The geometry of dependence: solitary bee larvae prioritize carbohydrate over protein in parentally provided pollen

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Acknowledgements

The authors thank Victor Swetez, Toby Bagnall, Fiona Tainsh and Shannon Woodmansey for logistical assistance; Stephen Simpson, Audrey Dussutour, Mathieu Lihoreau, Michal Filipiak, Lori Lawson Handley and Domino Joyce for valuable discussions and comments on the manuscript. The study was funded by the University of Hull.

Author Contributions

JDJG and AA conceived and designed the study. AA gathered and analysed all data. Both authors wrote and edited the manuscript.

Abstract

1. Most organisms must regulate their nutritional intake in an environment full of complex food choices. While this process is well understood for self-sufficient organisms, dependent offspring, such as bee larvae, in practice have limited food choices because food is provided by parents. Nutrient balancing may therefore be achieved by offspring, by parents on offspring's behalf, or cooperatively by both.
2. We used the Geometric Framework to investigate the capacity of dependent larval mason bees (*Osmia bicornis*) to regulate their intake of protein and carbohydrate. Herbivorous insect larvae are typically expected to balance protein and carbohydrate to maximise growth and reproduction.
3. Contrary to prediction, carbohydrate and not protein mediated both growth and survival to pupation. Accordingly, larvae prioritised maintaining a constant intake of carbohydrate while tolerating excesses and deficiencies of protein, and self-selected a relatively carbohydrate biased diet compared to other hymenopterans. Reasons for prioritising carbohydrate may include (1) the relative abundance of protein in their normal pollen diet, (2) the relative paucity of nectar in parental provisions making carbohydrate a scarce resource, or (3) the requirement for diapause for all *O. bicornis* larvae. Larvae were intolerant of moderate dietary dilution, reflecting an evolutionary history of nutrient-dense food.
4. Our results demonstrate that dependent *O. bicornis* offspring remain capable of balancing nutrition and thus play an active role in their own nutrient balancing, but they are nevertheless tolerant of wide swings in protein, an important macronutrient for development. Research should now aim to establish whether protein is regulated by parents, or by larvae via post-ingestive processing.

Keywords: bees, parental care, offspring provisioning, nutritional ecology, nutritional geometry, limiting nutrient, diapause, environmental change

Introduction

Most animals manage their nutrient intake by combining nutritionally different foods (Simpson and Raubenheimer 2012). However, the importance of this ability depends upon the nutritional variability of the animals' typical food (Despland and Noseworthy 2006; Raubenheimer et al. 2009); extreme specialists, for example, can lose the capacity to regulate nutrition (Warbrick-Smith et al. 2009; Poissonnier et al. 2018 Aug 19). One way in which organisms can experience limited nutritional choice is if they are dependent upon others for nutrition, or "alloregulation" (Lihoreau et al. 2014), such as dependent offspring of altricial birds, human toddlers, and many larval insects. Offspring tend to have different requirements from parents (Harper and Turner 2000; Michaelsen et al. 2003) - particularly for protein, given their elevated rates of somatic growth and development. Accordingly, parents often make different nutritional choices for their offspring versus when foraging for themselves (Royama 1970; Dussutour and Simpson 2009; Burt and Amin 2014). For example, granivorous birds usually provision young with insects, rather than the seed diets of adults, to fulfil protein requirements (Wiens and Johnston 2012). Under these circumstances, by what rules offspring regulate their own consumption should depend upon provisioning rules of parents. If parents alloregulate offspring nutrition tightly, then offspring should have no need for self-regulation, like extreme specialists (Poissonnier et al. 2018).

The Geometric Framework for Nutrition (GF) allows us to investigate foraging decisions made by animals in multi-dimensional "nutrient space" (Simpson and Raubenheimer 1993). The GF can be used to determine animals' nutritional choices relative to their "intake target" - the optimal amount and balance of multiple macronutrients - as well as their "rule of compromise" that governs their choices when restricted to suboptimal food (Raubenheimer and Simpson 1999a). The GF has provided insights into the nutritional ecology of a broad range of taxa (reviewed in Simpson and Raubenheimer 2012). Its application to dependent offspring, though, has typically been as part of studies of social insect systems (e.g. Helm et al. 2017) and often inferring offspring requirements from patterns of alloparental feeding in studies more broadly focused on adult foraging (see Dussutour and Simpson 2009; Cook et al. 2010; Vaudo et al. 2016). In such systems, multiple adults normally contact offspring, progressively feeding and adjusting nutrition in response to feedback (Field 2005; Schmickl and Karsai 2017), making the responses of individual larvae difficult both to follow and interpret.

In solitary bees, by contrast, typically females provision offspring individually with a pollen ball before sealing the cell and leaving. This behaviour makes solitary bees an ideal, manipulable model for directly studying the nutrition of dependent larvae (Strohm et al. 2002). Larvae of bees, like most aculeate hymenopterans, rely on parents or alloparents for nutrition (Field 2005). Nutritional requirements for bee adults and offspring differ, often radically (Weeks et al. 2004; Filipiak 2019); adults primarily feed on carbohydrate-rich nectar (although see Cane 2016) and larvae feed mostly on protein-rich pollen (Filipiak 2019). Solitary bees, along with most other hymenopterans and many other parental insects, typically have a simple one-to-one parent-offspring relationship whereby parents "mass provision" their young, providing a finite, fixed-mass food provision, and have no contact with their young during development (Costa 2006). Such systems are almost unstudied in a rigorous nutritional context (but see e.g. Roulston and Cane 2002). In these species, there is no opportunity for parents to adjust nutrition according to offspring feedback, and the larva must therefore make the best of what it is given. It may be that offspring regulate their own nutrition to compensate for variation, as in more independent insect larvae (Lee et al. 2002). Alternatively, they may have lost this capacity, like extreme specialists (Warbrick-Smith et al. 2009; Poissonnier et al. 2018). We know very little about how bee larvae deal with variable nutrition (but see Helm et al. 2017) - a knowledge gap with potentially important consequences, considering the proposed link between nutritional stress and bee declines (Roulston and Goodell 2011; Goulson et al. 2015).

In this study, we used a commercially important solitary bee species, *Osmia bicornis*, to investigate how dependent larvae cope with varying nutrition, and whether they can regulate their own intake. *O. bicornis* are pollen generalists (Falk 2015) and the pollen balls that parents provide to offspring are variable in species composition (Haider et al. 2014). Natural variation in pollen ball nutrient content is largely unquantified (although see Budde and Lunau 2007), so there is no prior expectation about the capacity of larvae to regulate their consumption. We used a classic GF design (e.g. Lee et al. 2008), focusing on protein and carbohydrate, with two experimental phases. In the first "no-choice" phase we raised *O. bicornis* larvae on fixed diets of differing protein to carbohydrate ratios to determine their rules of compromise and the diet composition that maximised fitness. In a second "choice" phase, we then provided larvae with targeted choices between sets of two imbalanced diets that differed in their protein:carbohydrate ratios to determine whether larvae defend an intake target. Given the central role of protein in

growth of insect larvae (Scriber and Slansky 1981; Behmer 2009), we predicted that (1) protein would be a key driver of fitness in larval *O. bicornis*, (2) larvae would accordingly aim for a relatively protein-biased intake target, and (3) larvae would prioritize regulating intake of protein over carbohydrate.

Methods

Study organism

Osmia bicornis is a common, cavity-nesting solitary bee (Falk 2015), and a commercially important pollinator (Jauker et al. 2012). *O. bicornis* larvae were obtained as diapausing adults in cocoons (Mauerbienen®). These were released at the nesting site at the University of Hull in April 2017, and emerging adults allowed to breed. Early trials revealed that fresh eggs and newly emerged larvae were too fragile for manipulation. Therefore, newly emerged larvae were left alone for two days before we transferred them to a single-occupancy nest and assigned each to an experimental treatment. Details of nesting apparatus and monitoring protocols are available in the supplementary methods.

Diet Formulation

Existing artificial diet protocols for solitary bees have met with limited success in terms of larval survival (Nelson et al. 1972; Fichter et al. 1981). We used six diets, consisting of three different protein:carbohydrate (P:C) ratios (Diet A = 1:1.2, Diet B = 1:2.3 & Diet C = 1:3.4) and two total macronutrient concentrations (concentration 1 = 90% nutrients, 10% diluent, or concentration 2 = 70% nutrients, 30% diluent; see table S1). Diet ratios were chosen based on a combination of the nutrient ratios in honeybee-collected pollen loads and published data for protein content of *O. bicornis* pollen balls (Budde and Lunau 2007). Diets were diluted with sporopollenin, the primary constituent of the exine of pollen (Mackenzie et al. 2015) an extraordinarily stable natural polymer. Sporopollenin is a novel dietary diluent for bees; we have demonstrated its suitability in a separate study (manuscript submitted). For a more detailed description of sporopollenin and its preparation, see supplementary methods.

Experiment 1: No-choice phase

Two-day-old larvae, randomized by parentage, were allocated to one of 6 treatments corresponding to our 6 artificial diets (n = 20/treatment). Provisions were made to resemble the size of natural provisions (mean initial artificial provision weight = 0.323g +/- 0.034g). Once provisioned, larvae were placed in an incubation chamber (Gallenkamp, IH-270) at 23°C and 80% RH. Provisions were replaced weekly, to avoid desiccation and mould formation, or when fully consumed by larvae, ensuring the diet was always available in excess. Weight of provision consumed was recorded upon provision replacement. Should they finish this provision, it was replenished with a fresh one. A "water control" group, containing artificial diets but no larvae, was used to track water loss from the diets, going through the same weighing regime as above with weight loss recorded at each swap. Nests were checked daily for mortality. Cocoon weight was recorded at the completion of pupation.

Experiment 2: Choice phase

In the choice experiment, 32 two-day-old larvae of mixed parentage were randomly divided among four treatments. Treatments consisted of strategic pairwise combinations (see Fig 1; Table 1) of four possible diets: A1 (1P:1.2C, 90%), A2 (1P:1.2C, 70%), C1 (1P:3.4C, 90%), C2 (1P:3.4C, 70%). Because *O. bicornis* larvae are sedentary and receive a single provision, it is not biologically appropriate to present choices between two diets simultaneously. Therefore, choices were offered temporally by swapping the provision every other day, presenting one diet at a time. This required the larvae to differentially feed over time to compensate for temporal imbalance, in order to converge on an intake target (see e.g. Raubenheimer and Jones 2006). All larvae were kept on the same treatment from two days post-hatching up to pupation, whereupon diet replenishment ceased. The diet that the larvae would be fed first was randomly assigned prior to the experiment.

Statistical Analysis

All analyses were conducted in R version 3.4.2 (R Core Team, 2017). For the no-choice experiment, we calculated total nutrients consumed (protein and carbohydrate) from raw diet consumption data for each swap, adjusted for water loss and dilution. Values were then summed for each larva.

To investigate consumption rules, including rules of compromise, we first asked whether diet ratio and concentration affected consumption of (a) the total provision, (b) protein, or (c) carbohydrate, using models of each respective variable with “ratio” and “concentration” as predictors. Rules of compromise can include nonlinear effects (Simpson and Raubenheimer 1993); we investigated linear effects first using ordinary linear models, assessing goodness of fit before progressing to evaluating nonlinear effects.

To assess fitness consequences of macronutrient consumption, we assessed cocoon weight at pupation and survival to pupation. We analysed cocoon weight using a linear model with “cocoon weight” as a predictor and “protein consumed”, “carbohydrate consumed” and their interaction as predictors. To analyse survival, we used parametric survival analysis in the *survival* package in R. In both cases, we first assessed the fit of linear models before attempting to fit more complex nonlinear effects. To visualise these fitness effects, we calculated response surfaces for cocoon weight and survival, and visualised them using non-parametric thin-plate splines.

In the choice experiment, the mean final consumption of each nutrient was investigated using linear models with diet combination, dilution and their interaction as predictors, and using Tukey’s post hoc tests to compare individual treatments against each other. Under a null expectation we would expect larvae to eat randomly from each diet (Fig 1). Thus, for each larva we calculated the deviation from this null expectation. We then tested whether these values systematically departed from zero for protein and carbohydrate, and whether these departures from random consumption differed by treatment group. We used a linear model with "deviation from random consumption" as the response variable and "treatment group" as a predictor.

Larvae that died pre-pupation were not used in the calculation of the mean protein and carbohydrate consumption for diets in either experiment, or for cocoon weight, but were used in analyses involving survival.

Results

No-choice phase

Dietary P:C ratio had a significant effect on the overall amount of provision consumed, with larvae consuming more provision on high P:C ratio diets (Fig. 2a). Total consumption was

185 also affected by diet concentration; larvae on less concentrated diets consumed more provision,
186 indicating compensatory feeding (Table 2a, Fig. 2a).
187 Dietary P:C ratio had a strong effect on the total amount of P eaten; more protein was
188 eaten by larvae raised on the higher P:C diets (Fig. 3). Diet concentration had no effect on the
189 amount of P consumed; neither was there a ratio:concentration interaction (Table 2b).
190 In contrast, larvae consumed similar amounts of C across all diets, with neither
191 concentration nor dietary P:C ratio having an influence on the amount of C consumed (Table 2c).
192 A median of 0.22 ± 0.06 g of C was consumed by (surviving) larvae across all diet treatments
193 (Fig. 3).
194 Cocoon weight varied differently with macronutrient intake depending on the overall
195 concentration of the diet (Table 3a). At 90% nutrient density, cocoon weight was correlated
196 positively with the amount of carbohydrate consumed, and negatively with protein (Fig. 4a). For
197 our range of diets, the greatest weights were obtained by larvae that ate above approx. 0.3g C and
198 below 0.15g P. In contrast, at 70% nutrient density, cocoons were lower in weight than on the
199 90% diets, and were fairly uniform in weight irrespective of macronutrient intake (Fig 4b). Note
200 that the linear model fitted well and no non-linear effects were observed for the range of diets
201 used - that is, we did not identify an optimal amount of P or C that maximised cocoon weight.
202 The relationship between survival and nutrition similarly depended upon dietary
203 concentration. Survival of larvae fed diets at 90% concentration depended primarily upon
204 carbohydrate consumption (Fig 5a). Those larvae that consumed high amounts of carbohydrate
205 saw the lowest mortality irrespective of how much protein was consumed. Interestingly,
206 however, at lower levels of carbohydrate, mortality increased with decreasing amounts of protein
207 (Table 3b). Survival of larvae raised on the more dilute diets was greater, and was not
208 substantially affected by intake of P or C (Fig 5). Again, all linear models showed a good fit, so
209 we did not look for non-linear patterns in larval survival.
210
211 *Choice phase*
212 We found no evidence of larvae defending a common intake target at the choice phase.
213 Nevertheless, they converged upon a P:C ratio of approx. 1:1.8 (i.e. a common line, or
214 "nutritional rail"; Fig. 6). The amount of protein consumed by larvae was significantly affected
215 by diet combination: more protein was consumed by individuals offered diet combinations that

were overall more concentrated (Table 4a, Fig 6). Similarly, carbohydrate consumption was significantly affected by diet combination (Table 4b). However, unlike with protein, this pattern appeared to be driven by the diets at the extreme, with significantly more carbohydrate eaten only by those in the most concentrated diet pair compared to the least concentrated pair (Fig 6); other pairwise comparisons were not significant.

Despite the lack of a common intake target, larvae were not consuming diets at random. For both carbohydrate and protein we saw differences in consumption from what would have been expected for each larva based on random consumption, and this effect was dependent on the specific set of diet choices (Fig 7a, Table 4c, d). When visualised as the amounts of protein and carbohydrate consumed during each 48h treatment period, it is clear that larvae were achieving a degree of homeostasis in carbohydrate consumption compared to what would be expected under random consumption of each diet choice, whereas their consumption of protein aligned closely with what would be expected under random consumption (Fig. 7b-d).

Discussion

Although we did not identify an optimal (fitness-maximising) intake for either carbohydrate or protein, carbohydrate was positively associated with both body size and survival in *Osmia bicornis* larvae (Figs. 4a, 5a). Accordingly, given a choice, larvae converged on a relatively carbohydrate-biased protein:carbohydrate ratio of 1:1.8 (Fig. 6). Moreover, larvae prioritised carbohydrate over protein intake, showing tighter control over carbohydrate consumption than over protein consumption (Fig 7), and pupating after eating about 0.22 g carbohydrate irrespective of protein and of dietary dilution (Fig 3). This carbohydrate target fell short of the amount of carbohydrate that maximised cocoon weight or survival to pupation. Yet achieving even this carbohydrate target carried costs for larvae on more dilute diets, which suffered greater mortality and lower cocoon weights (Figs 4b, 5b). Taken together, these results show that (1) larval *O. bicornis* are at least partially responsible for their own nutritional regulation, and (2) their performance and consumption rules suggest adaptation to a pattern of carbohydrate-limited growth and survival. In what follows, we suggest how and why these patterns in *O. bicornis* depart from expected results based on studies of related organisms, and more generally what these findings suggest about nutritional cooperation between parents and offspring in provisioning species.

247 Larvae grew and survived best on our highest carbohydrate (i.e. lowest
248 protein:carbohydrate) diets. Accordingly, across our range of diets, larvae maintained a constant
249 carbohydrate intake while tolerating excesses or deficiencies of protein (a "no-interaction" rule
250 of compromise; Raubenheimer and Simpson 1999a) - although it is conceivable that this
251 represented a narrow window on a more curved intake array that would be visible over a broader
252 diet range. Both these patterns are unusual because insect herbivores are generally considered to
253 be limited by protein (e.g. Bernays and Chapman 2007; although see Le Gall and Behmer 2014).
254 In the few existing studies involving larval bees, e.g. honeybees (Helm et al. 2017), bumblebees
255 (Kraus et al. 2019) and sweat bees (Roulston and Cane 2002), protein and not carbohydrate
256 mediated larval growth and/or survival. More broadly, insect larvae often grow and survive best
257 on balanced or moderately high protein:carbohydrate ratios (Roeder and Behmer 2014;
258 Rodrigues et al. 2015), although low protein:carbohydrate ratios are associated with longevity in
259 adults (e.g. Lee et al. 2008). Moreover, animals generally prioritise regulation of the nutrient that
260 is typically limiting in their normal diet, and tolerate variation in nutrients that are abundant
261 (Raubenheimer and Simpson 1999b). Tolerance of wide variation in protein is thus usually seen
262 in predators (e.g. Raubenheimer et al. 2007; Kohl et al. 2015). In contrast, herbivores often
263 regulate protein more tightly than carbohydrate (Lee et al. 2002; Le Gall and Behmer 2014;
264 VanOverbeke et al. 2017).
265 During the choice phase, when allowed to self-select diets, *O. bicornis* larvae converged
266 on a protein:carbohydrate ratio of 1:1.8. This ratio is considerably more carbohydrate-biased than
267 that preferred by bumblebees foraging on behalf of microcolonies (1:0.25, Vaudo et al. 2018;
268 1:0.08, Kraus et al. 2019), and (to a lesser extent) than ants foraging for colonies with offspring
269 (1:1.5, Dussutour and Simpson 2009). It is also more carbohydrate-biased than that selected by
270 reproductive, solitary phytophages such as grasshopper adults and lepidopteran larvae (1:0.25 -
271 1:1.4, reviewed in Behmer 2009) and is closer to diets selected by *Drosophila* larvae (1:2,
272 Rodrigues et al. 2015). Notably, though, 1:1.8 was more protein-biased than the ratio that we
273 found maximised both cocoon weight and survival (1:3.4), suggesting *O. bicornis* larvae may
274 choose diets that favour other fitness-related quantities (such as reproduction and/or
275 developmental time) over body size/survival, as in *Drosophila* (Lee et al. 2008; Rodrigues et al.
276 2015).

277 Two main features of *O. bicornis'* ecology may help to explain their prioritization of
278 carbohydrate, and their relative preference for this macronutrient compared to what we know of
279 related taxa. First, the relative paucity of carbohydrate in *O. bicornis'* larval diet may help to
280 explain these findings. Despite being herbivorous, *Osmia* larvae are unlikely to be protein-
281 limited, because pollen is among the most protein-rich of plant tissues (Mattson 1980).
282 Moreover, in *Osmia* specifically, nectar constitutes only a tiny fraction of the pollen ball, less
283 than 4% (Maddocks and Paulus 1987; see Radmacher and Strohm 2010), in contrast to many
284 other bees where nectar is a principal source of carbohydrate for larvae (e.g. Kraus et al. 2019).
285 *O. bicornis* larvae may therefore be limited more by the amount of digestible carbohydrate
286 within pollen than by dietary protein (see Roulston and Cane 2000). Second, *O. bicornis* is (to
287 our knowledge) the first truly solitary hymenopteran studied under the GF; other studies have
288 concerned individuals likely to become workers of social species. Unlike social hymenopterans,
289 *O. bicornis* offspring are all reproductive and undergo diapause (Fliszkiewicz et al. 2012) - both
290 activities dependent on the fat body, where carbohydrate-derived fat is stored (Kawooya and
291 Law 1988; Ziegler and Van Antwerpen 2006; Hahn and Denlinger 2007; Wasielewski et al.
292 2013). Thus, *O. bicornis* larvae may have additional requirements for carbohydrate over and
293 above those of developing nonreproductive, nondiapausing hymenopteran workers. These
294 contrasting findings reinforce the idea that bees' nutritional needs may be just as diverse as their
295 ecologies.
296 Although larvae retained the ability to regulate carbohydrate by over- or under-eating
297 protein, they nevertheless coped very poorly with dietary dilution (Fig 4b, 5b), despite displaying
298 compensatory feeding behaviour (Fig 2) that suggests they both detected and responded to such
299 dilution. The dilution was not excessive (70% nutrient density) compared to similar studies
300 offering very highly dilute diets (14%, Raubenheimer and Simpson 1993; 16.8%, Lee et al.
301 2004). The locusts and caterpillars in those studies, though, are adapted for diets that vary greatly
302 in nutrient density, beginning dilute and becoming even more dilute over the season (Scriber and
303 Slansky 1981). By contrast, pollen is among the most consistently nutrient-rich parts of a plant
304 (Roulston and Cane 2000) and does not broadly vary in composition over a season (DeGrandi-
305 Hoffman et al. 2018). With a normal diet of unadulterated pollen and very little nectar, *Osmia*
306 larvae may have had no need to evolve mechanisms to cope with dilution. In comparison,
307 caterpillars reared on an invariant diet for generations lost the ability both to regulate intake and

308 to cope with dilution (Warbrick-Smith et al. 2009). *Osmia* larvae appear to have retained the
309 former capacity, but lost the latter, suggesting a normal diet that is dense in nutrients, but
310 variable in composition.

311 In systems where parents gather food for offspring from the environment, both parents
312 and offspring can be active participants in nutritional regulation. The lack of protein regulation
313 shown by *O. bicornis* larvae highlights the importance of understanding (a) whether mother *O.*
314 *bicornis* adjust protein content of provisions in response to imbalances in the landscape, and (b)
315 whether larvae have physiological adaptations (e.g. post-ingestive processing) for tolerating
316 protein imbalance. Budde & Lunau (2007) found that *O. bicornis* provisions contained about
317 19% protein regardless of pollen species used, suggesting a degree of homeostasis by parents.
318 Yet human activity is reducing floral diversity (Papanikolaou et al. 2017). Evidence is mixed
319 concerning whether, in practice, parent bees assess pollen nutrients at the flower (reviewed by
320 Nicholls and Hempel de Ibarra 2016). Both bumblebees and ants balance nutrition on behalf of
321 colonies (Dussutour and Simpson 2009; Vaudo et al. 2018), regulating more tightly when
322 foraging for offspring - protein in both taxa (Dussutour and Simpson 2009; Kraus et al. 2019)
323 and carbohydrate in ants (Dussutour and Simpson 2008; Cook et al. 2010). On the other hand,
324 protein gathered by honeybees varied passively with landscape usage (while maintaining
325 carbohydrate and lipid; Donkersley et al. 2014). Which regulatory strategy *Osmia* parents and
326 larvae collectively pursue may have important implications for their vulnerability to human-
327 induced landscape change, and so should now be a focus for research.

328 More broadly, understanding the relative roles of offspring (intake regulation and post-
329 ingestive processing) versus parents in nutrient balancing will be key to understanding the
330 nutritional ecology of species with parental provisioning. Such species include not just bees, but
331 other important ecosystem service providers such as dung beetles (Frank et al. 2017) and burying
332 beetles (Hopwood et al. 2013), as well as altricial birds (Wiens and Johnston 2012) and even
333 humans (Burt and Amin 2014). Alloregulation by parents is not a given; the relative roles of
334 parent and offspring in these groups are likely to reflect species' ecologies. Recent studies have
335 found nutritional mismatches between oviposition sites selected by parents and the nutritional
336 requirements of the offspring that will develop in those sites (Rodrigues et al. 2015; Lihoreau et
337 al. 2016). Parent sweat bees (*Lasioglossum zephyrum*) appear not to regulate protein in larval
338 provisions, despite protein mediating offspring performance (Roulston and Cane 2002). In *O.*

339 *bicornis*, we have shown that offspring retain the ability to regulate their nutritional intake
340 despite all food selection being done by parents. Larvae appeared to pay closest attention to
341 regulating dietary carbohydrate, consistent with this nutrient mediating both growth and survival.
342 Yet protein remains a key requirement for development; key now is to establish specifically how
343 protein balance is achieved, and whether parents or larvae carry that responsibility.
344
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518

519

520

521 **Tables & Figures**

522

523 **Table 1. Sample sizes for each diet combination used for choice phase (allocated by random**
524 **coin toss). "Order" refers to diet order - e.g. for A1C1, Order 1 would receive A1 first**
525 **whereas Order 2 would receive C1 first, determined by coin toss. Surviving larvae are in**
526 **parentheses.**

	Order 1	Order 2
A1C1	1 (1)	6 (5)
A1C2	5 (5)	4 (3)
A2C1	3 (2)	5 (5)
A2C2	5 (2)	3 (1)

527

528 **Table 2. Model tables for models of consumption in no-choice phase. Key: ratio = dietary**
529 **protein:carbohydrate ratio (A = 1P:1.2C, B=1P:2.3C, C=1P:3.4C). Conc=overall nutrient**
530 **concentration.**

(a) Total amount consumed (linear model)

	Coefficients of minimal model		**Effect of dropping term from full model**			
Model term	**Estimate**	**Std. Error**	**df1**	**df2**	**F**	**p**
Intercept (ratio A, conc 90%)	0.67	0.03				
ratio	**-**	**-**	**2**	**80**	**10.86**	**<0.0001**
ratio B	-0.13	0.05				
ratio C	-0.20	0.04				
conc	**-**	**-**	**1**	**79**	**14.04**	**<0.001**
conc 70%	0.15	0.04				
ratio × conc	-	-	2	78	0.4	0.67

(b) Protein consumed (linear model)

Model term	**Estimate**	**Std. Error**	**df1**	**df2**	**F**	**p**
Intercept (ratio A)	0.18	0.01				
ratio	-	-	**2**	**80**	**75.87**	**<0.0001**
ratio B	-0.07	0.01				
ratio C	-0.11	0.01				

conc	-	-	1	79	1.85	0.1
ratio × conc	-	-	2	78	0.0032	0.99

(c) Carbohydrate consumed (linear model)

Model term	Estimate	Std. Error	df1	df2	F	p
Intercept	0.23	0.01				
ratio	-	-	2	80	1.52	0.22
conc	-	-	1	79	2.97	0.09
ratio × conc	-	-	2	78	0.20	0.82

534

Table 3. Model tables for models of fitness correlates in no-choice phase

(a) Cocoon weight (linear model)

	Coefficients of minimal model		Effect of dropping term from full model			
Model term	**Estimate**	**Std. Error**	**df1**	**df2**	**F**	**p**
Intercept (conc 90%)	0.032	0.015	-	-	-	-
Protein	-0.317	0.065	-	-	-	-
Carbohydrate	0.408	0.066	-	-	-	-
Conc 70%	-0.011	0.027	-	-	-	-
Protein:Carbohydrate	-	-	1	72	0.145	0.705
Protein:Conc 70%	**0.392**	**0.126**	**1**	**72**	**8.942**	**0.004**
Carbohydrate:Conc 70%	**-0.337**	**0.132**	**1**	**72**	**6.310**	**0.014**

(b) Survival to pupation (parametric survival regression)

	Coefficients of minimal model		Effect of dropping term from full model			
Model term	**Estimate**	**Std. Error**	**df1**		**$\chi 2$**	**p**
(Intercept)	2.114	0.556	-	-	-	-
Protein	6.009	2.257	-	-	-	-

Carbohydrate	9.236	3.188	-	-	-	-
Conc 70%	0.467	0.543	-	-	-	-
Protein:Carbohydrate	**-18.505**	**8.426**	**1**		**-3.977**	**0.046**
Protein:Conc 70%	-	-	1		-0.072	0.789
Carbohydrate:Conc 70%	**-6.279**	**3.138**	**1**		**-4.875**	**0.027**

539

Table 4. Model tables for models of consumption in choice phase. For details of diet combinations, see text.

(a) Protein consumed (amount)

	Coefficients of minimal model		Effect of dropping term from full model			
Model term	**Estimate**	**Std. Error**	**df1**	**df2**	**F**	**p**
Diet combination	**-**	**-**	**3**	**23**	**7.43**	**0.002**
A1C2	0.19	0.01				
A2C1	0.15	0.01				
A1C1	0.21	0.01				
A2C2	0.12	0.02				

(b) Carbohydrate consumed (amount)

	Coefficients of minimal model		Effect of dropping term from full model			
Model term	**Estimate**	**Std. Error**	**df1**	**df2**	**F**	**p**
Diet combination	**-**	**-**	**3**	**23**	**4.58**	**0.013**
A1C2	0.32	0.02				
A2C1	0.28	0.02				
A1C1	0.37	0.02				

A2C2	0.23	0.03				

(c) Protein consumed (departure from random)

	Coefficients of minimal model		Effect of dropping term from full model			
Model term	**Estimate**	**Std. Error**	**df1**	**df2**	**F**	**p**
Diet combination	**-**	**-**	**4**	**20**	**19.67**	**<0.0001**
A1C2	0.007	0.002				
A2C1	0.006	0.002				
C1A1	0.013	0.002				
C2A2	-0.001	0.003				

(d) Carbohydrate consumed (departure from random)

	Coefficients of minimal model		Effect of dropping term from full model			
Model term	**Estimate**	**Std. Error**	**df1**	**df2**	**F**	**p**
Diet combination	**-**	**-**	**4**	**20**	**51.647**	**<0.0001**
A1C2	-0.001	0.003				
A2C1	-0.035	0.003				
C1A1	-0.020	0.003				

C2A2	0.002	0.004

543 **Figure captions**

544

545 **Figure 1.** Expected protein and carbohydrate consumption if larvae ate indiscriminately between
546 two diets. Diet choices are pairwise combinations of diets A, B, C and D, which each contain
547 protein and carbohydrate at different ratios and concentrations. Solid lines represent P:C ratios;
548 black points represent actual nutrient content of each diet, which depends upon dilution as well
549 as P:C ratio. Red points represent expected consumption if larvae eat randomly (i.e. equally)
550 from each of a choice of two diets (choices denoted by the red point labels).

551

552 **Figure 2.** Amount of provision in grams consumed by larvae raised on the 3 different P:C ratio
553 artificial diets at the 2 different macronutrient concentrations (90% and 70% macronutrient
554 content).

555

556 **Figure 3**. Mean total (+/- 1 SD) amount of P and C consumed in grams by larvae on each
557 diet before pupation. Solid lines and letters represent three P:C ratios (A = 1:1.2, B = 1:2.3,
558 C = 1:3.4). Numbers following letters denote diet concentration (1 = 90%, 2 = 70%).

559

560 **Figure 4.** Effects of P and C consumption upon cocoon weight (g) in larvae fed diets at (a)
561 90% and (b) 70% nutrient density. Transition from blue to red indicates heavier cocoons.
562 For context, mean total consumption of P and C for each diet is plotted (data as in Fig. 2).
563 Solid lines and letters represent three P:C ratios (A = 1:1.2, B = 1:2.3, C = 1:3.4).

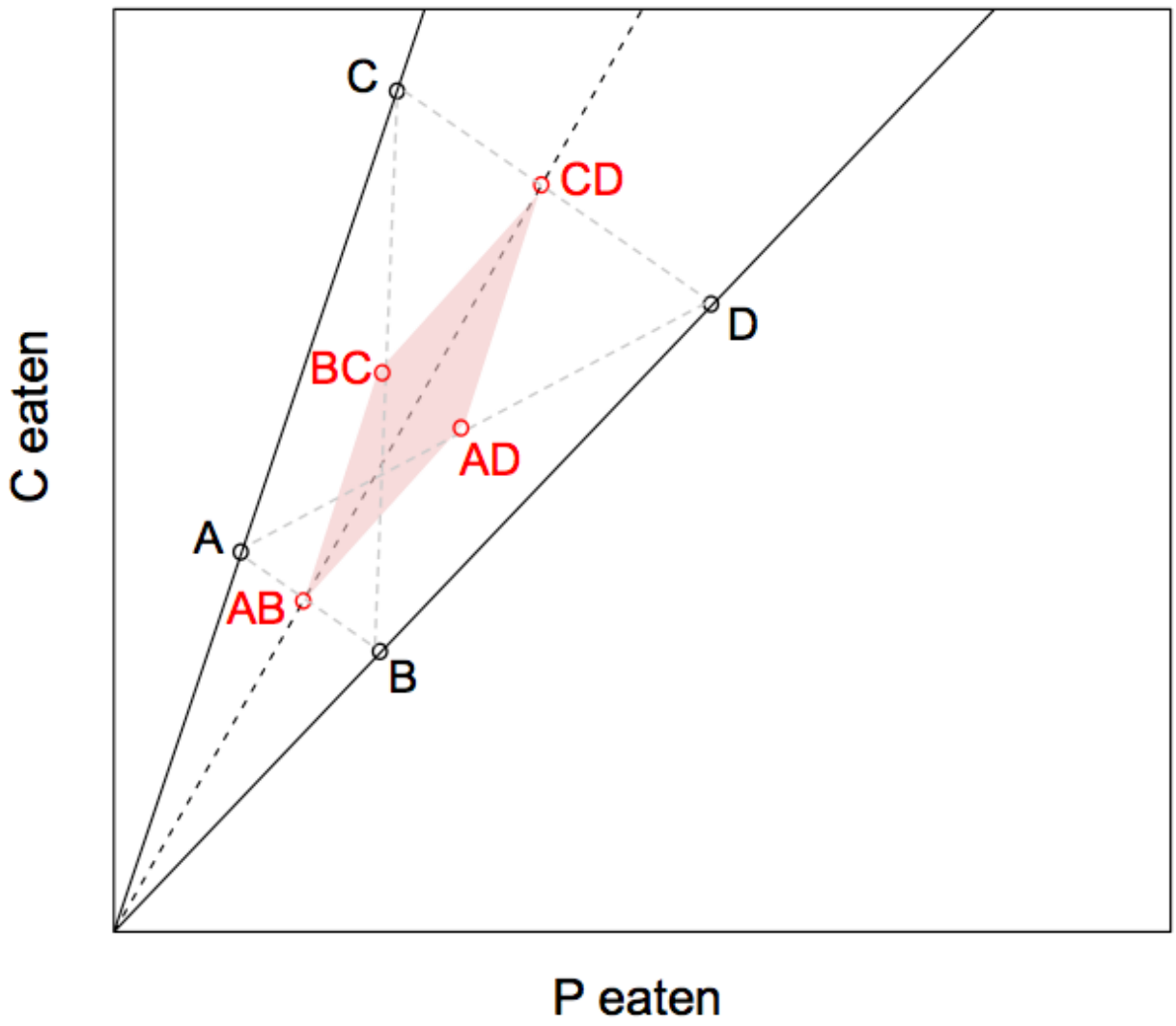
564

565 **Figure 5.** Effects of P and C consumption upon survival probability (black = died, white =
566 pupated) and duration (colour; transition from blue to red indicates longer survival
567 duration) in larvae fed diets at (a) 90% and (b) 70% nutrient density. For context, mean
568 total consumption of P and C for each diet is plotted (data as in Fig. 2). Solid lines and
569 letters represent three P:C ratios (A = 1:1.2, B = 1:2.3, C = 1:3.4).

570

571 **Figure 6.** The mean (+/- SE) amount of protein (P) and carbohydrate (C) eaten by larvae in
572 the choice experiment. Each point label denotes a choice of two diets, one A and one C;
573 black labels show observed intake, red labels show expected intake under random

574 consumption. Letters represent diet P:C ratio (A = 1:1.2, C = 1:3.4); numbers represent diet
575 concentration (1 = 90%, 2 = 70%). Solid lines represent dietary P:C ratios (Top line = Diet
576 A, Bottom line = Diet C). Dashed red line shows expected average P:C ratio based on
577 random consumption. Dashed black line shows average P:C ratio of observed intake across
578 larvae. Black bars show comparisons *among* treatment groups' observed intake (Tukey's
579 post-hoc comparisons; for details of linear model see text) in P and C.
580
581 **Figure 7.** (a) Deviation from random intake of protein and carbohydrate for larvae in
582 different treatment groups during the choice phase. Bars with similar letters displayed
583 above or below are not statistically significantly different (Tukey's post-hoc comparisons).
584 (b, c) Mean (+/- 1SD) observed intake of protein and carbohydrate over successive diet
585 swaps, irrespective of the concentrations of the diet choices, for larvae starting on diet A
586 (b) or diet C (c), versus expected nutrient intake under random consumption of diets (d,
587 showing larvae starting on diet C, chosen arbitrarily). For calculations of expected
588 consumption, see text.



589

590 Fig. 1.

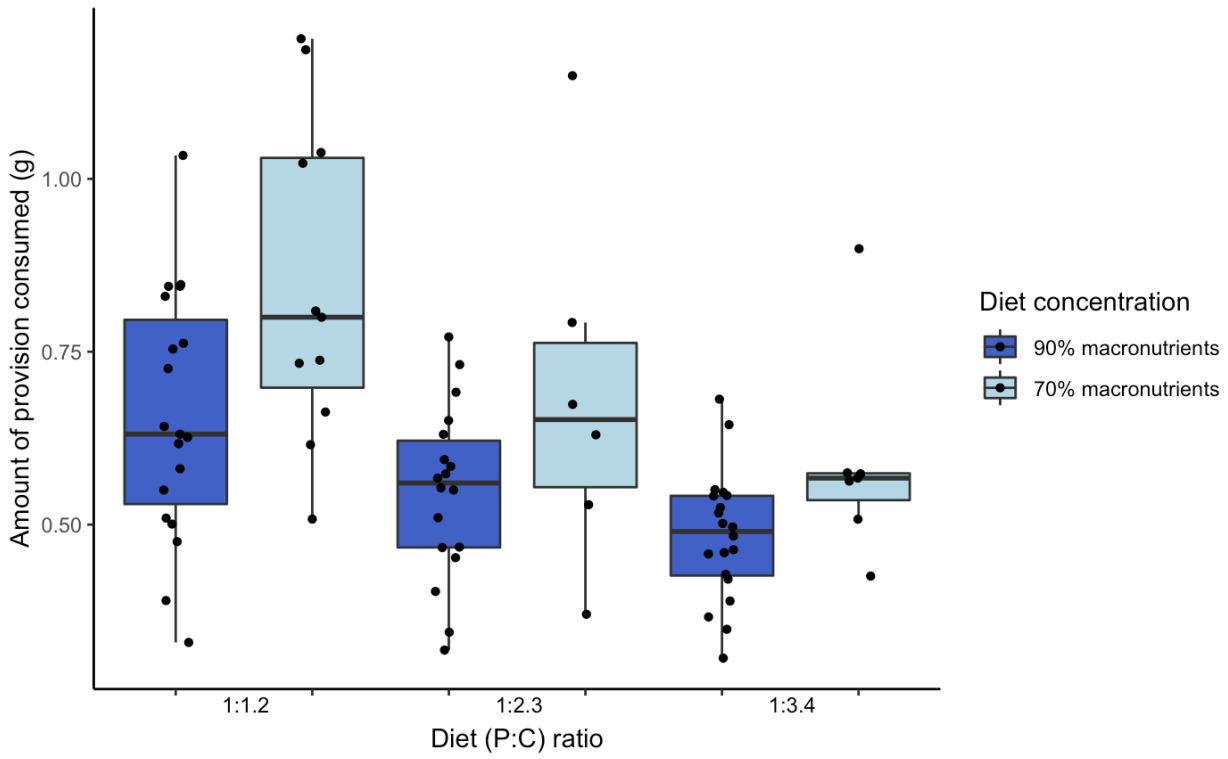

Amount of provision consumed (g)
1.00
0.75
0.50
1:1.2
1:2.3
1:3.4
Diet (P:C) ratio
Diet concentration
90% macronutrients
70% macronutrients

Fig. 2.

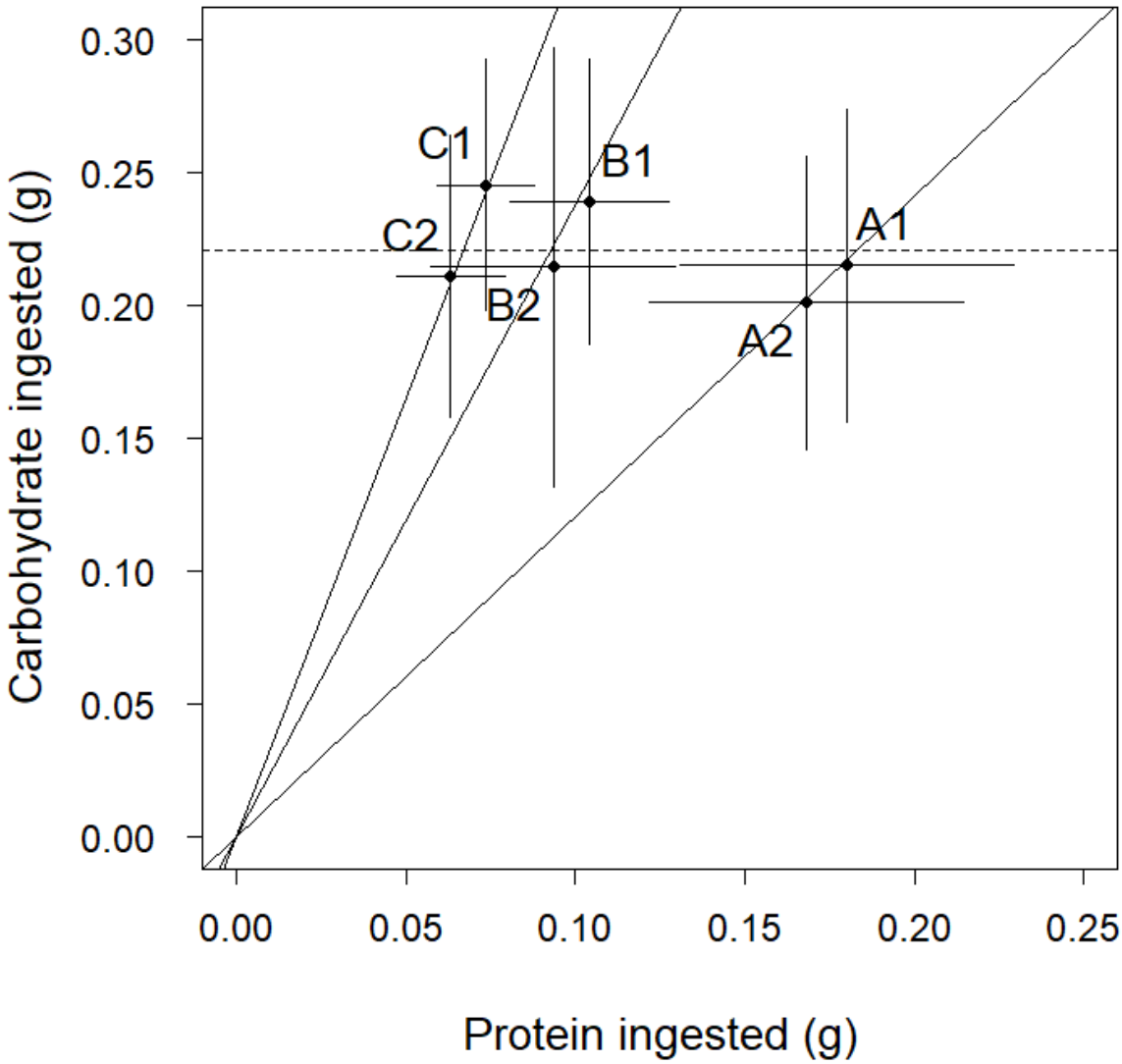

0.30
0.25
0.20
0.15
0.10
0.05
0.00
Carbohydrate ingested (g)
C1
B1
C2
A1
B2
A2
0.00
0.05
0.10
0.15
0.20
0.25
Protein ingested (g)

Fig. 3.

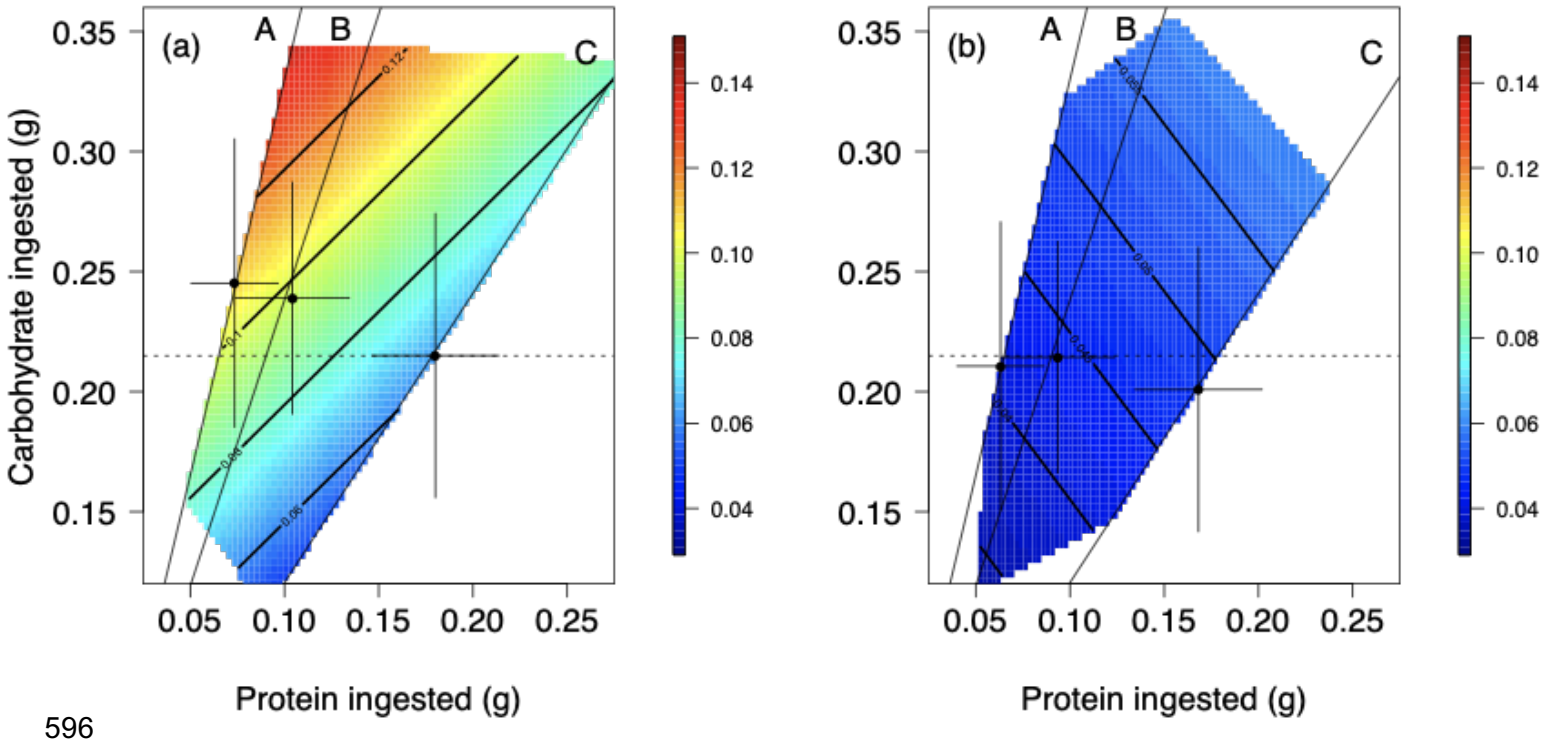

(a)
A
B
C
0.35
0.30
0.25
0.20
0.15
Carbohydrate ingested (g)
0.05
0.10
0.15
0.20
0.25
Protein ingested (g)
0.14
0.12
0.10
0.08
0.06
0.04
(b)
A
B
C
0.35
0.30
0.25
0.20
0.15
0.05
0.10
0.15
0.20
0.25
Protein ingested (g)
0.14
0.12
0.10
0.08
0.06
0.04

Fig. 4.

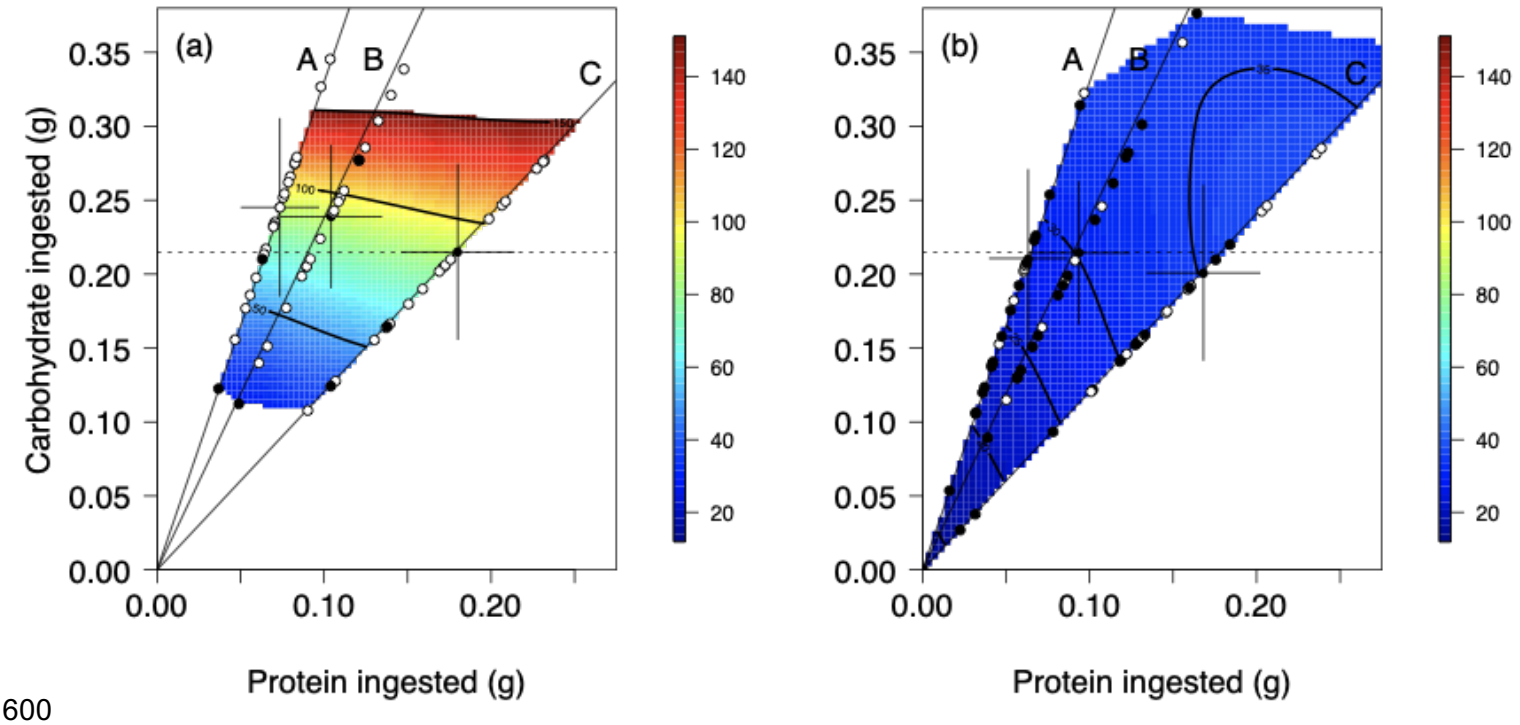
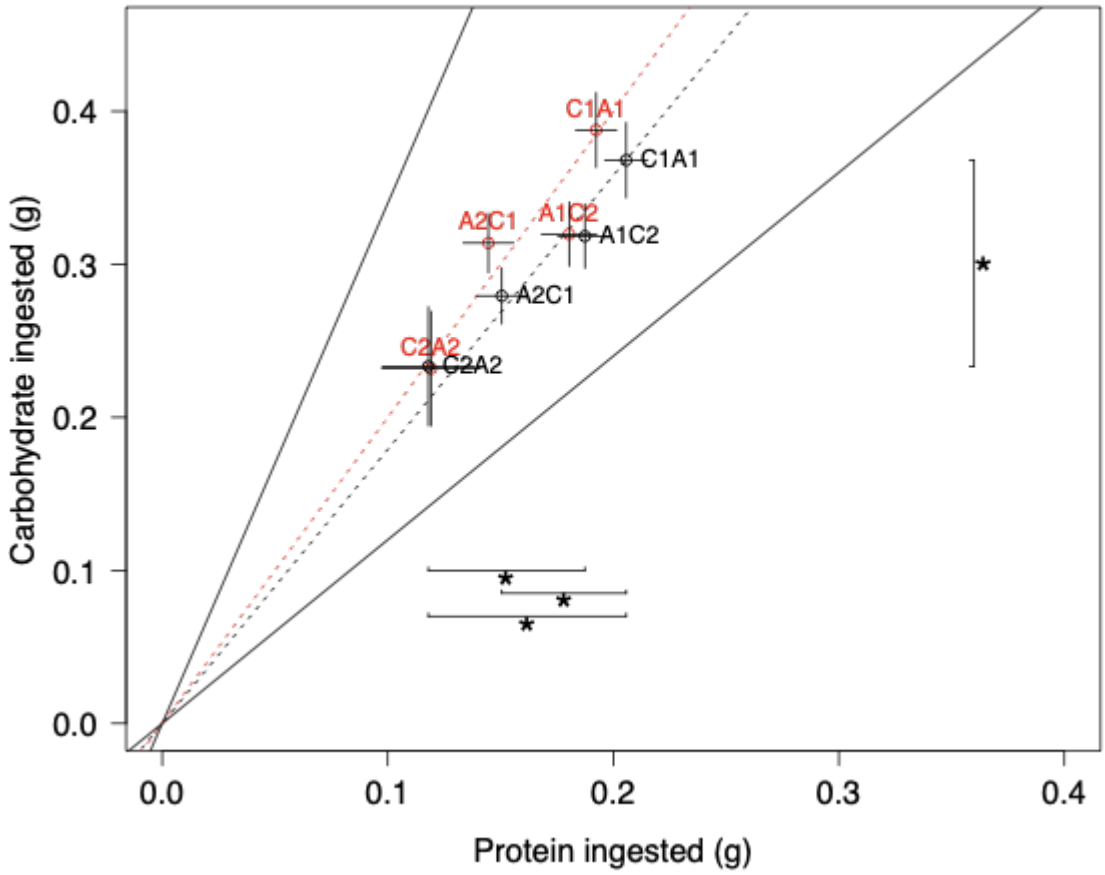

(a)
A
B
C
Carbohydrate ingested (g)
Protein ingested (g)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.00
0.10
0.20
100
140
120
100
80
60
40
20
(b)
A
B
C
Protein ingested (g)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.00
0.10
0.20
140
120
100
80
60
40
20

Fig. 5.



603

604 Fig. 6.

605

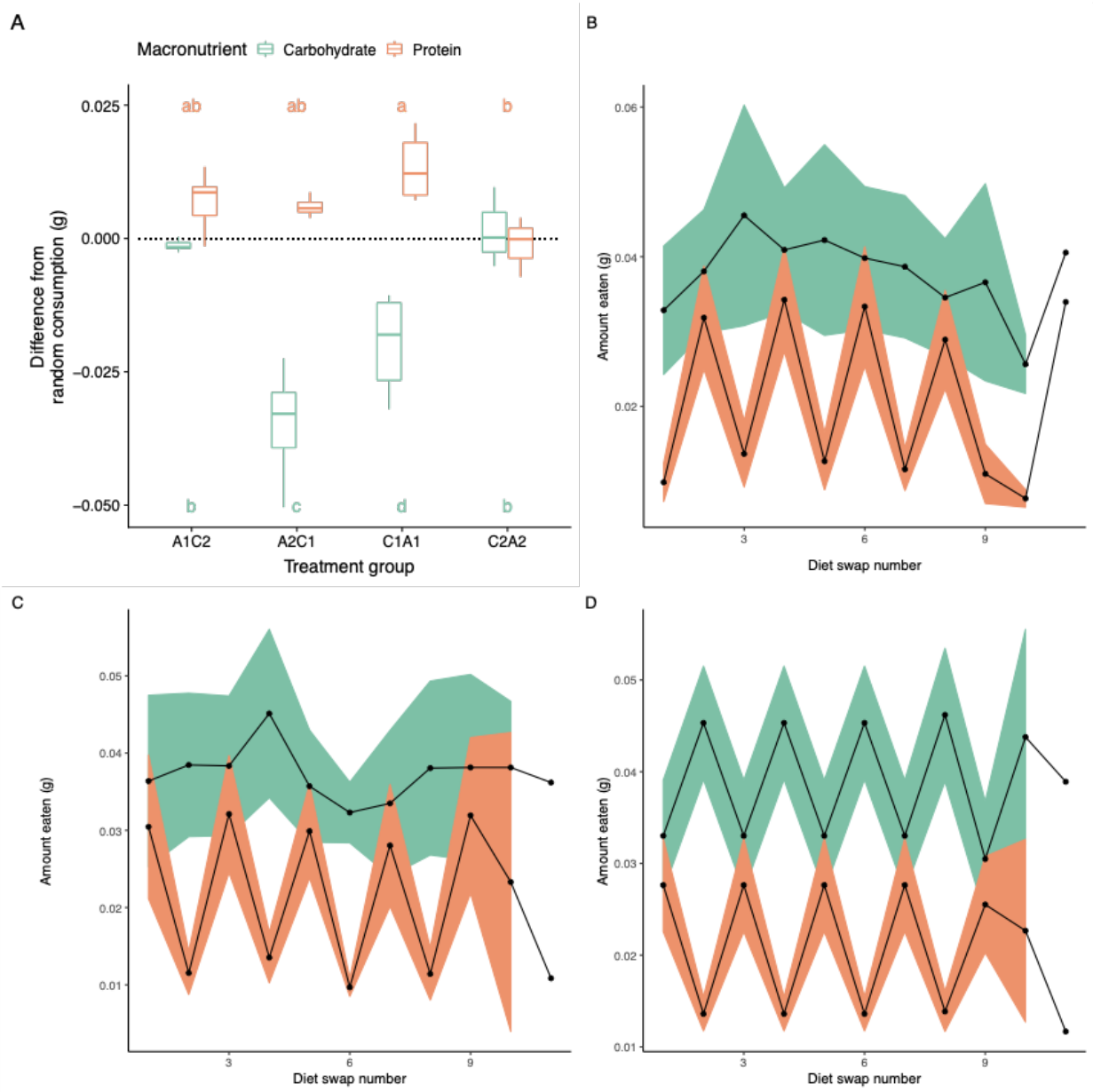

A
Macronutrient
Carbohydrate
Protein
Difference from random consumption (g)
0.025
0.000
−0.025
−0.050
ab
ab
a
b
b
c
d
b
A1C2
A2C1
C1A1
C2A2
Treatment group
B
Amount eaten (g)
0.06
0.04
0.02
3
6
9
Diet swap number
C
Amount eaten (g)
0.05
0.04
0.03
0.02
0.01
3
6
9
Diet swap number
D
Amount eaten (g)
0.05
0.04
0.03
0.02
0.01
3
6
9
Diet swap number

Fig. 7.