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

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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.

48   3. We simultaneously manipulated acquisition (through dietary nutrition) and access to

49   potential mates (as a proxy for manipulating sexual trait allocation) in male decorated

50   crickets (*Gryllodes sigillatus*), while measuring age- and signalling effort-mediated changes in

51   energy storage components.

52   4. Increased diet quality caused increased signalling effort and energy storage, while access to

53   females increased both the likelihood of and time spent signalling. Males with lower

54   resource budgets signalled less, but still suffered energetic storage loss and viability costs.

55   5. Our results suggest that energetic constraints, rather than strategic resource accumulation,

56   reduced signalling levels in males with lower resource acquisition ability. Our findings imply

57   a non-adaptive explanation for age-dependent variation in sexual signalling, and an

58   important role for energetic constraints in maintaining the honesty of costly behavioural

59   displays.

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63   **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

89   real life history trade-offs between expensive traits (Van Noordwijk & De Jong 1986; De Jong & Van

90   Noordwijk 1992; Reznick, Nunney & Tessier 2000; Hunt *et al.* 2004b).

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

92   The resource pool itself cannot be measured directly via phenotypic traits: many such traits may

93   covary with an individual's resource budget, but must themselves have been constructed using

94   resources and therefore will necessarily be traded against other life history traits (Hunt *et al.* 2004b;

95   Houslay & Bussière 2012). Residuals of body mass over a fixed measure of body size are commonly

96   used as a proxy for condition (e.g., Peig and Green 2009), yet are unlikely to be consistently

97   adequate (Wilder, Raubenheimer & Simpson 2015; Barnett *et al.* 2015): for example, energetically

98   expensive trait expression (such as sexual display) is fuelled by sugars that are supplied by fat stores

99   (Tomkins *et al.* 2004). Consequently, condition indices can only be used to reliably compare

100   individuals of different sizes if we know how fat content scales with size (Kotiaho 1999). Ideally,

101   direct physiological analyses (rather than genetic or phenotypic approaches) are needed to study the

102   mechanisms mediating energy storage and use, and their consequences for variation in sexual trait

103   expression (Zera & Harshman 2001).

104   The problems inherent in assessing resource trade-offs are compounded when an individual

105   expresses a sexual trait repeatedly over its lifetime. Such individuals face trade-offs not only

106   between the focal trait and other components of life history, but also between current and future

107   expression of the sexual trait: a male that invests many resources when young has fewer resources

108   to spend when old, and vice versa (Badyaev & Qvarnström 2002). To save resources for critical

109   periods, males may elect not to signal at certain ages, or to delay maturation until later in life (Kokko

110   1997). These trade-offs create variation between individuals in patterns of age-dependent

111   expression of sexual traits, and impede females from using signal expression as an index of a male's

112   overall acquisition ability. In other words, investment in sexual signalling at any given age is less

113   likely to be an honest indicator of quality than if this trait remained constant across his lifetime

114   (Kokko 1997).

115   The honesty of sexual traits has long been of interest to students of sexual selection (Zahavi 1975;

116   Andersson 1982; Nur & Hasson 1984; Grafen 1990; Johnstone 1995; Kokko 1997). Strict honesty in

117   sexual trait expression may not always be fulfilled in a population (Candolin 1999; Hunt *et al.* 2004a),

118   but the 'on-average' honesty enforced by life history trade-offs maintains stability in systems where

119   male advertisement is subject to within-individual variation over lifetimes (Kokko 1997). Identifying

120   costs associated with sexual trait expression is therefore crucial to determining variation in age-

121   dependent signalling, and pinpointing mechanisms enforcing honesty in such systems.

122   A primary model for studying investment in age-dependent sexual expression is acoustic signalling

123   (Andersson 1994). In crickets, males produce an advertisement call through stridulation of their

124   forewings, and the amount of time a male spends calling ('calling effort') is a strong predictor of

125   mating success in nature (Hunt *et al.* 2004a; Bentsen *et al.* 2006; Rodriguez-Munoz *et al.* 2010).

126   Calling is highly energetically expensive (e.g., Kavanagh 1987; Hunt et al. 2004a; Ophir et al. 2010;

127   Mowles 2014), and may increase mortality risk from both intrinsic (calling diverts energy from other

128   functions, such as somatic maintenance, Hunt et al. 2004a) and extrinsic sources (male calls attract

129   predators, Walker 1979, or parasitoids, Cade 1975). Among crickets, calling is likely fuelled by

130   aerobic metabolism (Prestwich & Walker 1981; Bailey *et al.* 1993; Prestwich 1994; Hoback & Wagner

131   1997), with variation in calling effort linked to the ability to acquire and mobilise combinations of

132   glycogen, carbohydrates and lipids (*Acheta domesticus,* Bertram *et al.* 2011; *Gryllus assimilis, Gryllus*

133   *texensis,* Thomson, Darveau & Bertram 2014). Studies that manipulated resource acquisition have

134   shown that male condition strongly affects the timing and magnitude of calling effort in several

135   different cricket species (*Gryllus pennsylvanicus,* Judge, Ting & Gwynne 2008; *A. domesticus,* Bertram

136   *et al.* 2009; *Teleogryllus commodus,* Maklakov *et al.* 2009; Zajitschek *et al.* 2009, 2012; *Gryllodes*

137   *sigillatus,* Houslay *et al.* 2015), and also that high-condition males sometimes invest so heavily in

138   sexual displays that they suffer heightened mortality compared to lower-condition individuals (*T.*

139   *commodus*, Hunt et al. 2004a).

140   However, manipulating acquisition alone may not necessarily reveal differential costs of sexual trait

141   expression, as males in good condition have more resources to allocate to all traits (Kotiaho 2001).

142   Testing for differential costs requires manipulating both acquisition and trait investment, then

143   studying their effects on other life history traits (e.g., future reproductive effort or survival).

144   Moreover, behavioural signals are highly phenotypically plastic, which may allow males to react

145   quickly to changes in the local environment so as to maximise fitness (Komers 1997; Bretman, Gage

146   & Chapman 2011). We do not know the importance of resource trade-offs relative to other factors in

147   causing distinct age-specific investment strategies (live fast and die young vs. slow and steady). Male

148   crickets respond plastically to the social environment: juveniles reared in environments indicating

149   high competition for females invest more in reproductive tissues (*T. oceanicus*, Bailey et al. 2010)

150   and age-specific calling effort (*T. commodus*, Kasumovic et al. 2012a). During adulthood, rival male

151   presence can cause plastic shifts towards increased calling effort (*T. commodus*, Callander et al.

152   2013), while the introduction of females can affect call frequency (*Gryllus bimaculatus*, Simmons

153   1986) and timing (*G. sigillatus, Gryllus veletis,* Burpee & Sakaluk 1993). The simultaneous

154   manipulation of diet and the socio-sexual environment can therefore be used to evaluate both the

155   energetic and life history consequences of trait investment, and reveal whether plasticity in the male

156   signal is due to the strategic saving of resources or resource constraints.

157   In this study, we measure changes in allocation to sexual signalling and resource storage as a

158   function of both acquisition and the demands of prior signalling in male decorated crickets (*G.*

159   *sigillatus*). We measure calling effort, and use biochemical assays to estimate the energetic resource

160   budgets of separate subsets of males before and after manipulating resource acquisition and access

161   to potential mates. Furthermore, we assess the consequences of our experimental manipulations on

162   longevity. Our measurements of male daily calling behaviour reflect two processes: a male's decision

163 as to whether or not he calls, and – given a positive decision – his calling effort. The presence of

164 females should present universal incentive in the decision to call, enabling us to separate this choice

165 from the more resource-dependent component (the non-zero amount of calling effort). By isolating

166 components of male investment decisions in this way, we can disentangle variation due to

167 constraints from variation due to adaptive planning. If future planning is the primary force shaping

168 an individual's response to social conditions, low levels of calling early in life among males with

169 reduced resource acquisition ability should also be associated with increased energy reserves and

170 greater calling later in life (Kokko 1997; Hunt *et al.* 2004a). If, however, there is no evidence of

171 greater energy reserves for allocation at a later point, it is likely that low calling levels are due simply

172 to a lack of energy for current allocation to reproductive effort. In the latter case, we would also

173 expect all males to respond to the availability of potential mates by investing their resources more

174 heavily in calling effort, rather than saving for the future.

175

Materials and Methods

176

CRICKET HUSBANDRY

178 We used *G. sigillatus* (Figure 1) descended from a 2001 collection of 500 adult crickets in Las Cruces,

179 New Mexico, which had been used to initiate a laboratory culture maintained at a population size of

180 approximately 5000 crickets and allowed to breed panmictically (Ivy & Sakaluk 2005). Crickets were

181 housed in 15-litre plastic containers in an environmental chamber maintained at 32±1∘C on a

182 reversed 14:10 hours light/dark cycle. We provided juvenile crickets with egg cartons for shelter, *ad*

183 *libitum* water in cotton wool-plugged test tubes, and a standard diet of ground cat food (Friskies Go-

184 Cat Senior®, Purina, London, UK). We provided moistened cotton wool pads to adults as an

185 oviposition substrate. We maintained crickets at a density of approximately 300 indviduals per

186 container.

EXPERIMENTAL PROTOCOL

188   We manipulated male resource acquisition through diet treatment, and also manipulated access to

189   females (as a proxy for manipulating resource allocation). We investigated variation in allocation to

190   four different energy storage components (carbohydrates, glycogen, lipids and proteins; details

191   given below) by sacrificing subsets of the experimental population (by placing individuals in a -80°C

192   freezer) at three different ages: immediately at eclosion (baseline variation); one week post-

193   eclosion (effect of resource acquisition manipulation); and two weeks post- eclosion (effects of both

194   resource acquisition and female-access manipulations). A fourth group of males were allowed to die

195   naturally, to estimate how acquisition and prior signalling investment affected lifespan.

196   We separated 630 male cricket nymphs from laboratory stock cultures during the final instar. Upon

197   eclosion, we photographed individuals (Motic SMZ-168 with Moticam 2000) and measured

198   pronotum length using NIH ImageJ v1.48 (Schneider, Rasband & Eliceiri 2012). We measured body

199   weight using a high-precision electronic balance (Denver Instrument, model PI-225DA) at eclosion

200   and weekly intervals thereafter. At eclosion, we assigned males randomly to one of four groups:

201   those to be sacrificed immediately ($n = 58$), after one week ($n = 56$), after two weeks ($n = 256$), or

202   allowed natural lifespan ($n = 260$). We transferred male crickets that were not immediately sacrificed

203   to individual clear plastic containers (5×5×5cm), each containing plastic mesh as substrate, a cotton

204   wool-plugged water bottle, and the food treatment. We cleaned containers and provided fresh food

205   and water weekly.

206   We quantified allocation to the male sexual trait as 'calling effort', the duration (in seconds) that

207   each individual spent broadcasting his sexual advertisement call. We measured each male overnight

208   from 2pm-9am (dusk until afternoon of the following day on the reversed light cycle; lights off

209   2.30pm-12.30am, with 30 min rampdown) every day from the day after eclosion up to a maximum

210   of 35 days. We mounted a microphone (C1163, Dick Smith Electronics) in the lid of each individual

211   container, placing that container into a hollowed-out cube of soundproofing foam (15×15×15cm) to

212   minimise outside disturbance and prevent crosstalk between containers during recording. We

213   removed a corner of the foam lid to maintain crickets on the set light cycle. An electronic acoustic

214   recording system (Bertram and Johnson 1998) sampled from each microphone of each 10 times per

215   second to determine whether or not a male was calling (see Hunt et al. 2004a).

CREATION OF ARTIFICIAL DIETS

217   We manipulated resource acquisition by creating seven artificial, dry, granular diets that varied in

218   the amount of total nutritional content (see Appendix S1). All diets had a protein:carbohydrate ratio

219   of 1:8, based on the effect of a high-carbohydrate diet in maximising calling effort and longevity in

220   this species (J. Hunt, unpublished data). Diets ranged from 12-84% (in 12% increments) in total

221   protein and carbohydrate content (referred to throughout as nutritional content).

FEMALE-ACCESS TREATMENT

223   During the second week post-eclosion, we allowed male crickets access to a single different adult

224   female cricket during the recording period each night for between 0 and 7 consecutive nights

225   (always beginning on the first day of the second week post-eclosion; i.e., eight distinct treatment

226   groups). As logistical constraints prevented us from providing virgin females of similar age each day,

227   we instead controlled female 'experience' to be similar to the male with which she would be placed:

228   on the first day virgin males had access to virgin females, on the second day a male would gain

229   access to a female that had had a single night's access to males, and so forth. When not housed with

230   an experimental male in his container, females were maintained in single-sex groups according to

231   experience, and provided with water, shelter, and stock diet. Female experience was manipulated to

232   the necessary level by housing them overnight with random stock males. Before being placed with

233   an experimental male, females were also given at least a day without access to a male (considered

234   sufficient as female *G. sigillatus* have a high remating rate, averaging 22 times over a 20-day period;

235   Sakaluk 1987). During the week that access to females was manipulated, the food was removed

236   from each male's container during the call recording period (regardless of female presence). Males

237   were given access to food whenever calling effort was not being recorded (9am – 2pm).

238   During the female-access treatment period, we allowed paired crickets to mate freely. In many

239   cricket species, males stop calling after mating until they have generated a new spermatophore and

240   are ready to mate again (Huber 1962; Loher 1974; Cade & Wyatt 1984; Wagner 2005); this is also the

241   case in *G. sigillatus* (T. Houslay, personal observation). Males transfer a 2-part spermatophore,

242   comprising the sperm ampulla and a spermatophylax 'nuptial gift' (Sakaluk 1984). While these take

243   an average of 3.25 hours to compose (Sakaluk 1985), they consist of 80-85% water and have little or

244   no nutritional value (Will & Sakaluk 1994; Warwick *et al.* 2009), and as such the metabolic costs of

245   their generation are uncertain. We anticipate that one major effect of mating will be a reduction in

246   calling, with the concomitant saving in stored resources that might otherwise be spent on calling

247   activity. Our large sample size and use of continuous (rather than categorical) treatments enable us

248   to capture diet- and calling-mediated changes in energy storage despite any additional effects of

249   mating on males.

250   **MEASURING ENERGY STORAGE**

251   We used a modified version of Foray et al. (2012) to measure the major components of energy

252   storage in each cricket: carbohydrates, glycogen, lipids and proteins (see Appendix S2). These can be

253   assumed to comprise an individual's entire current energy budget. Cricket flight is fuelled by lipid

254   breakdown (Zhao & Zera 2002), and the nature of the underlying pathways makes it likely that lipids

255   are the major energy store for stridulatory calling effort. Their presence is likely to be greatest in

256   males that have access to high quality resources. Glycogen is the storage form of glucose, and can be

257   broken down in quick response to the need for energy in high-intensity activity (Campbell & Farrell

258   2003). High circulating glycogen levels may indicate males that are prepared to engage in sustained

259   calling bouts, while carbohydrate content shows the existence of free sugars that can be used to fuel

260   energetically expensive signalling. Protein contains the same energy content per gram as

261 carbohydrates, but takes longer to break down and is not typically used as a short-term energy

262 source. The relative value of these energy storage forms for sexual signalling is currently unclear;

263 lipids provide more energy than sugars on a gram-for-gram basis (Campbell & Farrell 2003), yet

264 mobilisation of shorter-term energy forms is probably important for plastic responses to

265 reproductive opportunities. If males were future planners, such that low-acquisition males restrict

266 early signalling in favour of storage for use later in life, we would expect these males to show greater

267 storage of lipids relative to glycogen and carbohydrate.

268 **STATISTICAL ANALYSIS**

269 We performed all statistical analyses using R 3.1.1 (R Core Team 2014). Independence between

270 linear and quadratic forms of numeric predictors (e.g., diet and $diet^2$) was achieved by centring the

271 input variable before squaring (Gelman & Hill 2007). Diet treatments were centred and scaled to

272 single-unit deviations, such that model coefficients show the effect on the response variable

273 equivalent to one unit of change in diet treatment. In analyses of daily calling effort (discussed

274 further below), male age post-eclosion was also centred but not scaled (coefficients show effect

275 equivalent to one day's change). Continuous covariates (e.g., measurements of energy storage when

276 used as predictors) were standardised by mean-centring and scaling (dividing by 1 standard

277 deviation), putting them on a common scale and aiding interpretation of main effects (Gelman & Hill

278 2007; Schielzeth 2010). Unless otherwise stated, we performed model simplification by dropping

279 non-significant higher-order terms (interactions and quadratic effects) from the full model

280 sequentially, and using likelihood ratio tests to compare nested models. We retained more complex

281 models whenever simplification resulted in a significant increase in model deviance. We observed no

282 cases in which non-significant main effects became significant during model simplification steps that

283 did not involve the deletion of an interaction term including that particular main effect.

284 *Daily calling decisions and effort*

285   Daily calling effort data were over-dispersed and zero-inflated; for analyses of these data, we used

286   the R package MCMCglmm (Hadfield 2010) to fit a zero-altered Poisson (ZAP) model: a two-part

287   model that includes a logistic regression for the zeroes in the data and a Poisson regression for the

288   zero-truncated counts (see Appendix S3). We accounted for repeated observations on individuals by

289   specifying a random effect of cricket ID. The ZAP model enabled us to ask two distinct questions

290   within a single statistical structure (Atkins *et al.* 2013): what factors influenced whether a male

291   called or not (*i.e.*, non-zero or zero), and, if he did call, what factors influenced the magnitude of

292   calling (Houslay *et al.* 2015)?

293   For the first week post-eclosion, the full model included predictor variables of linear and quadratic

294   terms for both diet treatment and days post-eclosion (corresponding to male age, modelled as a

295   continuous variable), and all interactions (except those between the linear and quadratic forms of

296   the same predictor, e.g. day × day^2, as such interactions are not interpretable).

297   For analysis of daily calling effort across the second week post-eclosion, female-access treatment

298   could not be entered in the model as a single predictor because the differences among treatment

299   groups only become apparent as the week progresses. Instead, we use both a binary predictor of

300   'female presence' (indicating whether a male had access to a female on the current day of

301   observation) and a continuous 'prior female access' variable (the number of days prior to the current

302   one in which a male had access to females). The full model for daily calling over the second week

303   post-eclosion therefore included predictor variables of female presence (binary variable), linear and

304   quadratic terms for both diet treatment and male age (in days post-eclosion), prior female access,

305   and up to three-way interactions between these (except those featuring linear and quadratic forms

306   of the same predictor).

307   *Changes in body mass*

308   We used multiple regression to assess the linear and quadratic effects of diet treatment on changes

309   in individual body mass (using the scaled mass index, SMI; Peig and Green 2009) over the first week

310   post-eclosion. SMI is a better index of the relative size of energy reserves (Peig & Green 2010), and a

311   better correlate of standardised mass in crickets (*A. domesticus, G. texensis;* Kelly, Tawes &

312   Worthington 2014), than other commonly-used proxies.

313   To estimate the effects of both acquisition and allocation on the change in SMI (ΔSMI) over week 2,

314   our predictors were diet treatment and log-transformed total calling effort. We used a separate

315   model to investigate the effects on ΔSMI of diet treatment, female access treatment, and their

316   interaction. Given that our individuals had already been subject to their diet treatments for the first

317   week post-eclosion, we corrected ΔSMI over week 2 for the effect of regression to the mean, using

318   the method outlined by Kelly and Price (2005).

319   *Changes in energy storage*

320   For those individuals sacrificed at eclosion, we used linear regression models to test whether

321   carbohydrate, glycogen, lipid or protein content predicted an individual's scaled body mass (SMI).

322   We log-transformed SMI so that model residuals conformed to a normal distribution. We excluded

323   one individual because its SMI was over 4 standard deviations from the mean (all others were within

324   ± 2.2 standard deviations).

325   We also used linear regression models to investigate the causes of variation in energy storage

326   components at the end of the first week post-eclosion. For each response variable (protein, lipid,

327   glycogen and carbohydrate), we estimated approximate changes over this period by subtracting the

328   mean concentration of each storage form at eclosion from every individual's measurements. We

329   then tested for the effect of diet treatment on these changes. The values representing changes in

330   carbohydrates, glycogen and lipids were log-transformed prior to analysis. We also tested whether

331   the changes in each form of energy storage predicted changes in SMI.

332   We estimated approximate changes in energy storage components over the second week by

333   subtracting the diet-specific mean content of each storage form at the end of week 1 from each

334   individual's measurements. We then tested whether these estimated changes in energy storage

335   were associated with individual changes in SMI. We also tested whether individual energy storage

336   measurements at the end of week 2 predicted SMI at the same stage.

337

338   *Results*

339   As expected (because treatments began after males reached eclosion), there were no significant

340   differences between diet or female-access treatment groups in pronotum length, raw body mass, or

341   scaled body mass (scaled mass index, SMI) at eclosion (all $P > 0.37$). Of the four energy storage forms

342   measured, only lipids were a significant predictor of SMI at eclosion (lipid coefficient = 0.065 ± 0.030,

343   $t_{1,50} = 2.19$, $P = 0.033$).

344   **MANIPULATION OF RESOURCE ACQUISITION IN EARLY ADULTHOOD**

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

346   The simplified ZAP model for days 2-7 post-eclosion included predictor variables of diet, linear and

347   quadratic terms for the day of calling (i.e., the age of an individual post-eclosion), and an interaction

348   between diet and the linear day term. The likelihood of calling increased significantly along with

349   both nutritional diet content and age over the first week (Table 1, Fig. 2(a)). The age-related increase

350   in the likelihood of calling was less pronounced towards the end of the week. When males did call,

351   there was a significant two-way interaction between day and diet on the amount of calling effort:

352   effort increased over the course of the week, especially when males were fed higher quality diets

353   (Table 1, Fig. 2(b)). The main effects of age and diet were to increase calling effort during this period,

354   age acted in a non-linear manner. This non-linear effect of age can be seen in the change in the

355   intercept (centred at 48% diet) across panels in Fig. 2(b): the age-related change in the intercept

356   does not increase at a constant rate, with the rate of increase dropping at higher values of male age.

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

358   The change in SMI among individuals increased with improving dietary nutrition, although this

359   increase levelled off at higher diets (negative quadratic effect of diet; Table 2(a)). We estimated

360   changes in energy storage content by comparing individual measurements of each storage

361   component to averages taken from the subset of crickets sacrificed at eclosion, enabling us to

362   investigate the underlying physiological response to our dietary manipulation. Improving dietary

363   nutrition significantly increased carbohydrate, glycogen and lipid reserves, but not protein (Table

364   2(b)). Separate models also showed that the estimated changes in lipid and glycogen were significant

365   predictors of changes in SMI over the first week post-eclosion (carbohydrate = 0.015 ± 0.004, $t_{1,43}$ =

366   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 =

367   0.024; protein < -0.001 ± 0.003, $t_{1,48}$ = -0.01, P = 0.992).

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

369   The significant interaction term (Table 3) indicated that the relationship between total calling effort

370   and change in SMI over week 1 was strongly dependent upon diet, switching from a negative to a

371   positive relationship as diet improved (Figure 3): increased dietary nutrition meant males that called

372   more were also better able to increase body mass.

373   **MANIPULATION OF RESOURCE ACQUISITION AND ACCESS TO POTENTIAL MATES**

374   In the first part of our study, males on lower quality diets called less often and less intensely than

375   those on higher-quality diets (Fig. 2). While total calling effort and storage appeared to covary

376   negatively among those low-acquisition males, those males did not invest more in energy reserves

377   relative to higher-acquisition individuals (Fig. 3). To demonstrate 'future planning', low-acquisition

378   males should remain less likely to call even in the presence of females, instead conserving energy

379   reserves for greater calling later in life.

380   *Males are more likely to call when females are present*

381   The simplified ZAP model for daily calling effort during the female-access treatment period included

382   predictor variables of diet, female presence on the current day, the age of an individual at the time

383   of measurement (in days post-eclosion), and prior female access (the number of days the male had

384   access to a female prior to the current measurement). Multiple interactions were retained in the

385   model, including a three-way interaction between prior female access, diet, and day of

386   measurement.

387   Female presence significantly increased the likelihood of calling (Table 4, Fig. 4(a)). Males were less

388   likely to call as the week progressed, but this decrease was not observable when females were

389   present ('Female presence × day' interaction in Table 4; Fig. 4(a)). Finally, call likelihood was

390   significantly reduced in males that had greater access to females prior to the current measurement

391   ('Prior female access', Table 4). Diet had no effect on the likelihood of calling: low-acquisition males

392   were just as likely to call as high-acquisition males.

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

394   Calling effort increased significantly when a female was present, but the effect decreased over the

395   week ('Female presence × day' interaction term in Table 4; Fig. 4(b)). Dietary nutrition also

396   significantly increased calling effort (Fig. 5), but this effect was reduced in males with greater access

397   to females as the week progressed ('Prior female access × diet × day' interaction in Table 4; Fig. 5).

398   These effects are visualised in Fig. 5, which shows the relationship between diet treatment and

399   calling effort for the first (left panels) and final (right panels) days of the female-access manipulation,

400   for those given no female access (lower panels) or daily female access (upper panels).

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

402   Having excluded males that did not call or that cannibalised females during the female-access

403   treatment period, we found that increased total calling effort during week 2 was associated with

404   decreased SMI (log total calling = -0.0018 ± 0.0007, $t_{1,307}$ = -2.55, P = 0.011). Diet treatment increased

405   individual SMI, but this effect was reduced at higher diets (diet = 0.0021 ± 0.0006, $t_{1,307}$ = 3.51, P <

406   0.001; diet^2 = -0.0018 ± 0.0003, $t_{1,307}$ = -3.76, P < 0.001). Separately, we found no effect of diet on the

407   frequency with which males cannibalised females during the female access treatment period (χ^2_6 =

408   1.10, P = 0.98).

409   Table 5 shows the minimum adequate model for a separate analysis of the effects of our

410   experimental manipulations on the change in SMI over week 2 (again, excluding males that

411   cannibalised females). The effect of diet depended on female-access treatment; in the absence of

412   females, males on high-nutrition diets tended to gain more body mass during this period than those

413   on low diets; however, high-nutrition males with consistent access to potential mates lost more

414   mass ('Diet × Female-access' interaction term in Table 5; Fig. 6).

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

416   We found that the estimated change in carbohydrate and glycogen reserves were significant

417   predictors of changes in individual SMI over the second week post-eclosion (carbohydrate = 0.0045 ±

418   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 ±

419   0.0009, $t_{1,142}$ = -0.72, P = 0.470; protein = 0.0007 ± 0.0009, $t_{1,139}$ = 0.70, P = 0.486).

420   We also tested whether any of our energy storage measurements correlated with individual SMI at

421   the end of week 2; only lipid content significantly predicted SMI (lipid = 0.014 ± 0.005, $t_{1,142}$ = 2.96, P

422   = 0.004).

423   **EFFECTS OF RESOURCE ACQUISITION AND ACCESS TO POTENTIAL MATES ON SURVIVAL**

424   Due to the high proportions of deaths in the two lowest diet treatments (100% and 60.5%

425   respectively), we excluded individuals in those treatments from our analysis of the likelihood of

426   dying during the female-access period. Greater female access significantly increased the risk of death

427   during this period, while diet significantly reduced it (diet = -0.225 ± 0.113, $t_{1,377}$ = -1.99, P = 0.047;

428   female-access = 0.226 ± 0.074, $t_{1,377}$ = 3.04, P = 0.002; Fig. S2). A model retaining the excluded diet

429   treatments gave qualitatively similar results, which are not presented here.

430   Our female-access treatment had a significant effect on lifespan after the conclusion of the female

431   access treatment period (i.e., after the end of the second week post-eclosion); males with greater

432   access to females during week 2 had shorter lifespans (female-access = -3.913 ± 0.937 days, $t_{1,145}$ = -

433   4.18, P = 0.001; Fig. S3(a)). Lifespan was significantly increased by greater dietary nutrition, although

434   this effect disappeared at higher quality diets (diet = 11.823 ± 1.734 days, $t_{1,145}$ = 6.82, P <0.001; diet^2

435   = -2.491 ± 0.869, $t_{1,145}$ = -2.87, P = 0.005; Fig. S3(b)).

436

437   *Discussion*

438   Male sexual signals are often condition-dependent, with trait expression closely associated with the

439   size of the resource budget (e.g. Andersson 1994; Johnstone 1995). Few studies have simultaneously

440   manipulated resource acquisition and access to reproductive opportunities while also examining the

441   resultant patterns of energy storage and use (Kotiaho 2000). Recently, it has become clear that traits

442   used to measure condition are themselves condition-dependent (and are built using the resources

443   that make up condition), meaning that traits reflecting condition must inevitably trade-off with one

444   another (Tomkins *et al.* 2004; Houslay & Bussière 2012). This fact prescribes careful interpretation of

445   covariances between proxies for condition and other life history traits, and highlights the need for

446   more direct measurements of energy reserves (Morehouse 2014; Wilder *et al.* 2015). Despite the

447   inherent challenges, identifying relationships between trait investment and stored energy reserves

448   can illuminate mechanisms leading to changes in resource storage and use, and therefore help

449 clarify the adaptive significance of variation in resource allocation among males as well as the

450 consequences of energetic trade-offs for honesty in sexual trait expression.

451 By manipulating dietary nutrition in male crickets, we showed that greater resource acquisition led

452 to an increase in both the likelihood and amount of signalling in early adulthood (Table 1, Fig. 2), as

453 well as greater investment in energy stores (lipid, glycogen and carbohydrate) during this period

454 (Table 2). The relationship between allocation to scaled body mass (as scaled mass index, SMI) and

455 calling effort depended on diet quality, switching from negative covariance when diet quality was

456 low to positive covariance when high (Table 3, Fig. 3). While low-acquisition males called less often,

457 and less intensely, during this period than did higher-acquisition males, we found no evidence that

458 such a suppression of signalling effort led to increased energy reserves. If low calling effort among

459 low-acquisition males was due to energy constraints rather than future planning, we predicted that

460 these males would respond to the presence of potential mates by investing more in calling instead

461 of saving energy for the future.

462 Sexual trait expenditure was highly plastic depending on the availability of a potential mate: males

463 responded strongly to the presence of a female by increasing both the likelihood of calling and time

464 spent calling (Table 4, Fig. 4). During the female-access treatment period, diet did not affect the

465 probability of signalling on any given day. It did, however, have a strong positive effect on the

466 amount of time spent calling (Fig. 5), mediated by female-access: the positive effect of diet on calling

467 effort decreased with greater exposure to females over the course of the week (Fig. 5). The change

468 in male scaled body mass (SMI) as a function of diet during this period was non-linear and also

469 depended on access to females (Table 5, Fig. 6). In the absence of females, males fed higher

470 nutrition diets tended to increase or maintain body mass more than those on lower-nutrition diets.

471 However, greater female-access effectively reversed this relationship: males on high-nutrition diets

472 tended to lose more SMI than those on lower-nutrition diets when given daily access to females over

473 the treatment period. Diminishing calling effort by the end of week 2, even by males on high-quality

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

475 5).

476 Indeed, the change in male body mass over week 2 covaried negatively with total calling effort, and

477 the likelihood of calling decreased as previous exposure to females increased (Fig. 4(a), Table 4) –

478 both results irrespective of diet. Greater exposure to potential mates also increased the risk of dying

479 during week 2, although this effect was mitigated by diet quality (Fig. S2). There were lasting

480 consequences of our experimental manipulations: longevity following week 2 was increased by

481 dietary nutritional quality, and decreased by greater prior exposure to females (Fig. S3). However,

482 this reduced viability cannot be considered a true cost unless it is accompanied by a net decrease in

483 fitness, because any decrease in lifespan may be offset in fitness terms by increased early

484 reproductive success (Kotiaho 2001; Hunt et al. 2004a,b).

485 **FUTURE PLANNING OR BUDGET CONSTRAINTS?**

486 Crickets are ideal for testing hypotheses concerning age-related reproductive effort because we can

487 precisely quantify sexual advertisement effort by males. Several studies have manipulated resource

488 acquisition to investigate its effects on life history traits, with largely consistent results: males fed

489 higher-quality diets typically increase their calling effort with age, and longer-lived males call more

490 (Judge *et al.* 2008; Maklakov *et al.* 2008; Zajitschek *et al.* 2009, 2012; Houslay *et al.* 2015). One

491 exception is the study of Hunt et al. (Hunt *et al.* 2004a), in which males fed a high-protein diet called

492 earlier and far more intensely than those fed lower protein diets; high-protein diet males also

493 suffered survival costs such that they died earlier than low-protein diet males. This pattern of age-

494 related allocation may reflect status-dependent plasticity in allocation to reproduction and survival

495 in some cases (Kokko 1997, 1998; Höglund & Sheldon 1998; Vinogradov 1998). In particular, low-

496 condition males can benefit from suppressing signalling at early ages and investing in longevity and

497 sexual advertisement later in life (Kokko 1997). If this adaptive plasticity in investment applied to *G.*

498   *sigillatus*, males restricted to lower-nutrient diets should have allocated resources preferentially to

499   energy storage instead of early signalling.

500   Males fed lower nutrition diets in our study called less, and the correlation between allocation to

501   storage (individual change in SMI) and calling effort was negative during week 1 among males on

502   lower-quality diets (Table 2, Fig. 3). However, while diet-restricted males were less likely to call over

503   the first week (Table 1, Fig. 2(a)), this effect was not evident during week 2 (Table 4). If males on

504   low-quality diets had continued to show a depressed propensity to call, and had increased body

505   mass over this period, that would be more consistent with crickets suppressing advertisement in

506   favour of increased future investment. Instead, it appears more likely that variation in calling effort

507   was due simply to budget constraints: i.e., having less energy to allocate to signalling. The non-

508   adaptive explanation for this variation among males is given further credence by dietary nutrition

509   being a strong predictor of survival, with males fed lower-nutrient diets suffering reduced longevity.

510   The fact that a male's current signalling effort is primarily governed by his acquisition ability and

511   previous expenditure also supports previous assertions that energetic trade-offs provide a

512   mechanism for maintaining honesty on average in male behavioural display traits (Kokko 1997).

513   **VARIATION IN USAGE AND IMPORTANCE OF ENERGY STORAGE FORMS**

514   Increased resource acquisition from higher-nutrient diets enabled male crickets to allocate more

515   resources to storage after eclosion in the forms of greater lipid, glycogen, and carbohydrate content

516   (Table 2(b)). These appear to represent the major forms of energy stores; protein levels did not vary

517   over the three time points that these stores were measured, indicating that males seem to maintain

518   protein content rather than increase it (although the nutrient ratio in our diet treatment was heavily

519   in favour of carbohydrate rather than protein, the variation in total nutrient content would enable

520   individuals to differ in allocation if required). We hypothesised that 'future planning' would result in

521   low-acquisition males prioritising storage in the form of lipids; the positive effect of diet on this form

522   indicates that this was not the case. Our results further corroborate findings in other cricket species,

523 in which calling effort covaried with the ability to acquire, metabolise and mobilise these three

524 storage forms (Bertram *et al.* 2011; Thomson *et al.* 2014).

525 Males tended to lose scaled body mass (SMI) over week 2 (Table 5), and individual SMI change in this

526 period was associated with estimated changes in glycogen and carbohydrate reserves – both of

527 which are quick-release forms of energy. Absolute SMI at the end of that period was associated with

528 current lipid reserves. Together, these results suggest that glycogen and carbohydrate are more

529 freely expendable forms of energy, while lipids are more carefully conserved. Such variation in the

530 conservation of storage forms indicates that lipids differ from carbohydrate and glycogen in usage

531 and importance, and highlights the importance of estimating multiple metabolic reserves when

532 examining life-history trade offs. The patterns of change in individual body mass and energy reserves

533 over weeks one and two suggest that resource acquisition early in adulthood tended to be mobilised

534 for fuelling calling effort as soon as the social circumstances favour it.

535 **ACQUISITION CAN AFFECT COVARIANCES BETWEEN EXPENSIVE LIFE HISTORY TRAITS**

536 Variation in resource acquisition ability among individuals can lead to positive correlations between

537 life history traits, as predicted by Van Noordwijk and De Jong (1986) and often found in nature

538 (Jennions *et al.* 2001). Fig. 3 demonstrates clearly how such a correlation can arise when some males

539 have acquired a greater amount of resources and can then allocate to two competing traits.

540 Increased nutrient availability can mask any negative correlation between two traits because

541 individuals have more resources to allocate to both, as seen in the increasingly positive relationship

542 between calling effort and body mass when dietary nutrition increases. We found that the mean

543 change in body mass also increased with diet and with total calling effort, indicating that individuals

544 allocate more resources to both storage and current reproductive effort as acquisition increases.

545 These results have implications for how males are likely to sample their social environment through

546 calling effort: males probably call irregularly early in life, and the extent to which this activity affects

547 the accumulation of resources depends on nutrient intake. The accumulation of body mass with

548   increases in both diet and total calling effort may be due partly to those individuals on higher quality

549   diets having a greater resource intake, and also because of the smaller marginal costs of increased

550   advertisement to higher condition males, as predicted by models of sexual selection under

551   condition-dependence (Grafen 1990; Rowe & Houle 1996). These findings mirror interspecific (Judge

552   *et al.* 2008) and intraspecific (Hunt *et al.* 2004a) differences in the covariance between expensive

553   traits under contrasting conditions, although it is not clear the extent to which our results are related

554   to selection in the lab rather than in the wild. We hope to see more studies in more taxa that directly

555   assess the storage and use of multiple energy forms over time – particularly in the face of varying

556   resource acquisition regimes, and with varying reproductive opportunities.

557   **SOCIALLY MEDIATED PLASTICITY IN REPRODUCTIVE EFFORT**

558   Male decorated crickets responded strongly to female presence, with significant increases in both

559   the likelihood and the time spent calling. To date, most work on male responses to the social

560   environment in crickets have focused on adaptive plasticity for anticipating future conditions

561   (Kasumovic & Brooks 2011): manipulations of juveniles' perceived density and future competition

562   can affect adult life history traits (*T. oceanicus,* Bailey et al. 2010; *T. commodus,* Kasumovic et al.

563   2012a,b). Manipulations of the social environment in adult crickets have thus far been less common.

564   Callander et al (2013) measured the calling effort of male Australian black field crickets (*T.

565   commodus*) that were either kept isolated or housed with a rival male after reaching maturity.

566   However, all males in that study were isolated for the measurement period itself, which might

567   explain the lack of behavioural plasticity across treatments (Callander *et al.* 2013). The extreme

568   phenotypic flexibility of behavioural traits means individuals can adjust trait expression rapidly in

569   response to social cues (Ghalambor, Angeloni & Carroll 2010; Bretman *et al.* 2011); indeed, female

570   presence has previously been shown to affect the frequency and timing of cricket calling (Simmons

571   1986; Burpee & Sakaluk 1993; although see Bertram, Orozco & Bellani 2004). Storage of resources in

572   'quick-release' form as sugars (carbohydrate and glycogen) may provide male crickets the flexibility

573 to signal more effectively in the presence of females, explaining the increased calling effort by males

574 on higher-quality diets during our female-access treatment period.

575 **HOW STRONG IS THE EVIDENCE FOR ADAPTIVE PLASTICITY IN AGE-SPECIFIC REPRODUCTIVE**

576 **EFFORT?**

577 An unresolved question from our study relates to the decrease in calling effort over the second week

578 due to greater previous exposure to females (Fig. 5). Early theoretical models concluded that

579 reproductive investment should increase with age (Williams 1966), but recent studies using game

580 theory and life history theory paint a more complex picture (Kokko 1997, 1998; Lindström *et al.*

581 2009). Kokko (1998) illustrated three phenomena that can prevent positive correlations between life

582 history traits: 1) trade-offs acting over lifetimes, such that fitness benefits accrued early in life

583 outweigh late life fitness (Hansen & Price 1995); 2) increasing marginal gains in fecundity could

584 favour decreased survival and intense sexual advertisement for high-quality males; and/or, 3)

585 suboptimal allocation. The last point is of particular interest: if individuals can plastically vary trait

586 expression in heterogeneous environments, and trait expression is under selection, shouldn't we

587 generally expect adaptive plasticity for continuous traits (Scheiner & Holt 2012)? One explanation for

588 suboptimal allocation is that the genetic and regulatory machinery required for controlling allocation

589 across traits and over time is expensive to maintain, and may not confer a sufficiently high selection

590 advantage over a simpler set of rules: spend resources if you have them, and if not, focus on

591 acquiring more resources instead. The differences we find in individuals that signal at high and low

592 levels early in life may be primarily determined by budget constraints, as opposed to adaptive

593 perception of future opportunities.

594

595 **AUTHOR CONTRIBUTIONS**

596   TMH, LFB and JH conceived the ideas and designed the experiment; JR advised on design and

597   created the artificial diets; TMH performed the experiment and collected the data; KFH performed

598   the energy storage estimation assays; TMH analysed the data; TMH, LFB and JH led the writing of the

599   manuscript. All authors contributed critically to manuscript drafts and gave final approval for

600   publication.

601

609

610   **DATA ARCHIVING**

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

612

613 **LITERATURE CITED**

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Figures

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

818 Figure 2: The effects of diet treatment (x-axis) and day of measurement (equivalent to male age

819 post-eclosion; modelled as a continuous variable and shown here as distinct panels) on male sexual

820 advertisement during the first week post-eclosion. (a) The likelihood of male calling, with fitted

821 regression line taken from coefficients from the zero-altered part of the daily calling analysis; (b) the

822 amount of calling effort (plotted on log-scale y-axis), given a male called, with fitted regression line

823 from the Poisson part of the daily calling analysis. The first day post-eclosion was excluded from the

824 analysis as no males called.

825 Figure 3: The relationship between total calling effort over the first week post-eclosion (log-

826 transformed) and the change in scaled mass index (SMI) during this period, plotted separately for

827 each diet treatment (note that diet was modelled as a continuous predictor). Lines are predicted

828 slopes from a linear regression model, and shaded areas represent 95% confidence intervals.

829 Figure 4: The partial effect of day of measurement (equivalent to male age post-eclosion) on male

830 sexual advertisement during week 2, plotted separately for whether a female was absent or present

831 on the day of measurement. (a) The likelihood of male calling, with fitted regression line taken from

832 coefficients from the zero-altered part of the daily calling analysis; (b) the amount of calling effort

833 (plotted on log-scale y-axis), given a male called, with fitted regression line from the Poisson part of

834 the daily calling analysis. Measurements from males fed 12% nutrition diet have been excluded.

835 Figure 5: 'Small multiples' show how the relationship between diet and daily calling effort changes as

836 a function of day and female access treatment. Each individual panel plots the predicted slope from

837 the Poisson part of our MCMCglmm ZAP model of daily calling effort against dietary nutrition (24-

838 84%). Here we present a subset of panels, showing how the effect of diet on calling effort (plotted

839 on log-scale y-axis) changes from the first to last day of the female access treatment period (male

840   ages of 8 and 14 days post-eclosion, overall horizontal axis), among males that had either no access

841   to females (lower panels) or daily access to females (upper panels). See text for model details; Figure

842   S1 presents the complete set of panels for all combinations of day and female availability treatment.

843   Figure 6: Predicted quadratic slopes from a linear regression model show the effect of diet

844   treatment on the change in individual scaled mass index (SMI) over week 2, separately for each

845   female access treatment. All males on the lowest nutrition (12%) diet died before the end of the

846   week and were thus excluded; we also excluded males that cannibalised females. Shaded areas

847   represent 95% confidence intervals. Values for the change in SMI have been corrected for the effect

848   of regression to the mean.

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850

Tables

852 Table 1: MCMCglmm zero-altered Poisson (ZAP) analysis of male nightly calling effort over the first

853 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	(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)	

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856 Table 2: Linear regression models showing the effect of diet over the first week post-eclosion on (a)

857 individual changes in body mass, as scaled mass index (SMI), and (b) approximated changes in four

858 different energy reserve forms. Values representing changes in carbohydrate, glycogen and lipid

859 were log-transformed prior to analysis.

	Response	Predictor	df	t	Estimate ± S.E.	P
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

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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
Log week 1 total calling effort × diet	2.88	0.0013 ± 0.0004	0.004

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867 Table 4: MCMCglmm zero-altered Poisson (ZAP) analysis of male nightly calling over the second

868 week post-eclosion. Males fed the 12% nutrition diet were excluded as they all died prior to the end

869 of the week. Also excluded were males that cannibalised a female during the female-access

870 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)	

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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

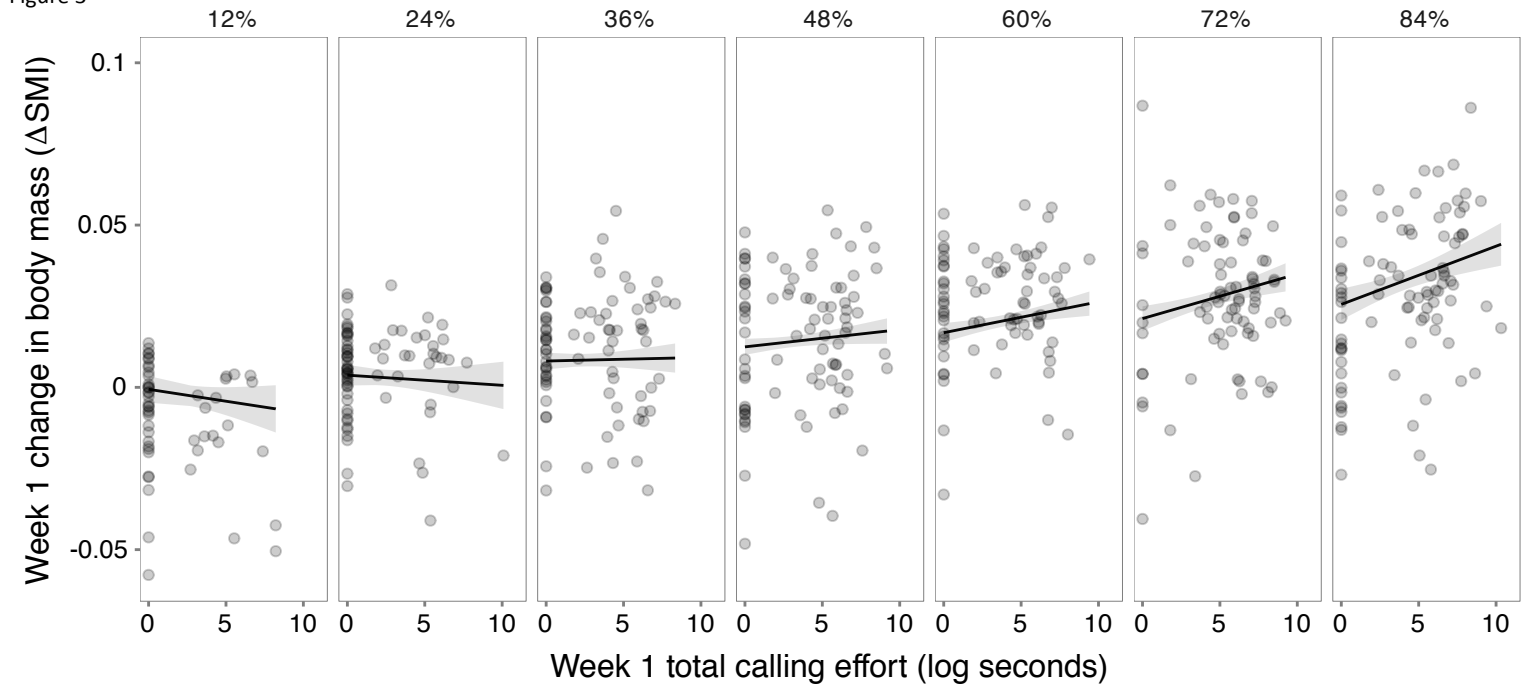|	t	Estimate ± S.E.	*P*
(Intercept)	1.34	0.0019 ± 0.0014	0.182
Diet	3.5	0.0031 ± 0.0008	<0.001
Female-access	0.09	0.0001 ± 0.0003	0.927
Diet^2	-3.72	-0.0011 ± 0.0003	<0.001
Diet × Female-access	-2.16	-0.0004 ± 0.0002	0.032

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Figure 1

Figure 2

Figure 3

Week 1 change in body mass (ΔSMI) vs. Week 1 total calling effort (log seconds), across panels labelled 12%, 24%, 36%, 48%, 60%, 72%, 84%.

Figure 4

(a)

Female absent | Female present

Likelihood of calling

(b)

Daily calling effort (seconds)

Age at measurement (days)

Figure 5

Figure 6