, K.N. & Walker, T.J. (1981) Energetics of singing crickets: Effects of temperature in three
758 trilling species (Orthoptera: Gryllidae). *Journal of Comparative Physiology*, **143**, 199–212.

759 R Core Team. (2014) R: A language and environment for statistical computing.

760 Reznick, D.N., Nunney, L. & Tessier, A. (2000) Big houses, big cars, superfleas and the costs of
761 reproduction. *Trends in ecology & evolution*, **15**, 421–425.

762 Rodriguez-Munoz, R., Bretman, A., Slate, J., Walling, C.A. & Tregenza, T. (2010) Natural and Sexual
763 Selection in a Wild Insect Population. *Science*, **328**, 1269–1272.

764 Rowe, L. & Houle, D. (1996) The Lek Paradox and the Capture of Genetic Variance by Condition
765 Dependent Traits. *Proceedings of the Royal Society B: Biological Sciences*, **263**, 1415–1421.

766 Sakaluk, S.K. (1984) Male crickets feed females to ensure complete sperm transfer. *Science (New*
767 *York, N.Y.)*, **223**, 609–10.

768 Sakaluk, S.K. (1985) Spermatophore size and its role in the reproductive behaviour of the cricket,
769 *Gryllobates supplicans* (Orthoptera: Gryllidae). *Canadian Journal of Zoology*, **63**, 1652–1656.

770 Sakaluk, S.K. (1987) Reproductive behaviour of the decorated cricket, *Gryllobates supplicans*
771 (Orthoptera: Gryllidae): calling schedules, spatial distribution, and mating. *Behaviour*, **100**,
772 202–225.

773 Scheiner, S.M. & Holt, R.D. (2012) The genetics of phenotypic plasticity. X. Variation versus
774 uncertainty. *Ecology and evolution*, **2**, 751–67.

775 Schielzeth, H. (2010) Simple means to improve the interpretability of regression coefficients.
776 *Methods in Ecology and Evolution*, **1**, 103–113.

777 Schneider, C.A., Rasband, W.S. & Eliceiri, K.W. (2012) NIH Image to ImageJ: 25 years of image
778 analysis. *Nature Methods*, **9**, 671–675.

779 Simmons, L.W. (1986) Inter-male Competition and Mating Success in the Field Cricket, *Gryllus*
780 *Bimaculatus* (De Geer). *Animal Behaviour*, **34**, 567–579.

781 Stearns, S.C. (1989) Trade-Offs in Life-History Evolution. *Functional Ecology*, **3**, 259–268.

782 Stearns, S.C. (1992) *The Evolution of Life Histories*. Oxford University Press, New York.

783 Thomson, I.R., Darveau, C.-A. & Bertram, S.M. (2014) Body morphology, energy stores, and muscle
784 enzyme activity explain cricket acoustic mate attraction signaling variation. *PloS one*, **9**, e90409.

785 Tomkins, J.L., Radwan, J., Kotiaho, J.S. & Tregenza, T. (2004) Genic capture and resolving the lek
786 paradox. *Trends in ecology & evolution*, **19**, 323–8.

787 Vinogradov, A.E. (1998) Male reproductive strategy and decreased longevity. *Acta biotheoretica*, **46**,
788 157–60.

789 Wagner, W.E. (2005) Male field crickets that provide reproductive benefits to females incur higher
790 costs. *Ecological Entomology*, **30**, 350–357.

791 Walker, T.J. (1979) Calling crickets (*Anurogryllus arboreus*) over pitfalls: Females, males, and
792 predators. *Environmental entomology*, **8**, 441–443.

793 Warwick, S., Vahed, K., Raubenheimer, D. & Simpson, S.J. (2009) Free amino acids as
794 phagostimulants in cricket nuptial gifts: support for the “Candymaker” hypothesis. *Biology*
795 *letters*, **5**, 194–6.

796 Wilder, S.M., Raubenheimer, D. & Simpson, S.J. (2015) Moving beyond body condition indices as an
797 estimate of fitness in ecological and evolutionary studies (ed KP Lee). *Functional Ecology*, **30**,
798 108–115.

799 Will, M.W. & Sakaluk, S.K. (1994) Courtship feeding in decorated crickets - is the spermatophylax a
800 sham? *Animal Behaviour*, 1309–1315.

801 Williams, G.C. (1966) Natural Selection, the Costs of Reproduction, and a Refinement of Lack’s
802 Principle. *American naturalist*, **100**, 687–690.

803 Zahavi, A. (1975) Mate selection-a selection for a handicap. *Journal of theoretical biology*, **53**, 205–
804 14.

805 Zajitschek, F., Hunt, J., Jennions, M.D., Hall, M.D. & Brooks, R.C. (2009) Effects of juvenile and adult
806 diet on ageing and reproductive effort of male and female black field crickets, *Teleogryllus*
807 *commodus*. *Functional Ecology*, **23**, 602–611.

808 Zajitschek, F., Lailvaux, S.P., Dessmann, J. & Brooks, R.C. (2012) Diet, sex, and death in field crickets.
809 *Ecology and Evolution*, **2**, 1627–1636.

810 Zera, A.J. & Harshman, L.G. (2001) The Physiology of Life History Trade-Offs in Animals. *Annual*
811 *Review of Ecology and Systematics*, **32**, 95–126.

812 Zhao, Z. & Zera, A.J. (2002) Differential lipid biosynthesis underlies a tradeoff between reproduction
813 and flight capability in a wing-polymorphic cricket. *Proceedings of the National Academy of*
814 *Sciences of the United States of America*, **99**, 16829–34.

815

Figures

Figure 1: A male *Gryllodes sigillatus* calls to a female (photograph by T. Houslay).

Figure 2: The effects of diet treatment (x-axis) and day of measurement (equivalent to male age post-eclosion; modelled as a continuous variable and shown here as distinct panels) on male sexual advertisement during the first week post-eclosion. (a) The likelihood of male calling, with fitted regression line taken from coefficients from the zero-altered part of the daily calling analysis; (b) the amount of calling effort (plotted on log-scale y-axis), given a male called, with fitted regression line from the Poisson part of the daily calling analysis. The first day post-eclosion was excluded from the analysis as no males called.

Figure 3: The relationship between total calling effort over the first week post-eclosion (log-transformed) and the change in scaled mass index (SMI) during this period, plotted separately for each diet treatment (note that diet was modelled as a continuous predictor). Lines are predicted slopes from a linear regression model, and shaded areas represent 95% confidence intervals.

Figure 4: The partial effect of day of measurement (equivalent to male age post-eclosion) on male sexual advertisement during week 2, plotted separately for whether a female was absent or present on the day of measurement. (a) The likelihood of male calling, with fitted regression line taken from coefficients from the zero-altered part of the daily calling analysis; (b) the amount of calling effort (plotted on log-scale y-axis), given a male called, with fitted regression line from the Poisson part of the daily calling analysis. Measurements from males fed 12% nutrition diet have been excluded.

Figure 5: 'Small multiples' show how the relationship between diet and daily calling effort changes as a function of day and female access treatment. Each individual panel plots the predicted slope from the Poisson part of our MCMCglmm ZAP model of daily calling effort against dietary nutrition (24-84%). Here we present a subset of panels, showing how the effect of diet on calling effort (plotted on log-scale y-axis) changes from the first to last day of the female access treatment period (male

ages of 8 and 14 days post-eclosion, overall horizontal axis), among males that had either no access to females (lower panels) or daily access to females (upper panels). See text for model details; Figure S1 presents the complete set of panels for all combinations of day and female availability treatment.

Figure 6: Predicted quadratic slopes from a linear regression model show the effect of diet treatment on the change in individual scaled mass index (SMI) over week 2, separately for each female access treatment. All males on the lowest nutrition (12%) diet died before the end of the week and were thus excluded; we also excluded males that cannibalised females. Shaded areas represent 95% confidence intervals. Values for the change in SMI have been corrected for the effect of regression to the mean.

Tables

Table 1: MCMCglmm zero-altered Poisson (ZAP) analysis of male nightly calling effort over the first week post-eclosion. Day 1 was excluded from the analysis as no males called.

Fixed effects		Estimate	95% CI (lower, upper)	pMCMC
Likelihood of calling	(Intercept)	-5.636	(-5.926, -5.374)	<0.001
	Diet	0.187	(0.081, 0.297)	<0.001
	Day	0.420	(0.306, 0.540)	<0.001
	Day^2	-0.147	(-0.198, -0.088)	<0.001
	Diet × day	0.036	(-0.006, 0.082)	0.120
Calling effort				
Calling effort	(Intercept)	3.636	(3.391, 3.876)	<0.001
	Diet	0.183	(0.094, 0.276)	<0.001
	Day	0.191	(0.115, 0.265)	<0.001
	Day^2	-0.074	(-0.108, -0.042)	<0.001
	Diet × day	0.060	(0.033, 0.089)	<0.001
Variance components		Estimate	95% CI (lower, upper)	
Likelihood of calling	ID	1.128	(1.009, 1.258)	
Calling effort	ID	1.559	(1.190, 1.979)	
	Residual	5.000	(3.990, 6.086)	

Table 2: Linear regression models showing the effect of diet over the first week post-eclosion on (a) individual changes in body mass, as scaled mass index (SMI), and (b) approximated changes in four different energy reserve forms. Values representing changes in carbohydrate, glycogen and lipid were log-transformed prior to analysis.

	Response	Predictor	df	t	Estimate \pm S.E.	<i>P</i>
a)	Δ SMI	Diet	1,536	13.97	0.0062 ± 0.0004	<0.001
		Diet ²	1,536	-3.62	-0.0009 ± 0.0002	<0.001
b)	Δ Carbohydrate	Diet	1,43	3.39	0.050 ± 0.015	0.001
	Δ Glycogen	Diet	1,47	3.90	0.135 ± 0.035	0.003
	Δ Lipid	Diet	1,49	2.67	0.075 ± 0.028	0.010
	Δ Protein	Diet	1,48	0.53	0.100 ± 0.189	0.597

862 Table 3: Multiple regression showing how individual change in body condition over the first week of
 863 adulthood (SMI day 7 – SMI at eclosion) is affected by how much a male called over week 1 (log-
 864 transformed and standardised) and diet.

	t	Estimate ± S.E.	P
(Intercept)	16.34	0.0143 ± 0.0009	<0.001
Log week 1 total calling effort	1.80	0.0016 ± 0.0009	0.073
Diet	12.87	0.0058 ± 0.0004	<0.001
865 Log week 1 total calling effort × diet	2.88	0.0013 ± 0.0004	0.004

866

Table 4: MCMCglmm zero-altered Poisson (ZAP) analysis of male nightly calling over the second week post-eclosion. Males fed the 12% nutrition diet were excluded as they all died prior to the end of the week. Also excluded were males that cannibalised a female during the female-access treatments.

Fixed effects		Estimate	95% CI (lower, upper)	pMCMC
Likelihood of calling	(Intercept)	-4.617	(-5.034, -4.179)	<0.001
	Female presence	3.007	(2.491, 3.564)	<0.001
	Diet	0.031	(-0.149, 0.196)	0.732
	Day	-0.120	(-0.244, -0.005)	0.058
	Prior female access	-0.647	(-0.871, -0.429)	<0.001
	Female presence × day	0.779	(0.566, 1.001)	<0.001
	Prior female access × diet	0.055	(-0.018, 0.144)	0.185
	Prior female access × day	0.014	(-0.041, 0.067)	0.607
	Prior female access × diet × day	0.017	(-0.010, 0.044)	0.223
Calling effort	(Intercept)	4.065	(3.717, 4.381)	<0.001
	Female presence	0.742	(0.498, 0.971)	<0.001
	Diet	0.385	(0.281, 0.489)	<0.001
	Day	-0.195	(-0.264, -0.130)	<0.001
	Prior female access	-0.080	(-0.214, 0.058)	0.247
	Female presence × day	-0.210	(-0.335, -0.082)	0.001
	Prior female access × diet	0.024	(-0.012, 0.058)	0.191
	Prior female access × day	0.054	(0.028, 0.079)	<0.001
	Prior female access × diet × day	-0.020	(-0.032, -0.007)	<0.001
Variance components		Estimate	95% CI (lower, upper)	
Likelihood of calling	ID	5.365	(3.982, 6.876)	
Calling effort	ID	2.108	(1.694, 2.553)	
	Residual	1.283	(1.183, 1.394)	

874 Table 5: Simplified multiple regression model showing factors affecting individual change in scaled
 875 body mass over the second week of adulthood (SMI day 14 – SMI day 7, corrected for the effect of
 876 regression to the mean). Males that cannibalised a female during this period were excluded from the
 877 analysis.

878

	t	Estimate ± S.E.	<i>P</i>
(Intercept)	1.34	0.0019 ± 0.0014	0.182
Diet	3.5	0.0031 ± 0.0008	<0.001
Female-access	0.09	0.0001 ± 0.0003	0.927
Diet ²	-3.72	-0.0011 ± 0.0003	<0.001
879 Diet × Female-access	-2.16	-0.0004 ± 0.0002	0.032

880

881 **Supporting information**

882 Appendix S1: Specifications for the creation of artificial diets.

883 Appendix S2: Details of methods used for estimating energy storage components.

884 Appendix S3: MCMCgImm Zero-Altered Poisson (ZAP) model specification for the analysis of daily
885 calling effort.

886 Figure S1: The effect of diet on calling effort as a function of day and female-access treatment.

887 Figure S2: The effects of diet and female access on the likelihood of death during female-access
888 period.

889 Figure S3: The effects of diet and female access on male lifespan.

890

Figure 1



Figure 2

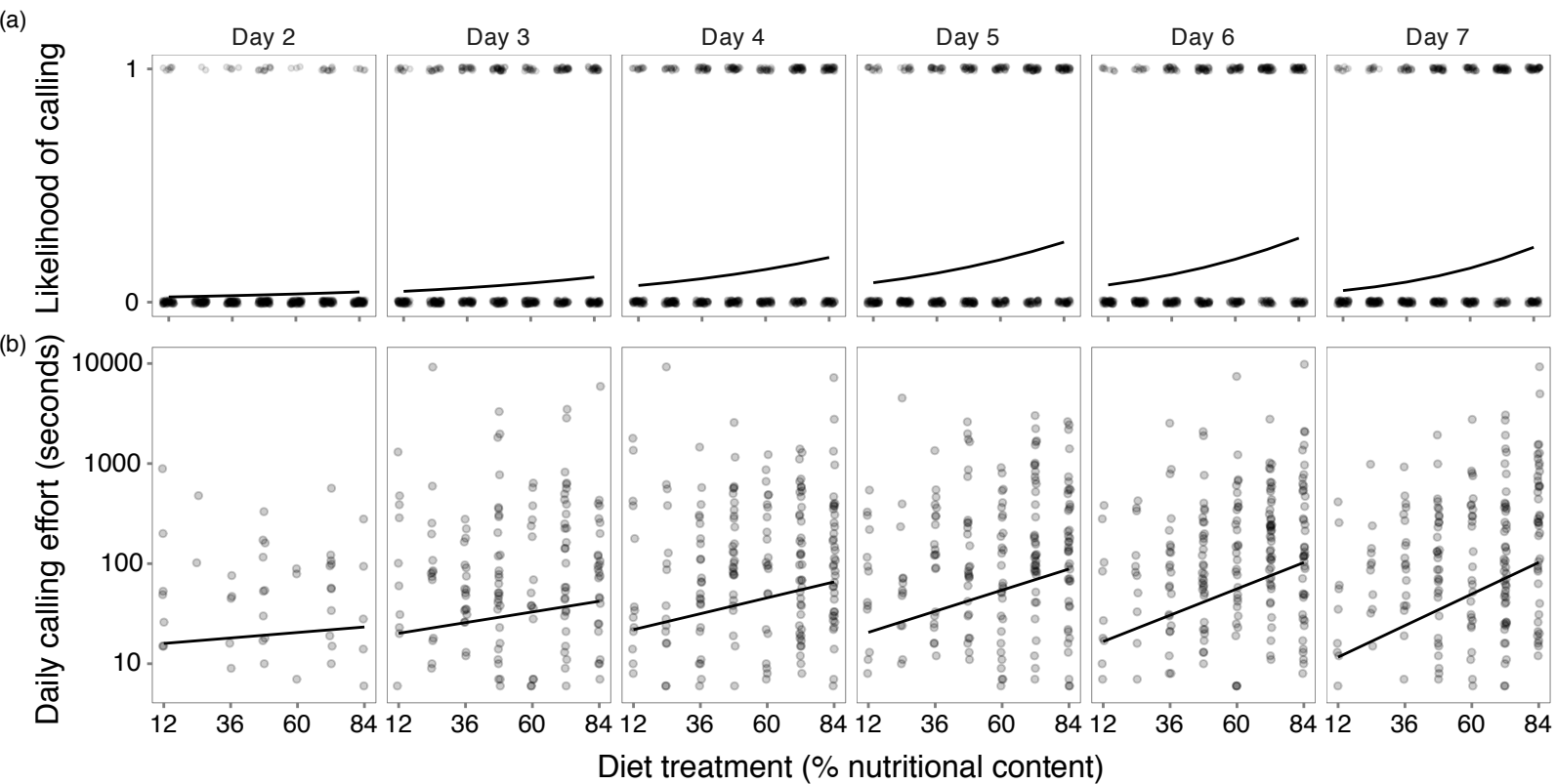


Figure 3

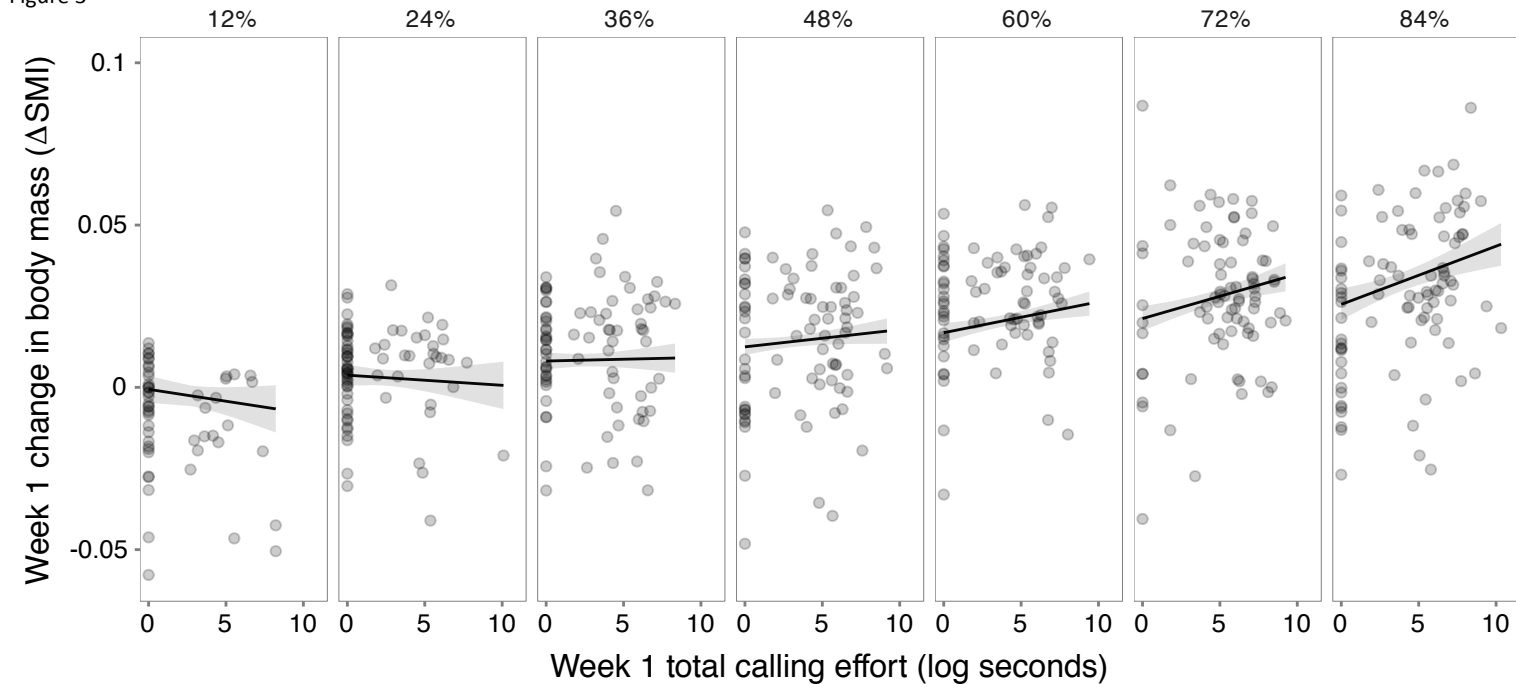


Figure 4

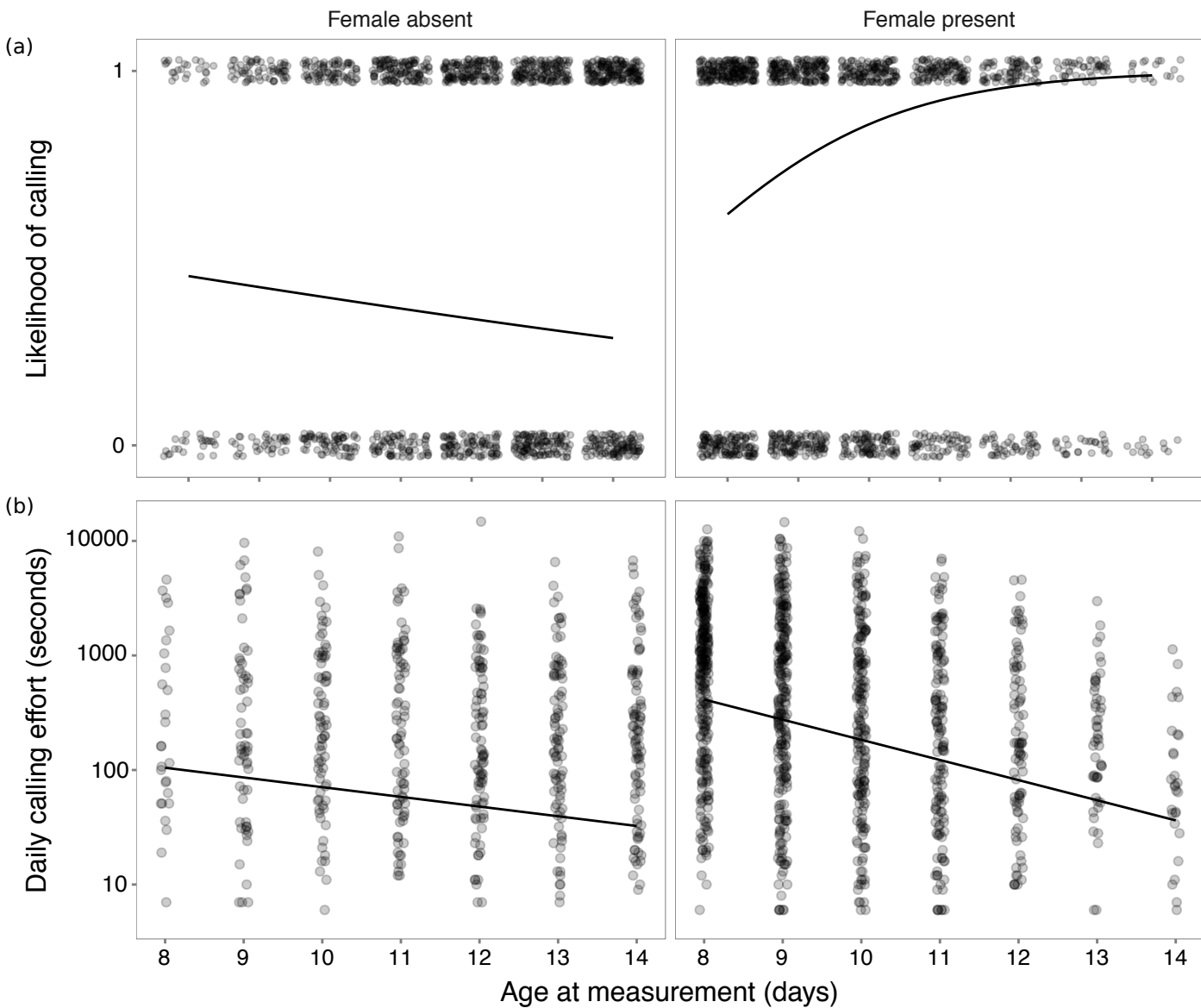


Figure 5

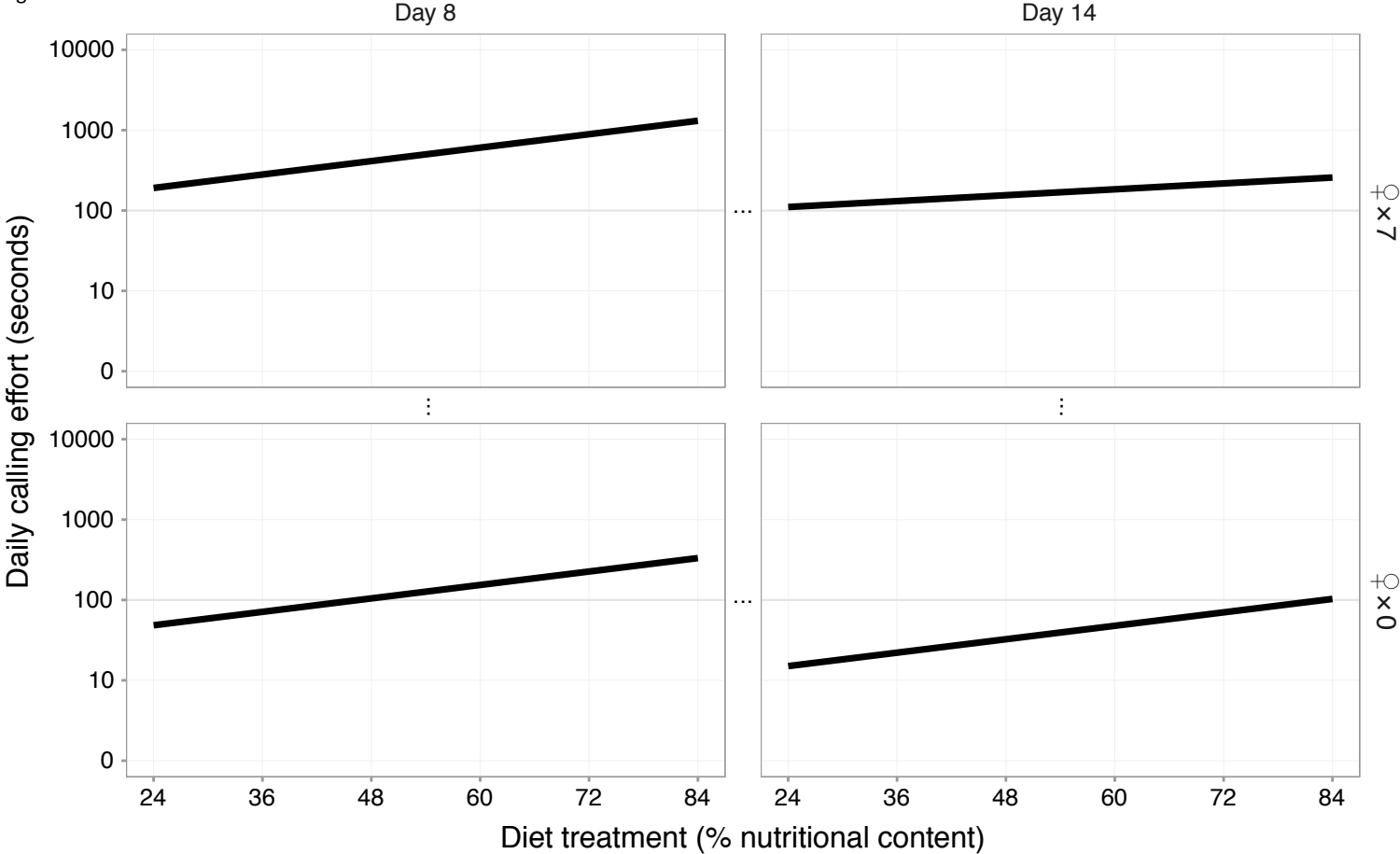


Figure 6

