1   **Solitary bee larvae prioritize carbohydrate over protein in parentally provided pollen**

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3   Alexander J. Austin[1,2†] and James D. J. Gilbert[1]

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5   [1]Department of Biological and Marine Sciences, University of Hull, UK

6   [2]Strategy & Environment, Ku-ring-gai Council, NSW, Australia

7   †To whom correspondence should be addressed. Email: alexander.j.austin17@gmail.com

8

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16

17   **Author Contributions**

18   JDJG and AA conceived and designed the study. AA gathered all data. Both authors analysed the

19   data, and wrote and edited the manuscript.

20

21   **Data Accessibility**

22   All data in this study will be made available upon acceptance via a digital repository such as

23   DRYAD, Zenodo, Open Science Framework etc.

24

25   **Abstract**

26   1. Most organisms must regulate their nutritional intake in an environment full of complex
27   food choices. While this process is well understood for self-sufficient organisms,
28   dependent offspring, such as bee larvae, in practice have limited food choices because
29   food is provided by parents. Nutrient balancing may therefore be achieved by offspring,
30   by parents on offspring's behalf, or by both, whether cooperatively or in conflict.

31   2. We used the Geometric Framework to investigate the capacity of dependent larval mason
32   bees (*Osmia bicornis*) to regulate their intake of protein and carbohydrate. Female *Osmia*
33   seal eggs individually inside cells they have provisioned with pollen, and have no contact
34   with developing offspring, allowing offspring choices to be studied in isolation.
35   Herbivorous insect larvae are typically expected to balance protein and carbohydrate to
36   maximise growth and reproduction.

37   3. Contrary to prediction, carbohydrate and not protein mediated both growth and survival
38   to pupation. Accordingly, larvae prioritised maintaining a constant intake of carbohydrate
39   and self-selected a relatively carbohydrate biased diet compared to other hymenopterans,
40   while tolerating wide excesses and deficiencies of protein, rendering them potentially
41   vulnerable to dietary change or manipulation. Reasons for prioritising carbohydrate may
42   include (1) the relative abundance of protein in their normal pollen diet, (2) the relative
43   paucity of nectar in parental provisions making carbohydrate a scarce resource, or (3) the
44   requirement for diapause for all *O. bicornis* larvae. Larvae were intolerant of moderate
45   dietary dilution, likely reflecting an evolutionary history of nutrient-dense food.

46   4. Our results demonstrate that dependent offspring can remain active participants in
47   balancing their own nutrients even when sedentary, and, moreover, even in mass
48   provisioning systems where parents and offspring have no physical contact. Research
49   should now focus on whether and how evolutionary interests of parent and dependent
50   offspring coincide or conflict with respect to food composition, and the implications for
51   species' resilience to changing environments.

52

53

54   Keywords: pollination, foraging ecology, agriculture, nutritional geometry, limiting nutrient,
55   diapause, ecological trap, environmental change, bee health

56

57 **Introduction**

58   Most animals manage their nutrient intake by combining nutritionally different foods

59 (Simpson & Raubenheimer 2012). However, the importance of this ability depends upon the

60 nutritional variability of the animals' typical food (Despland & Noseworthy 2006;

61 Raubenheimer, Simpson & Mayntz 2009). Extreme specialists, for example, can lose the

62 capacity to regulate nutrition (Warbrick-Smith *et al.* 2009; Poissonnier *et al.* 2018). One way in

63 which organisms can experience limited nutritional choice is if they are dependent upon others

64 for nutrition, or "alloregulation" (Lihoreau *et al.* 2014), such as dependent offspring of altricial

65 birds, human toddlers, and many larval insects. Under these circumstances, by what rules

66 offspring regulate their own consumption should depend upon provisioning rules of parents. On

67 the one hand, offspring tend to have different requirements from parents (Harper & Turner 2000;

68 Michaelsen *et al.* 2003) - particularly for protein, given their elevated rates of somatic growth

69 and development. Accordingly, parents often make different nutritional choices for their

70 offspring versus when foraging for themselves (Royama 1970; Dussutour & Simpson 2009; Burt

71 & Amin 2014). For example, granivorous birds usually provision young with insects, rather than

72 the seed diets of adults, to fulfil protein requirements (Wiens & Johnston 2012). If parents

73 alloregulate offspring nutrition tightly, then offspring should have no need for self regulation,

74 like extreme specialists (Poissonnier *et al.* 2018). On the other hand, parents may provide

75 suboptimal nutrition for offspring - either through inefficiency (e.g. Seidelmann 2006), or if

76 parents' and offspring's evolutionary interests do not coincide (Trivers 1974). Here, offspring

77 may be able to use nutritional regulation to mitigate costs arising from their parents' nutritional

78 choices. While there has been much research into evolutionary compromises involving offspring

79 solicitation and corresponding parental responses (e.g. Smiseth, Wright & Kölliker 2008), less is

80 known about whether or how offspring may exert control by discriminating among parental

81 provisions.

82   The Geometric Framework for Nutrition (GF) allows us to investigate foraging decisions

83 made by animals in multi-dimensional "nutrient space" (Simpson & Raubenheimer 1993). The

84 GF can be used to determine animals' nutritional choices relative to their "intake target" - the

85 optimal amount and balance of multiple macronutrients - as well as their "rule of compromise"

86 that governs their choices when restricted to suboptimal food (Raubenheimer & Simpson 1999b).

87 The GF has provided insights into the nutritional ecology of a broad range of taxa (reviewed in

88 Simpson & Raubenheimer 2012). Its application to dependent offspring, though, has typically

89 been as part of studies of social insect systems (e.g. Helm *et al.* 2017) and studies have often

90 inferred offspring requirements indirectly from patterns of alloparental feeding in studies more

91 broadly focused on adult foraging (see Dussutour & Simpson 2009; Cook *et al.* 2010; Vaudo *et*

92 *al.* 2016). In such systems, multiple adults normally contact offspring, progressively feeding and

93 adjusting nutrition in response to feedback (Field 2005; Schmickl & Karsai 2017), making the

94 responses of individual larvae difficult both to follow and interpret.

95   In solitary bees, by contrast, typically females provision offspring individually with a

96 pollen ball before sealing the cell and leaving. This behaviour makes solitary bees an ideal,

97 manipulable model for directly studying the nutrition of dependent larvae (Strohm *et al.* 2002)

98 independently of provisioning decisions made by parents. Larvae of bees, like most aculeate

99 hymenopterans, rely on parents or alloparents for nutrition (Field 2005). Nutritional requirements

100 for bee adults and offspring differ, often radically (Weeks *et al.* 2004; Filipiak 2019); adults

101 primarily feed on carbohydrate-rich nectar (although see Cane 2016) while larvae feed mostly on

102 protein-rich pollen (Filipiak 2019). Solitary bees, along with most other hymenopterans and

103 many other parental insects, typically have a simple one-to-one parent-offspring relationship

104 whereby parents "mass provision" their young, providing a finite, fixed-mass food provision, and

105 have no contact with their young during development (Costa 2006). Such systems are almost

106 unstudied in a rigorous nutritional context (but see e.g. Roulston & Cane 2002). In these species,

107 there is no opportunity for parents to adjust nutrition according to offspring feedback, and the

108 larva must therefore make the best of what it is given. It may be that offspring regulate their own

109 nutrition to compensate for variation, as in more independent insect larvae (Lee *et al.* 2002), or

110 possibly to mitigate costs imposed by parents. Alternatively, they may have lost this capacity,

111 like extreme specialists (Warbrick-Smith *et al.* 2009; Poissonnier *et al.* 2018). We know very

112 little about how bee larvae deal with variable nutrition (but see Helm *et al.* 2017) - a knowledge

113 gap with potentially important consequences, considering the proposed link between nutritional

114 stress and bee declines (Roulston & Goodell 2011; Goulson *et al.* 2015).

115   In this study, we used a commercially important solitary bee species, *Osmia bicornis*, to

116 investigate how dependent larvae cope with varying nutrition, and whether they can regulate

117 their own intake. *O. bicornis* are pollen generalists (Falk 2015) and the solid, roughly spherical

118 pollen balls that parents provide to offspring are variable in species composition (Haider *et al.*

119   2014). Although *O.bicornis* larvae are sedentary, they are capable of limited movement, in

120   principle allowing them to preferentially consume specific parts of a fixed provision (note that

121   other invertebrates are also capable of extracting and consuming preferred nutrients from

122   nutritionally complex food items (Mayntz *et al.* 2005). The species is entirely solitary with no

123   known tendency for offspring to "help at the nest" as in other bees (Hogendoorn & Velthuis

124   1993; Rehan *et al.* 2014) so there is no reason to believe mothers would alter offspring nutrition

125   to force them to help, as in other systems (Lawson, Helmreich & Rehan 2017) and therefore no

126   obvious potential for parent-offspring conflict over offspring nutrition. Natural variation in

127   pollen ball nutrient content is largely unquantified (although see Budde & Lunau 2007), so there

128   is no prior expectation about the capacity of larvae to regulate their consumption. We used a

129   classic GF design (e.g. Lee *et al.* 2008), focusing on protein and carbohydrate, with two

130   experimental phases. In the first "no-choice" phase we raised *O. bicornis* larvae on fixed diets of

131   differing protein to carbohydrate ratios to determine their rules of compromise and the diet

132   composition that maximised fitness. In a second "choice" phase, we then provided larvae with

133   targeted choices between sets of two imbalanced diets that differed in their protein:carbohydrate

134   ratios to determine whether larvae defend an intake target. Given the central role of protein in

135   growth of insect larvae (Scriber & Slansky 1981; Behmer 2009), and following Hunt & Nalepa's

136   (1994) exhortation to "follow the protein", we predicted that (1) protein would be a key driver of

137   fitness in larval *O. bicornis*, (2) larvae would accordingly aim for a relatively protein-biased

138   intake target, and (3) larvae would prioritize regulating intake of protein over carbohydrate.

139

140   **Methods**

141

142   *Study organism*

143   *Osmia bicornis* is a common, cavity-nesting solitary bee (Falk 2015), and a commercially

144   important pollinator (Jauker *et al.* 2012). *O. bicornis* larvae were obtained as diapausing adults in

145   cocoons (Mauerbienen®). These were released at the nesting site at the University of Hull in

146   April 2017, and emerging adults allowed to breed. Early trials revealed that fresh eggs and

147   newly emerged larvae were too fragile for manipulation. Therefore, newly emerged larvae were

148   left alone for two days before we transferred them to a single-occupancy nest and assigned each

149   to an experimental treatment. Details of nesting apparatus and monitoring protocols are available

150   in the supplementary methods.

151

152   *Diet Formulation*

153   Existing artificial diet protocols for solitary bees have met with limited success in terms

154   of larval survival (Nelson, Roberts & Stephen 1972; Fichter, Stephen & Vandenberg 1981). We

155   used six diets, consisting of three different protein:carbohydrate (P:C) ratios (Diet A = 1:1.2,

156   Diet B = 1:2.3 & Diet C = 1:3.4) and two total macronutrient concentrations (concentration 1 =

157   90% nutrients, 10% diluent, or concentration 2 = 70% nutrients, 30% diluent; see table S1). Diet

158   ratios were chosen based on a combination of the nutrient ratios in honeybee-collected pollen

159   loads and published data for protein content of *O. bicornis* pollen balls (Budde & Lunau 2007).

160   Diets were diluted with sporopollenin, the primary constituent of the exine of pollen (Mackenzie

161   *et al.* 2015), an extraordinarily stable natural polymer. Sporopollenin is a novel dietary diluent

162   for bees; its suitability has been demonstrated in a separate study (Tainsh *et al.* 2020). For a more

163   detailed description of sporopollenin and its preparation, see supplementary methods.

164

165   *Experiment 1: No-choice phase*

166   Two-day-old larvae, randomized by parentage, were allocated to one of 6 treatments

167   corresponding to our 6 artificial diets (n = 20/treatment). Provisions were made to resemble the

168   size of natural provisions (mean initial artificial provision weight = 0.323g +/- 0.034g). Once

169   provisioned, larvae were placed in an incubation chamber (Gallenkamp, IH-270) at 23°C and

170   80% RH. Provisions were replaced weekly to avoid desiccation and mould formation, or when

171   fully consumed by larvae, ensuring the diet was always available in excess. Weight of provision

172   consumed was recorded upon provision replacement. A "water control" group, containing

173   artificial diets but no larvae, was used to track water loss from the diets, going through the same

174   weighing regime as above with weight loss recorded at each swap. Nests were checked daily for

175   mortality. Cocoon weight was recorded at the completion of pupation.

176

177   *Experiment 2: Choice phase*

178   In the choice experiment, 32 two-day-old larvae of mixed parentage were randomly

179   divided among four treatments. Treatments consisted of strategic pairwise combinations (see Fig

180   1; Table 1) of four possible diets: A1 (1P:1.2C, 90%), A2 (1P:1.2C, 70%), C1 (1P:3.4C, 90%),

181   C2 (1P:3.4C, 70%). Because *O. bicornis* larvae are sedentary and receive a single provision, it is

182   not biologically appropriate to present choices between two diets simultaneously. Therefore,

183   choices were offered temporally by swapping the provision every other day, presenting one diet

184   at a time. This required the larvae to differentially feed over time to compensate for temporal

185   imbalance, in order to converge on an intake target (see e.g. Raubenheimer & Jones 2006). All

186   larvae were kept on the same treatment from two days post-hatching up to pupation, whereupon

187   diet replenishment ceased. The diet that the larvae would be fed first was randomly assigned via

188   coin toss prior to the experiment.

189

190   *Statistical Analysis*

191   All analyses were conducted in R version 3.4.2 (R Core Team, 2017). For the no-choice

192   experiment, we calculated total nutrients consumed (protein and carbohydrate) from raw diet

193   consumption data for each swap, adjusted for water loss and dilution. Values were then summed

194   for each larva.

195   To investigate consumption rules, including rules of compromise, we first asked whether

196   diet ratio and concentration affected consumption of (a) the total provision, (b) protein, or (c)

197   carbohydrate, using models of each respective variable with "ratio" and "concentration" as

198   predictors. Rules of compromise can include nonlinear effects, particularly curves around the

199   intake target (Simpson & Raubenheimer 1993). To account for potentially curvilinear

200   relationships we also added ratio^2 as a predictor, as well as two-way interactions between all

201   predictors.

202   To assess fitness consequences of macronutrient consumption, we analysed cocoon

203   weight at pupation and survival to pupation. For both analyses, to analyse potentially nonlinear

204   effects of nutrient consumption upon fitness, we used polynomial regression, fitting both first-

205   and second-order polynomial terms for "protein consumed [P]" and "carbohydrate consumed

206   [C]". We analysed cocoon weight using a linear model with "cocoon weight" as a response. The

207   full model contained linear (P and C) and quadratic effects for both nutrients (P^2 and C^2) and

208   their interaction (P $\times$ C), as well as diet concentration (high or low), and two-way interactions

209   between concentration and nutrients (conc $\times$ P, conc $\times$ P^2, conc $\times$ C, conc $\times$ C^2). We used

210   standard diagnostics to check the fit of models, and used a reverse stepwise process to determine

211   the minimal model, at each step dropping the least significant term until the model contained

212   only significant terms. To analyse survival, we used parametric survival analysis in the *survival*

213   package in R and fitted the same full model as described above. We assessed model fit

214   graphically by inspecting the Kaplan-Meier estimates of the residuals against the assumed

215   Weibull distribution. Again we used reverse stepwise selection to determine the minimal model,

216   comparing models with likelihood ratio tests against a chi squared distribution. To visualise these

217   fitness effects, we calculated response surfaces for cocoon weight and survival, and visualised

218   them using non-parametric thin-plate splines.

219   In the choice experiment, the mean final consumption of each nutrient was investigated

220   using linear models with diet combination, dilution and their interaction as predictors, and using

221   Tukey's post hoc tests to compare individual treatments against each other. Under a null

222   expectation we would expect larvae to eat randomly from each diet (Fig 1). Thus, for each larva

223   we calculated the deviation from this null expectation. We then tested whether these values

224   systematically departed from zero for protein and carbohydrate, and whether these departures

225   from random consumption differed by treatment group. We used a linear model with "deviation

226   from random consumption" as the response variable and "treatment group" as a predictor.

227   Larvae that died pre-pupation were not used in the calculation of the mean protein and

228   carbohydrate consumption for diets in either experiment, or for cocoon weight, but were used in

229   analyses involving survival.

230

231

232   **Results**

233   *No-choice phase*

234   Dietary P:C ratio had a significant effect on the overall amount of provision consumed,

235   with larvae consuming more provision on high P:C ratio diets ($F_{1,78}=21.55$, $p<0.0001$). Total

236   consumption was also affected by diet concentration ($F_{1,78}=14.03$, $p<0.001$); larvae on less

237   concentrated diets consumed more provision, indicating compensatory feeding. The quadratic

238   term was not significant (Fig. 2a; Table S2a).

239   Dietary P:C ratio had a strong effect on the total amount of P eaten ($F_{1,79}=146.93$,

240    $p<0.0001$); more protein was eaten by larvae raised on the higher P:C diets (Fig. 3). Diet

241 concentration had no effect on the amount of P consumed; neither was there a ratio:concentration
242 interaction, nor a quadratic effect of ratio (Table S2b).
243   In contrast, larvae consumed similar amounts of C across all diets, with neither
244 concentration nor dietary P:C ratio (linear or quadratic) having an influence on the amount of C
245 consumed (Table S2c). A mean of 0.23 ± 0.01 g of C was consumed by (surviving) larvae across
246 all diet treatments (Fig. 3).
247   Cocoon weight varied differently with macronutrient intake depending on the overall
248 concentration of the diet (carbohydrate $\times$ conc interaction, $F_{1,72}=6.50$, p=0.01; protein $\times$ conc
249 interaction, $F_{1,72}=4.82$, p=0.03). At 90% nutrient density, cocoon weight was correlated
250 positively with the amount of carbohydrate consumed, and negatively with protein (Fig. 4a). For
251 our range of diets, the greatest weights were obtained by larvae that ate above approx. 0.3g C and
252 below 0.15g P. In contrast, at 70% nutrient density, cocoons were lower in weight than on the
253 90% diets, and were fairly uniform in weight irrespective of macronutrient intake (Fig 4b). No
254 quadratic effects were observed, nor interactions involving quadratic effects, meaning that we
255 did not identify an optimal amount of P or C that maximised cocoon weight within the range of
256 diets we used (Table S3a).
257   The relationship between survival and nutrition similarly depended upon dietary
258 concentration (carbohydrate $\times$ conc interaction: $\chi_1=6.50$, p=0.01). Survival of larvae fed diets at
259 90% concentration depended primarily upon carbohydrate consumption (Fig 5a). Those larvae
260 that consumed high amounts of carbohydrate saw the highest survival irrespective of how much
261 protein was consumed. At lower levels of carbohydrate, interestingly, protein weakly mediated
262 survival (protein $\times$ carbohydrate interaction: $\chi_1=-4.88$, p=0.046). Survival of larvae raised on the
263 more dilute diets was much lower, and was not substantially affected by intake of P or C (Fig
264 5b). Again, there were no significant quadratic terms, whether as main effects or as part of
265 interactions (Table S3b).
266
267 *Choice phase*
268   We found no evidence of larvae defending a common intake target *sensu stricto*
269 *(Raubenheimer & Simpson 1993, 1999b)*, i.e. a common ratio *and* amount of nutrients
270 consumed, which would have been evident as all groups clustering at a common point in nutrient
271 space in Fig 6a. Nevertheless, consumption deviated from random so as to converge upon a

272   target P:C ratio (see e.g. Deans, Sword & Behmer 2019) represented by a common line, or

273   "nutritional rail", of approx. 1:1.8 (Fig. 6a). The amount of protein consumed by larvae was

274   significantly affected by diet combination: more protein was consumed by individuals offered

275   diet combinations that were overall more concentrated ($F_{3,23}=7.43$, $p<0.01$, Fig 6a; Table S4a).

276   Similarly, carbohydrate consumption was significantly affected by diet combination ($F_{3,23}=4.58$,

277    $p=0.01$, Fig 6a; Table S4b). Unlike with protein, though, this pattern appeared to be driven by the

278   diets at the extreme; only the most concentrated diet pair (C1A1) differed from the least

279   concentrated pair (C2A2; Fig 6a); other pairwise comparisons were not significant (Table S4).

280   Despite the lack of a common intake target, larvae were not consuming diets at random

281   (Fig 6b, Table S4c, d). For both carbohydrate and protein we saw differences in consumption

282   from what would have been expected for each larva based on random consumption, and this

283   effect was dependent on the specific set of diet choices (protein, $F_{4,20}=19.67$, $p<0.001$;

284   carbohydrate, $F_{4,20}=51.65$, $p<0.001$). When visualised as the amounts of protein and

285   carbohydrate consumed during each 48h treatment period (Fig. 7), it is clear that larvae were

286   achieving a degree of homeostasis in carbohydrate consumption (Fig 7b) compared to what

287   would be expected under random consumption of each diet choice (Fig 7a), whereas their

288   consumption of protein (Fig 7d) aligned closely with what would be expected under random

289   consumption (Fig 7c).

290

Discussion

291

292   We found that carbohydrate was positively associated with both body size and survival in

293   *Osmia bicornis* larvae (Figs. 4a, 5a), although within our range of dietary ratios we did not

294   specifically identify an optimum (fitness-maximising peak) in intake for either carbohydrate or

295   protein. Accordingly, given a choice, larvae converged on a relatively carbohydrate-biased

296   protein:carbohydrate ratio of 1:1.8 (Fig. 6a). Moreover, larvae prioritised carbohydrate over

297   protein intake, showing tighter control over carbohydrate consumption than over protein

298   consumption (Fig 7), and they pupated after eating about 0.23 g carbohydrate irrespective of

299   protein and of dietary dilution (Fig 3). Yet this carbohydrate target fell short of the amount of

300   carbohydrate that maximised cocoon weight or survival to pupation (Fig 4a, 5a). Dietary dilution

301   imposed costs upon larvae regardless of nutritional intake, in the form of greater mortality and

302   lower cocoon weights (Figs 4b, 5b). Taken together, these results show that (1) larval *O. bicornis*

303 are at least partially responsible for their own nutritional regulation, and (2) their performance

304 and consumption rules suggest adaptation to a pattern of carbohydrate-limited growth and

305 survival. In what follows, we suggest how and why these patterns in *O. bicornis* depart from

306 expected results based on studies of related organisms, and more generally what these findings

307 suggest about nutritional cooperation and/or conflict between parents and offspring in (mass)

308 provisioning species.

309 Larvae grew and survived best on our highest carbohydrate (i.e. lowest

310 protein:carbohydrate) diets. Accordingly, across our range of diets, larvae maintained a constant

311 carbohydrate intake while tolerating excesses or deficiencies of protein (a "no-interaction" rule

312 of compromise; Raubenheimer & Simpson 1999b) - although it is conceivable that alternative

313 rules of compromise, such as the "equal distance rule" more typically seen in generalist

314 herbivores (Raubenheimer & Simpson 1999b; Behmer 2009), might have been evident over a

315 broader array of diets. Both these patterns are unusual because insect herbivores are generally

316 considered to be limited by protein (e.g. Bernays & Chapman 2007; although see Le Gall &

317 Behmer 2014). In the few existing studies involving larval bees, e.g. honeybees (Helm *et al.*

318 2017), bumblebees (Kraus *et al.* 2019) and sweat bees (Roulston & Cane 2002), protein and not

319 carbohydrate mediated larval growth and/or survival. More broadly, insect larvae often grow and

320 survive best on balanced or moderately high protein:carbohydrate ratios (Roeder & Behmer

321 2014; Rodrigues *et al.* 2015), although low protein:carbohydrate ratios are associated with

322 longevity in adults (e.g. Lee *et al.* 2008). Moreover, animals generally prioritise regulation of the

323 nutrient that is typically limiting in their normal diet, and tolerate variation in nutrients that are

324 abundant (Raubenheimer & Simpson 1999a). Tolerance of wide variation in protein is thus

325 usually seen in predators (e.g. Raubenheimer *et al.* 2007; Kohl, Coogan & Raubenheimer 2015).

326 In contrast, herbivores often regulate protein more tightly than carbohydrate (Lee *et al.* 2002; Le

327 Gall & Behmer 2014; VanOverbeke, Thompson & Redak 2017). It is worth noting that protein

328 *did* weakly mediate survival in our larvae to some extent, although only at low carbohydrate -

329 possibly as a result of switching to protein as an energy source.

330 During the choice phase, when allowed to self-select diets, *O. bicornis* larvae converged

331 on a protein:carbohydrate ratio of 1:1.8. This ratio is considerably more carbohydrate-biased than

332 that preferred by bumblebees foraging on behalf of microcolonies (1:0.25, Vaudo *et al.* 2018;

333 1:0.08, Kraus *et al.* 2019), and (to a lesser extent) than ants foraging for colonies with offspring

334 (1:1.5, Dussutour & Simpson 2009). It is also more carbohydrate-biased than that selected by

335 reproductive, solitary phytophages such as grasshopper adults and lepidopteran larvae (1:0.25 -

336 1:1.4, reviewed in Behmer 2009) and is closer to diets selected by *Drosophila* larvae (1:2,

337 Rodrigues *et al.* 2015). Notably, though, 1:1.8 was more protein-biased than the ratio that we

338 found maximised both cocoon weight and survival (1:3.4), suggesting *O. bicornis* larvae may

339 choose diets that favour other fitness-related quantities (such as reproduction and/or

340 developmental time) over body size/survival, as in *Drosophila* (Lee *et al.* 2008; Rodrigues *et al.*

341 2015). As a cautionary note, the specific source of nutrients may also affect the preferred ratio:

342 for example, adult honeybees exhibited different target P:C ratios when fed different protein

343 sources (Altaye *et al.* 2010). Whether larvae are similarly sensitive is still unknown.

344   Two main features of *O. bicornis'* ecology may help to explain their prioritization of

345 carbohydrate, and their relative preference for this macronutrient, compared to what we know of

346 related taxa. First, the relative paucity of carbohydrate in *O. bicornis'* larval diet may help to

347 explain these findings. Despite being herbivorous, *Osmia* larvae are unlikely to be protein-

348 limited, because pollen is among the most protein-rich of plant tissues (Mattson 1980).

349 Moreover, in *Osmia* specifically, nectar constitutes only a tiny fraction of the pollen ball, less

350 than 4% (Maddocks & Paulus 1987; see Radmacher & Strohm 2010), in contrast to many other

351 bees where nectar is a principal source of carbohydrate for larvae (e.g. Kraus *et al.* 2019). *O.*

352 *bicornis* larvae may therefore be limited more by the amount of digestible carbohydrate within

353 pollen than by dietary protein (see Roulston & Cane 2000). Second, *O. bicornis* is (to our

354 knowledge) the first truly solitary hymenopteran studied under the GF; other studies have

355 concerned individuals likely to become workers of social species. Unlike social hymenopterans,

356 *O. bicornis* offspring are all reproductive and undergo diapause (Fliszkiewicz *et al.* 2012) - both

357 activities dependent on the fat body, where carbohydrate-derived fat is stored (Kawooya & Law

358 1988; Ziegler & Van Antwerpen 2006; Hahn & Denlinger 2007; Wasielewski *et al.* 2013). Thus,

359 *O. bicornis* larvae may have additional requirements for carbohydrate over and above those of

360 developing nonreproductive, nondiapausing hymenopteran workers. These contrasting findings

361 reinforce the idea that bees' nutritional needs may be just as diverse as their ecologies.

362   Although larvae retained the ability to regulate carbohydrate by over- or under-eating

363 protein, they nevertheless coped very poorly with dietary dilution (Fig 4b, 5b), despite displaying

364 compensatory feeding behaviour (Fig 2) that suggests they both detected and responded to such

365   dilution. The dilution was not excessive (70% nutrient density) compared to similar studies

366   offering very highly dilute diets (14%, Raubenheimer & Simpson 1993; 16.8%, Lee,

367   Raubenheimer & Simpson 2004). The locusts and caterpillars in those studies, though, are

368   adapted for diets that vary greatly in nutrient density, beginning dilute and becoming even more

369   dilute over the season (Scriber & Slansky 1981). By contrast, pollen is among the most

370   consistently nutrient-rich parts of a plant (Roulston & Cane 2000) and does not broadly vary in

371   composition over a season (DeGrandi-Hoffman *et al.* 2018). With a normal diet of unadulterated

372   pollen and very little nectar, *Osmia* larvae may have had no need to evolve mechanisms to cope

373   with dilution. In comparison, caterpillars reared on an invariant diet for generations lost the

374   ability both to regulate intake and to cope with dilution (Warbrick-Smith *et al.* 2009). *Osmia*

375   larvae appear to have retained the former capacity, but lost the latter, suggesting a normal diet

376   that is dense in nutrients, but variable in composition.

377   In systems where parents gather food for offspring from the environment, both parents

378   and offspring can be active participants in nutritional regulation. The lack of protein regulation

379   shown by *O. bicornis* larvae highlights the importance of understanding (a) whether mother *O.*

380   *bicornis* adjust protein content of provisions in response to imbalances in the landscape, and (b)

381   whether larvae have physiological adaptations (e.g. post-ingestive processing) for tolerating

382   protein imbalance. Budde & Lunau (2007) found that *O. bicornis* provisions contained about

383   19% protein regardless of pollen species used, suggesting a degree of homeostasis by parents.

384   Yet human activity is reducing floral diversity and quality (Ziska *et al.* 2016; Papanikolaou *et al.*

385   2017). Evidence is mixed concerning whether, in practice, parent bees assess pollen nutrients at

386   the flower (reviewed by Nicholls & Hempel de Ibarra 2016). Both bumblebees and ants balance

387   nutrition on behalf of colonies (Dussutour & Simpson 2009; Vaudo *et al.* 2018), regulating more

388   tightly when foraging for offspring - protein in the case of both taxa (Dussutour & Simpson

389   2009; Kraus *et al.* 2019) and carbohydrate in ants (Dussutour & Simpson 2008; Cook *et al.*

390   2010). On the other hand, protein gathered by honeybees varies passively with landscape usage

391   (while maintaining carbohydrate and lipid; Donkersley *et al.* 2014). Which regulatory strategy

392   *Osmia* parents and larvae collectively pursue may have important implications for their

393   vulnerability to human-induced landscape change, and so should now be a focus for research.

394   Additionally, the ability to discriminate among nutrients provided by parents may be one

395   tool offspring can use to exert some control over their nutrition, even in the absence of contact

396   with parents. *Osmia* parents may provide suboptimal resources simply because of inefficiency in
397   gathering pollen: efficiency drops across the season (Seidelmann 2006) and is lower in smaller-
398   bodied parents (Seidelmann, Ulbrich & Mielenz 2010). Moreover, less efficient parents actively
399   switch to producing male offspring (Seidelmann *et al.* 2010), so male and female offspring may
400   experience different selection for regulation. This is well documented in other groups (e.g.
401   Maklakov *et al.* 2008) and sex differences in larval regulation should now be a focus for
402   research. But it is also well known that the evolutionary interests of parents and offspring
403   frequently differ over how resources should be allocated (Trivers 1974; Crespi & Semeniuk
404   2004; Kilner & Drummond 2007; Haig 2010). The potential for offspring to use nutritional
405   regulation to mitigate parentally imposed costs has been relatively overlooked, as most research
406   to date has focused on parent-offspring conflict over *amount* of parental provisions, despite clear
407   potential for conflict over composition (e.g. in discus fish, Buckley *et al.* 2010). Among
408   primitively social Hymenoptera, some parents actively stunt offspring by restricting provisions
409   (Lawson *et al.* 2017), securing their help by forcing them to become workers (Craig 1983). But
410   the composition of food provided by parents is also critical to offspring fitness (e.g. Roulston &
411   Cane 2002) and in extreme cases caste-determining (Anderson 1984). *O. bicornis* are solitary
412   and lack castes, but this does not preclude parent-offspring disagreement over the optimal
413   balance of offspring nutrition, as in e.g. *Drosophila* (Rodrigues *et al.* 2015).
414   More broadly, understanding the relative roles of offspring (intake regulation and post-
415   ingestive processing) versus parents in nutrient balancing, as well as their evolutionary interests,
416   will be key to understanding the nutritional ecology of species with parental provisioning. Such
417   species include not just bees and other Hymenoptera, but other important ecosystem service
418   providers such as dung beetles (Frank *et al.* 2017) and burying beetles (Hopwood, Moore &
419   Royle 2013), as well as altricial birds (Wiens & Johnston 2012) and even humans (Burt & Amin
420   2014). Alloregulation by parents is not a given; the relative roles and interests of parent and
421   offspring in these groups are likely to reflect species' ecologies. Recent studies have found
422   nutritional mismatches between oviposition sites selected by parents and the nutritional
423   requirements of the offspring that will develop in those sites (Rodrigues *et al.* 2015; Lihoreau *et*
424   *al.* 2016). Parent sweat bees (*Lasioglossum zephyrum*) appear not to regulate protein in larval
425   provisions, despite protein mediating offspring performance (Roulston & Cane 2002). In *O.*
426   *bicornis*, we have shown that offspring retain the ability to regulate their nutritional intake

427 despite all food selection being done by parents whom they never meet. Larvae appeared to pay

428 closest attention to regulating dietary carbohydrate, consistent with this nutrient mediating both

429 growth and survival. Yet protein remains a key requirement for development; key now is to (a)

430 establish the nutritional rules used by parents when provisioning offspring, and whether these

431 coincide with or depart from those employed by larvae, and (b) establish specifically how protein

432 balance is achieved, and whether parents or larvae carry that responsibility.

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673   **Tables & Figures**

674

675   **Table 1. Sample sizes for each diet combination used for choice phase (allocated by random**

676   **coin toss). "Order" refers to diet order - e.g. for A1C1, Order 1 would receive A1 first**

677   **whereas Order 2 would receive C1 first, determined by coin toss. Surviving larvae are in**

678   **parentheses.**

	Order 1	Order 2	Total
A1C1	1 (1)	6 (5)	7 (6)
A1C2	5 (5)	4 (3)	9 (8)
A2C1	3 (2)	5 (5)	8 (7)
A2C2	5 (2)	3 (1)	8 (3)

679

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681

682   **Figure captions**

683

684   **Figure 1. (a)** Expected protein and carbohydrate consumption if larvae ate indiscriminately

685   between two diets. Diet choices are pairwise combinations of diets A1, A2, C1 and C2, which

686   each contain protein and carbohydrate at different ratios and concentrations. Solid lines represent

687   P:C ratios; black points represent actual nutrient content of each diet, which depends upon

688   dilution as well as P:C ratio. Red points represent expected consumption if larvae eat randomly

689   (i.e. equally) from each of a choice of two diets (choices denoted by the red point labels). **(b)**

690   schematic describing how larvae were assigned to each diet grouping. Coloured arrows show the

691   period in days that each larva was fed a particular diet.

692

693   **Figure 2.** Amount of provision in grams consumed by larvae raised on the 3 different P:C ratio

694   artificial diets at the 2 different macronutrient concentrations (90% and 70% macronutrient

695   content).

696

697   **Figure 3**. Mean total (+/- 1 SE) amount of P and C consumed in grams by larvae on each

698   diet before pupation. Solid lines and letters represent three P:C ratios (A = 1:1.2, B = 1:2.3,

699   C = 1:3.4). Numbers following letters denote diet concentration (1 = 90%, 2 = 70%). Dotted

700   lines show global mean consumption of each nutrient.

701

702   **Figure 4.** Effects of P and C consumption upon cocoon weight (g) in larvae fed diets at (a)

703   90% and (b) 70% nutrient density. Transition from blue to red indicates heavier cocoons.

704   For context, mean total consumption of P and C for each diet is plotted (white points; data

705   as in Fig. 2) alongside raw data (grey points). Solid lines and letters represent three P:C

706   ratios (A = 1:1.2, B = 1:2.3, C = 1:3.4).

707

708   **Figure 5.** Effects of P and C consumption upon estimated survival time (colour) in larvae

709   fed diets at (a) 90% and (b) 70% nutrient density. Transition from blue to red indicates

710   longer survival. Black points, dead larvae; white points, larvae surviving to pupation. For

711   context, mean total consumption of P and C for each diet is plotted (large white points; data

712   as in Fig. 2). Solid lines and letters represent three P:C ratios (A = 1:1.2, B = 1:2.3, C = 1:3.4).

713

714 **Figure 6. (a)** The mean (+/- SE) amount of protein (P) and carbohydrate (C) eaten by

715 larvae in the choice experiment. Each point label denotes a choice of two diets, one A and

716 one C; black labels show observed intake, red labels show expected intake under random

717 consumption. Letters represent diet P:C ratio (A = 1:1.2, C = 1:3.4); numbers represent diet

718 concentration (1 = 90%, 2 = 70%), hence, for example, "A2C1" represents the pairing of

719 diet A2 with diet C1. Solid lines represent dietary P:C ratios (Top line = Diet C, Bottom line

720 = Diet A). Dashed red line shows expected average P:C ratio based on random consumption.

721 Dashed black line shows average P:C ratio of observed intake across larvae. (b) Deviation

722 from random intake of protein and carbohydrate for larvae in different treatment groups

723 during the choice phase. Treatment groups are given in order of overall diet concentration.

724 Bars with similar letters displayed above or below are not statistically significantly

725 different (Tukey's post-hoc comparisons).

726

727 **Figure 7.** (a, c) Mean expected intake over successive diet swaps assuming random

728 consumption of diets (+/- 1 SE, inner ribbon, and SD, outer ribbon) of protein (red, lower

729 ribbons) and carbohydrate (blue, upper ribbons), irrespective of the concentrations of the

730 diet choices, for larvae starting on (a) diet A or (c) diet C. (b, d) Nutrient intake actually

731 observed for larvae starting on (b) diet A or (d) diet C (+/- 1 SE, inner ribbon, and SD, outer

732 ribbon). For details of calculations of expected consumption, see text. Swap 11 lacks

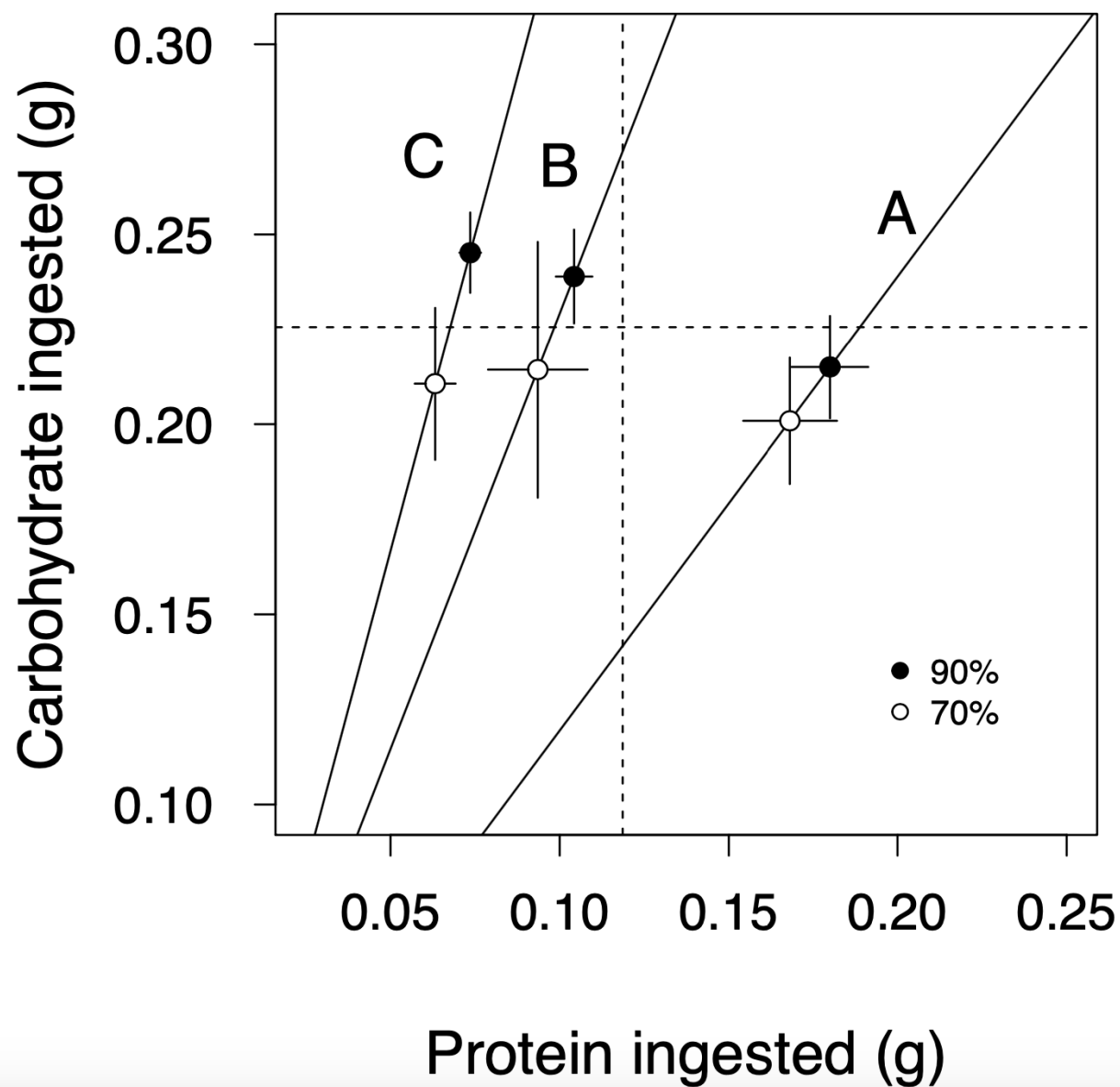733 confidence intervals because only one larva in each group reached this stage.
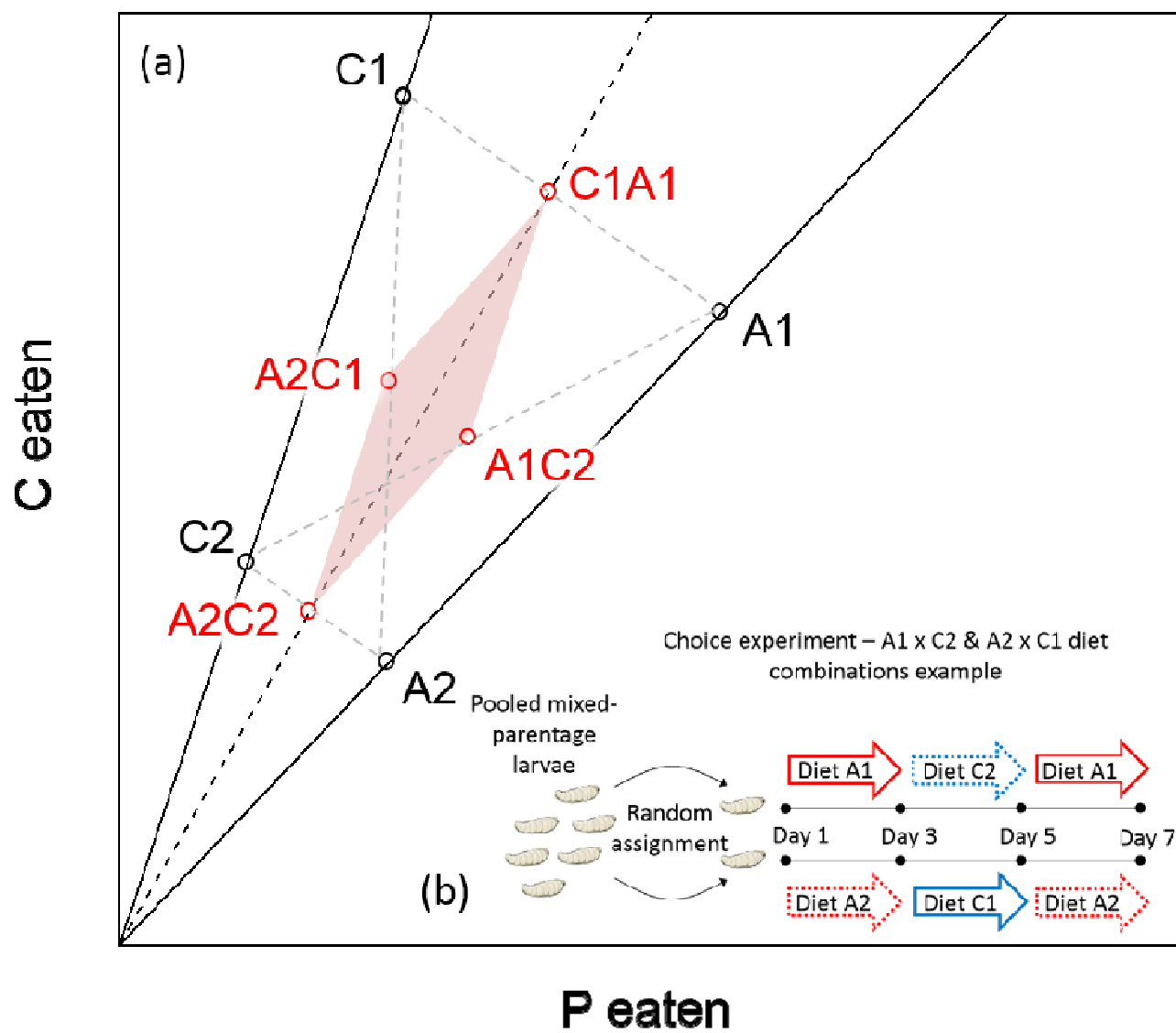
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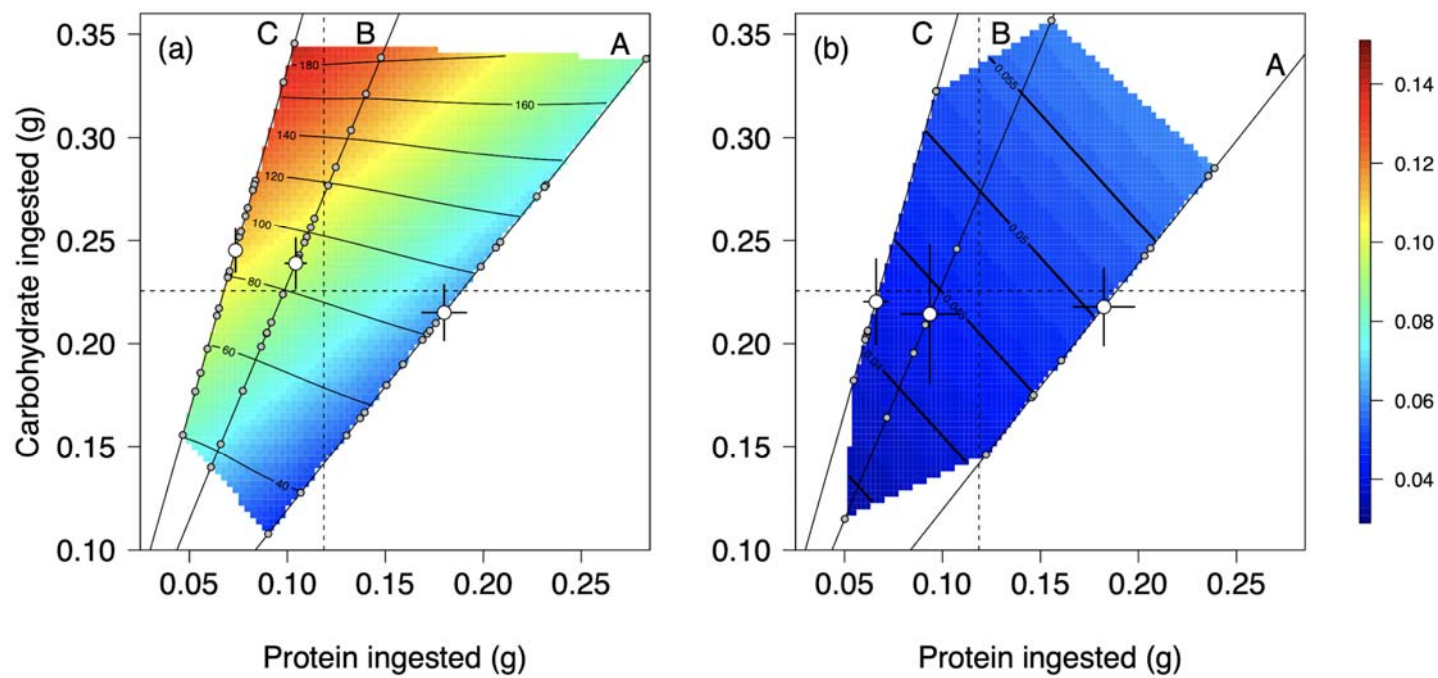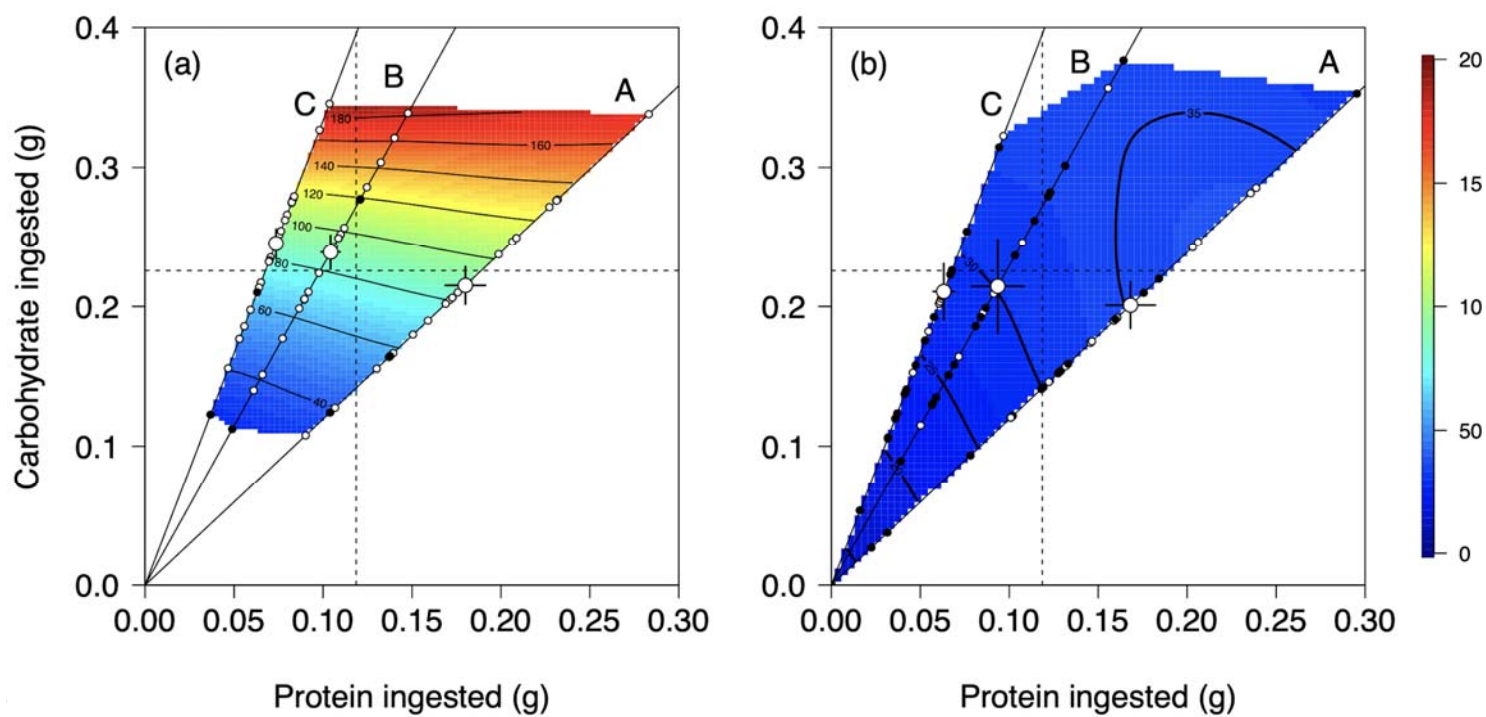735   Fig. 1.
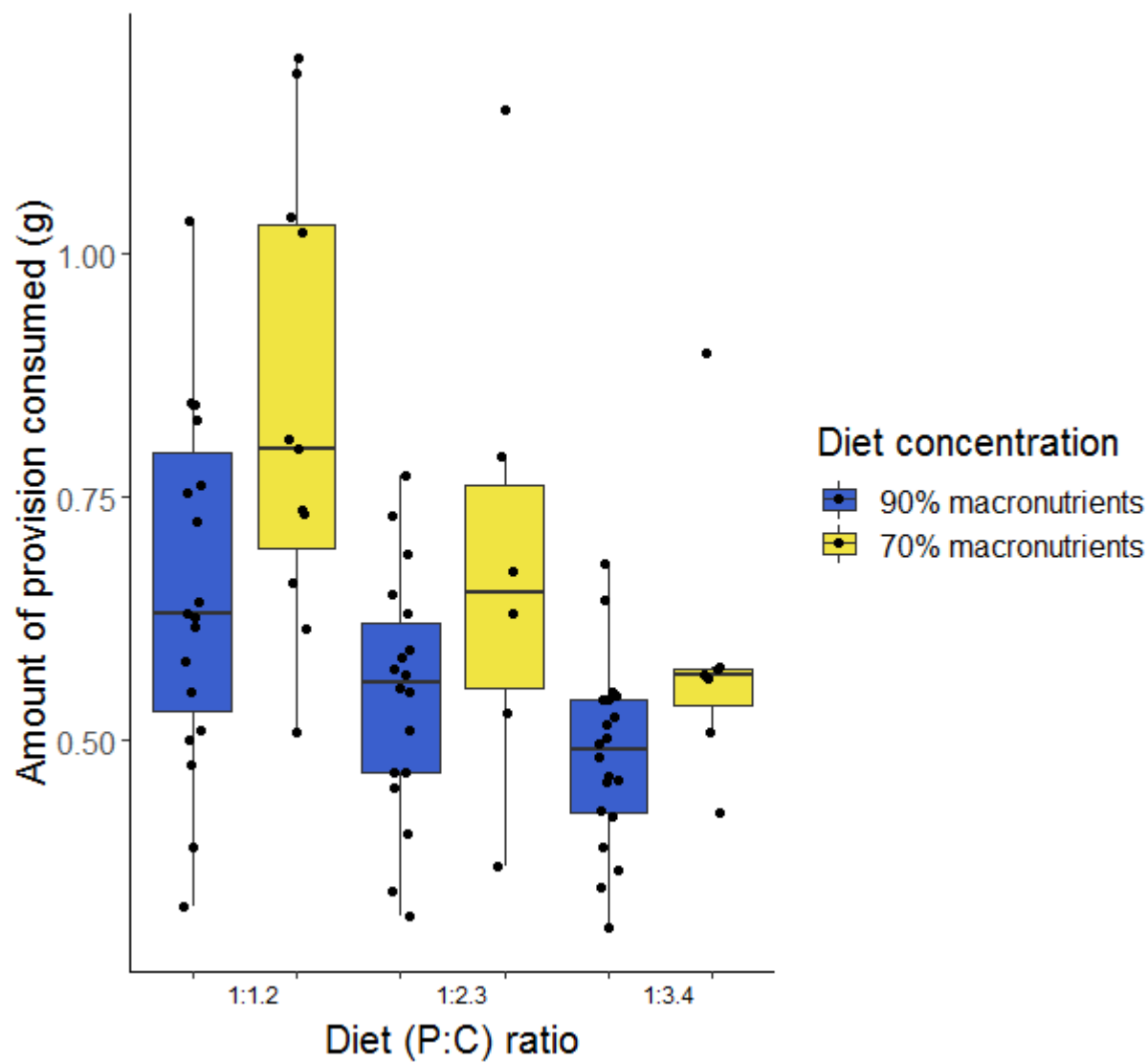
736
737   Fig. 2.
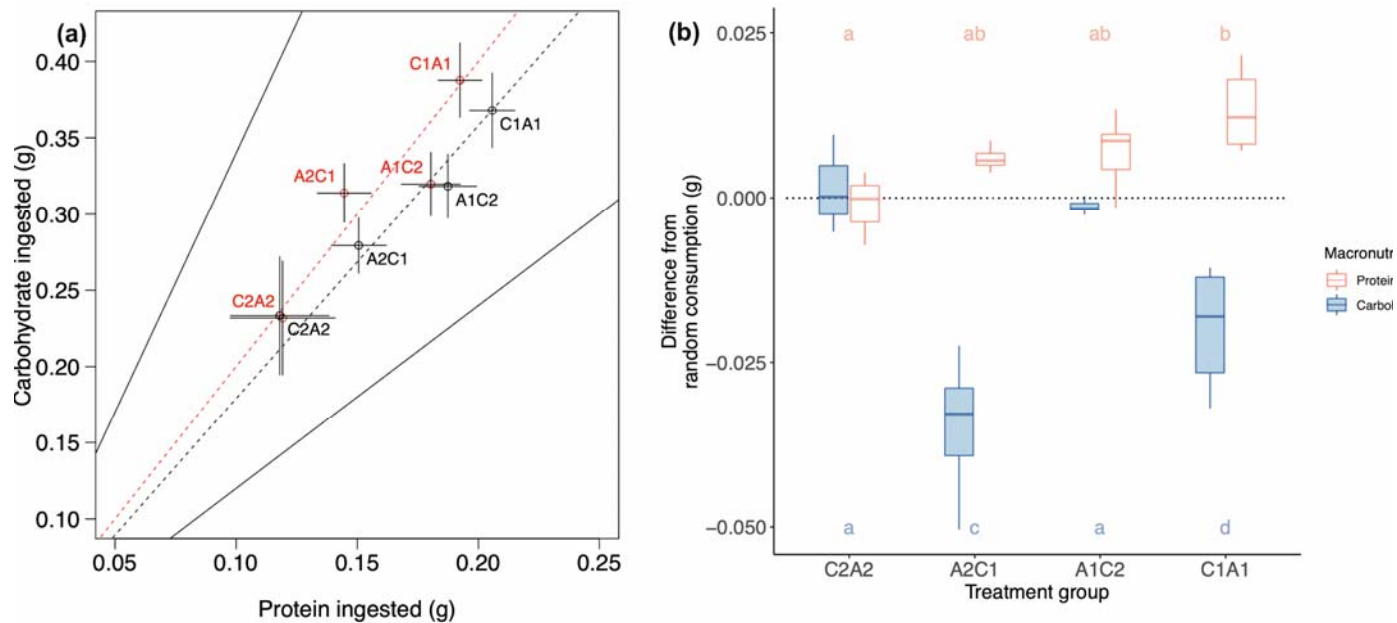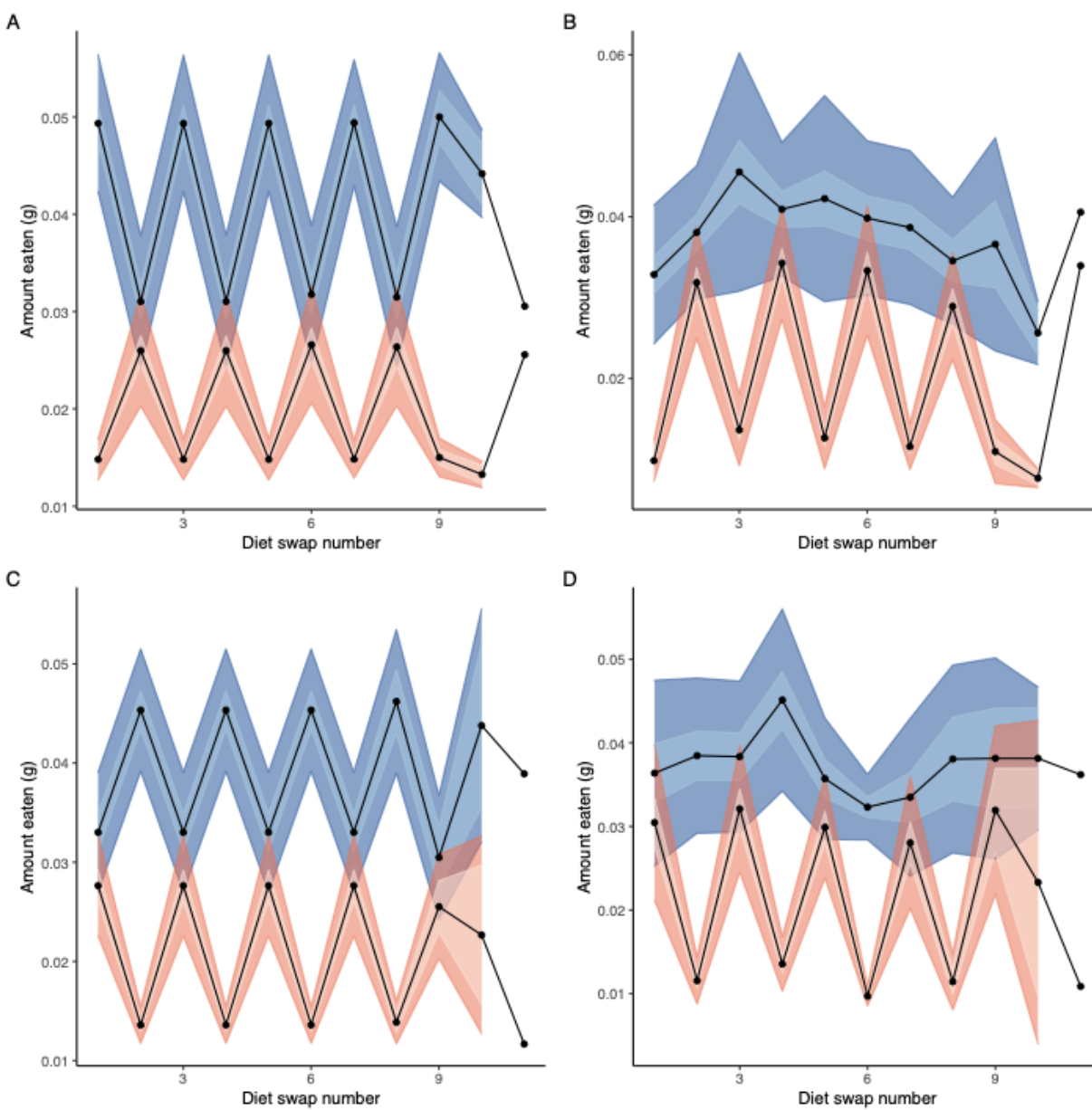738

739

740   Fig. 3.

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742
743 Fig. 4.

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747   Fig. 5.

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750 Fig. 6.
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754   Fig. 7.

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