“Nutritional Ecology of Farmland Bee Larvae Under A Changing Climate”

Toby Euan Bagnall - Biology BSc

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ABSTRACT

1. Bees are a major component of a healthy ecosystem, pollinating almost all globally important crops and flowering plants. Pollinator numbers are dropping drastically however, with human-induced landscape change and an enhanced greenhouse effect partially at fault. Effects of a changing nutritional landscape are evident in adults of pollinating species but information about their offspring respond is limited. The larval stages of social species are difficult to monitor however, solitary bees both outnumber and outperform social bees as pollinators.
2. *Osmia bicornis* is a species of mass-provisioning solitary bee that construct tube like nests with individual cells meaning larval nutrition can be manipulated and monitored. With this study I aimed to understand; (1) whether bees detect and respond to changes in the nutritional composition of food and (2) does a changing climate affect their ability to do so? I reared *O.bicornis* larvae under different ambient temperatures (15°C, 20°C, or 25°C), providing one of 6 fixed artificial diets (3 Protein:Carbohydrate ratios at 2 concentrations). Larvae could only control how much of the diet they consumed so I assessed macronutrient consumption, and fitness across groups under the Geometric Framework for Nutrition.
3. Larvae controlled carbohydrate consumption to meet an intake target while tolerating unmonitored protein consumption unless developing in 25°C where the opposite is true. Larval weight gain and time to death were maximised with high protein intake even while controlling carbohydrate intake and this effect was stronger with high diet concentration.

4. My study shows *O.bicornis* larvae carefully control carbohydrate intake in natural conditions but under predicted temperature rise protein consumption was linked to their time to death. High carbohydrate intake is linked to overwintering success however, beyond optimal temperatures larvae may be delaying diapause in favour of consuming protein to deal with the new nutritional requirements.

INTRODUCTION

Solitary bees make-up ~225 species out of 270 bee species in the UK (Falk, 2015); and outperform social bees as pollinators (Garibaldi et al., 2013; Winfree et al., 2007; Winfree et al., 2008; Woodcock et al, 2013). Ecosystem stability and global food security depends upon healthy populations of bees, our foremost pollinators, to the tune of many hundreds of billions of dollars annually. In 2009, the estimated global economic value of pollination reached €153 billion, or 9.5% of the value of global agricultural production used for human food in 2005 (Gallai et al., 2009; The European Commission, 2019). Pollination underpins 78% of global flowering plant reproduction and enhances production in 75% of globally important crops (Breeze et al., 2016). Our dependency on pollinators will only intensify as agriculture intensifies worldwide in response to growing human populations (Garibaldi et.al., 2013). Yet despite their value, bee populations are rapidly declining, with multiple causes (Biesmeijer, 2006). A two-year study by the Chicago Botanic Gardenfield and Northwestern University revealed 35 percent of bees kept under temperatures simulating future climates died in the first year and 70 percent died in the second, compared to a 1-2 percent in the control group (CaraDonna, Cunningham & Iler, 2018).

Temperature has an important role in insect development, affecting an animals' metabolic rate, nutritional requirements, physiology, digestion, nutrient assimilation, and nutritional choices (Radmacher, 2011). An increase in temperature has been shown to shorten development time for example however, beyond the organism's desired range (above or below) temperature can impose stress on the individual. Human activity is changing climates, the effects of which broadly include changing global temperature. The impact of climate change on pollinators could aggravate this pollinator crisis and have serious ecological as well as economic consequences (Radmacher, 2011). In nature, an animal's survival is impacted by levels of stress (Archer et al. 2014; Buchanan, 2000). Stress can be induced by a number of factors such as predation or changes in their environment (e.g. temperature changes or landscape change). Stress carries a cost, disrupting crucial systems like an individual's homeostasis often resulting in death (Buchanan, 2000).

A primary mechanism organisms use to combat stress is the targeted consumption of specific nutrition. An individual may expend resources to increase their survival while under stress, such as carbohydrate to fuel thermoregulation (Simpson & Raubenheimer 2012). Therefore, stress resistance is often directly connected to the amount (e.g. Bozinovic et al. 2007) or ratio of particular nutrients that an individual eats (Lee et al. 2006; Povey et al. 2009; Hawlena & Schmitz 2010). In cases where stress resistance relies on a specific nutrient, stressed animals need to consume more of that specific nutrient without reaching the point where it would begin to reduce their fitness. *Spodoptera littoralis* and *S. exempta,* for example, depend on a high intake of protein to improve immune responses when infected therefore, consuming a protein-rich diet would improve their fitness (Lee et al. 2006; Povey et al. 2009). Furthermore, organisms develop physiological and behavioural responses to counter the negative impacts of stress and increase their survival (Archer et al. 2014) but require resources to function. Povey et al. (2009) demonstrated how when offered a choice of diets, caterpillars under stress from a bacterial infection chose diets with a higher protein content than those chosen by healthy individuals. Diet quality is partly inferred by the amount of protein and carbohydrate obtained from the food and at what rate (Clissold and Simpson, 2015; Clissold, Coggan and Simpson, 2013). If temperature influences the supply of protein and carbohydrate then insects will use thermoregulation behaviours to optimise nutrient consumption (e.g. *Locusta migratoria* nymphs, see Clissold, Coggan and Simpson, 2013).

The Nutritional Geometric Framework (GF) technique allows us to investigate the foraging decisions made by an organism with relation to the multi-dimensional "nutrient space" (Simpson & Raubenheimer, 2012) and thereby enables detailed assessment of how an organism behaves nutritionally under stress. The GF is able to determine an organism's intake target (how much of what macronutrients to consume to maximise fitness; Simpson and Raubenheimer, 1993) as well as investigate the rule of compromise (i.e. the rules an animal follows to govern consumption when its intake target cannot be met with the nutritional options available (e.g. Lee et al., 2004; Simpson and Raubenheimer, 2001).

But what about species whose offspring rely on their parents for an optimal diet? Examples of parental care include seed-eating birds who feed young with insects, and humans as toddlers require food that is different from adult diets (British Nutrition Foundation, 2015). Although GF has uncovered a lot about the nutrition of some highly social insect species, including bees (Archer et al., 2014; Stabler et al., 2015; Vaudo et al., 2016), those studies primarily focused on adults because of the difficulty to investigate larval nutrition in social species. Effects of a changing nutritional landscape on the health of offspring in these species is already evident (Wright et al., 1998; Simpson & Raubenheimer, 2005; Han, Lawlor & Kimm, 2010; Anzman, Rollins & Birch, 2010) but little is known of the rules governing how any dependent offspring achieve balanced diets. For example, are offspring able to self-regulate or process a wide variety of diets? Or are they solely reliant on parents to select an optimal diet?.

Bees, like most animals, can sensibly balance their own diet when an adult (Stabler et al., 2015), but represent an extreme example of parental care. Adults (who have different nutritional requirements to their offspring) (Filipiak, 2018) supply young with pollen from the environment during their important growth phase. Therefore, larvae rely on the nutritional choices made on their behalf by their parents (Stabler et al., 2015, Vaudo et al., 2016). Pollen already varies hugely in nutritional quality, from 2% to 60% protein (Rouston, Cane & Buchmann, 2000) however, intensive agriculture is altering the nutritional landscape of the environment which may mean bees in agricultural landscapes are already feeding offspring a diet lower in protein (Donkersley et al., 2014; Ziska et al., 2016). This may reduce the fitness of their young and threaten human food security as pollinator numbers continue to drop and remaining pollinators are weaker/less efficient than previous generations. Unless bees are able to detect and correctly respond to these changes they would succumb to the “nutritional trap” i.e. the sudden changes to their nutritional environment strip away the bees’ evolved ability to detect those changes, resulting in a loss of fitness (Austin & Gilbert, 2018).

Solitary bee nutrition is poorly studied (Roulston & Cane, 2006). Developing our wealth of knowledge about pollinators can only benefit us, improving our ability to protect and maintain pollinator populations as our reliance on them grows while their numbers decline. Similarly, improving our understanding of global climate change as it becomes ever more extreme and unpredictable will prove crucial when devising strategies to aid pollinator resurgence. With that in mind, I proposed using careful manipulations within controlled-environment chambers to explore how rearing temperatures affect the functional responses of solitary bee larvae to multi-dimensional changes in dietary macronutrients.

I used *Osmia bicornis* as my model species, a cavity-nesting solitary bee commonly found across northern Europe and the UK (Falk 2015), with notable commercial importance (Jauker et al. 2012; Schulze et al. 2012). *O.bicornis*, like many solitary bees, breeds once a year; using a pre-existing cavity as their nest site (e.g. hollow plant stems, cavities in walls, etc.) (Giejdasz et al. 2016) creating a row of cells and filling each with a single pollen ball on which to deposit a single egg (see appendix). *O.bicornis* are a useful model species for experimental manipulation owing to their willingness to nest in artificial constructs and particular reproductive biology (Strohm et al. 2002) especially the fact that the pollen ball is manipulable after the parent has left.

I reared 258 *O.bicornis* larvae under 3 different ambient temperatures and, under the Nutritional Geometry Framework, provided one of an array of different diets varying in protein and carbohydrate content, using artificial pollen diets and rearing protocols previously developed and established by

Austin & Gilbert (2018). By assessing (1) the macronutrients eaten, and (2) the general fitness of each diet group (time to death and larval growth) I could investigate; (1) can bees detect and respond to changes in the nutritional composition of food? and (2) does a changing climate affect their ability to detect and respond to changes in the nutritional composition of food? Across an array of fixed diets, the different total amounts that individuals choose to consume can provide insight into the “rule of compromise” that the species employs, or how much of a deficiency/excess of nutrient Y they are prepared to tolerate in order to achieve a target amount of nutrient X (Simpson and Raubenheimer, 1993). If larvae are able to successfully detect and respond to the nutritional composition of the provision provided to them, I predict carbohydrate consumption to increase with an increase in temperature because of its role as an energy source and the link between heat and metabolic rates (Giejdasz & Fliszkiewicz, 2016). Miller et al., (2009) investigated how locusts select favourable temperatures to support growth and found the rate of food intake was significantly related to temperature where cooler temperatures resulted in slower intake rates. Similarly, I predict increasing temperature will lower survival as it may interfere with the evolved diapause behaviour, causing larvae to start spinning a cocoon before they are ready.

MATERIALS AND METHODS

Study Organism and Population Species of the solitary bee genus *Osmia* are managed in many countries, used to pollinate greenhouses of fruit and seed cultivations. The Red Mason Bee (*Osmia bicornis*) is one such species of solitary bee, common in Central Europe, that has become commercially available as a pollinator from February to July. *Osmia bicornis* (OB) is an early spring species, beginning its flight season in late March and ending in mid-June. Males are polygamous, where females are monandrous, and emerge a week earlier than females so they can spend the 3-4 weeks of their lives looking for mates. Once mated, a female will look for a cavity to build their nest, usually tube-shaped like the dry stem of a hollow plant or a cavity in a wall. Females show a preference for hollow plant stems like the common reed or dead wood with a diameter between 6-8 mm although this choice is ultimately determined by availability, the female’s size and the nesting resources available (Giejdasz & Fliszkiewicz, 2016). Cells are constructed along the length of the cavity by mixing mud with the female’s saliva and using it like a cement to build the cell walls and partitions between cells. Once constructed, the female collects pollen to store as a ball in the cell and lay an egg upon before sealing that cell and repeating until all the cells are full.

O.bicornis is a polylectic species, meaning they source food from a wide range of plants, however while nesting, females try to store pollen from only one species in a cell at a time (Giejdasz & Fliszkiewicz, 2016). The development of *Osmia bicornis* can be separated into several growth stages. In the spring the embryonic and larval development stages (which are further subdivided into five growth stages) take place, and then in the summer the prepupal and pupal stages occur (Giejdasz & Fliszkiewicz, 2016). The prepupal stage marks the beginning of reproductive diapause where the organism’s metabolic processes slow down, allowing it to survive harsh conditions before spinning a cocoon to develop through the winter period in.

I used a previously established population of *O.bicornis* at the University of Hull to source the larvae for this experiment, supplementing this sample with additional diapausing adults in cocoons (Mauerbienen®, Germany) to allow for adequate larval production. All cocoons were stored at 4°C and 70% relative humidity to overwinter until they were placed out in experimental nest constructs and allowed to hatch naturally in early May 2018. Nest constructs consisted of tube-like styrofoam blocks (Styrodur 3035CS) with a 9mm trough cut down the length, and a polycarbonate lid, which were housed within a wooden frame (Austin & Gilbert, 2018) (see appendix). Once females began laying, I checked nests daily to assess their progress, waiting until a nest was filled and sealed to bring back to the lab. However, I would also collect a nest if no more progress had been made on it during a week, under otherwise favourable conditions, taking this as an indication that the female using that nest had finished producing eggs. If any eggs hatched prior to nest completion I individually collected them along with their pollen provision. In response to an initial high larval mortality (attributed to premature manipulation), larvae were allowed to feed on their natural provision over the first few days post hatching (>3 days) before being allocated to treatment groups, and were then transferred to individual nest blocks (see appendix) on one of ten diet treatments. Once relocated and provided with a diet treatment, larvae were separated into 3 groups (explained below) and kept in complete darkness in an environmental chamber in 1 of 3 temperatures for the duration of larval development (see Treatments & Diet Formulation).

Treatments & Diet Formulation To investigate how larval *O. bicornis* manage their intake of protein and carbohydrates I used the Geometric Framework (GF). Using the GF, I can determine the decision rules (i.e. rules of compromise) an organism follows when provided with a food source with unbalanced specific nutrients (see Simpson and Raubenheimer (1993) for examples). Exploring the links between an organism's rule of compromise and a specific physiologically relevant nutrient could explain what governs animals foraging decisions (Simpson and Raubenheimer, 1993). Plus, the results can then be mapped against fitness proxies to show fitness peaks i.e. the point in nutrient space that should maximise fitness (Lee et al., 2008). In this study, I used GF to determine *O.bicornis* larvae's *rule of compromise* between protein and carbohydrates. Then using known fitness proxies, I map macronutrient consumption against them to predict a possible intake target for *O.bicornis* larvae; i.e. the optimal amount and ratio of specific macronutrients they require to maximise their fitness (Simpson and Raubenheimer, 1995).

I randomly assigned each of the 258 larvae to 1 of 6 protein:carbohydrate (P:C) groups and provided larvae with the corresponding diet which varied in macronutrient ratios and were at one of two different macronutrient concentrations, 47.5 and 40% (95% and 80%

before the addition of agar) (Table 1 & 2). Diets were created using honeybee-collected pollen and honey as a base, adding fixed amounts of protein (micellar casein, Sigma-Aldrich) and lipid (soy lecithin, Agros Organics) plus differing amounts carbohydrate (trehalose, trehalose.co.uk) to create the required P:C ratios. To create different concentrations I diluted the diets using sporopollenin (Austin & Gilbert, 2018), which is the indigestible outer wall of pollen (Roulston and Cane, 2000a). These components were premixed then set in equal volumes of 3% agar solution so they could be kept frozen until required.

To avoid dessication, degradation or mould all provisions were regularly replaced at intervals of ~3 days. All larvae were provided with similar provision masses however, the weight of provisions was not controlled with the intent to provide food in excess.

For the duration of the experiment, larvae were located in one of three temperature chambers at different temperatures; one group at 15°C, another at 20°C and the last at 25°C, (Sanyo MLR-351H; 15°C/20°C/25°C, 70% relative humidity). I decided on these temperatures by referencing previous and expected weather patterns to reflect the extremes of fluctuating weather *Osmia* could face, made more extreme by global warming. Records from the The Met Office show that temperatures during the *Osmia* mating period (May-July) in 2017 peaked at almost 20°C (Met Office, 2019) and the Met Office also predicts that UK summers could be over 5C warmer by 2070 (BBC, 2018) so I made the hottest temperature group 25°C.

Table 2. Diet Combinations

	Concentration 1 (95%)	Concentration 2 (80%)
P:C Ratio:		
A (1P:2C)	A1	A2
C (1P:4C)	C1	C2
E (1P:6C)	E1	E2

Table 1. Diet Protein and Carbohydrate Ratio

Diet Group	Protein:Carbohydrate Ratio
A	1:2
C	1:4
E	1:6

Experimentation Larvae were each allocated a diet and temperature treatment randomly then transferred to the relevant environmental chamber in an individual nest block with their artificial provision 4 days post hatching (Austin & Gilbert, 2018). Nest blocks, constructed out of styrofoam (Styrodur 3035CS) with a plastic scoop and lid, were weighed before use (see image in the appendix). First, the nest block itself was weighed, followed by the scoop, then the complete nest block with an added cube of diet. Finally, the larva was removed from its natal nest and placed on the cube of diet in the nest block and the complete nest block was weighed again with the larva on it. These measurements isolated the weight of each component of the nest boxes, allowing us to clearly track diet consumption. During each provision swap, the larva was temporarily removed and any frass was removed and put to one side. Next, the complete nest, including scoop and any remaining provision (if any is left) was weighed to calculate the amount consumed. Before supplying the larva with a new provision, I cleaned and re-weighed the scoop then placed it back in the nest block with a new cube of diet. Next, the complete nest block was weighed before adding the larva and weighing again, thus giving us a change in larval mass. All these measurements were recorded whenever the larvae's diet was swapped, using a 5 place balance (AND BM-252) to 0.1 mg.

Now with a provision, the larvae were placed into an environmental chamber at either 15°C, 20°C or 25°C at 70% relative humidity and left to develop in complete darkness for ~3 days before having their provisions swapped. To ensure larvae would not run out of diet or that the provision was still fresh they were swapped twice a week. However, I monitored the larvae daily to check their condition and for signs of key developmental stages (e.g. first defecation or the start of cocoon spinning). Once larvae began spinning a cocoon or they died I no longer continued swapping their diet and weighed them again when the cocoon was completed. At this stage, I removed the scoop and any waste material from the nest before returning the nest and cocoon to the environmental chamber it came from. To calculate total provision consumption, diet consumption was calculated per swap summed across diet swaps and protein and lipid/carbohydrate amounts were back-calculated from the final consumption value.

Alongside the treatments described above, two additional treatments were created; a control group and a group to monitor water loss in the diet provisions. Control diets consisted of natural *O.bicornis* provisions and diluted with sporopollenin (80% and 95%) before being set in agar like the rest of the artificial diets. Larvae were placed on these diets and processed the same as all other experimental groups. The second treatment had no larvae assigned to it and was set up to monitor loss of moisture in all diet provisions under the 3 different temperatures. These diets were monitored for 6 days, more than the maximum time between diet swaps in the experimental groups, and weighed each day.

Statistical Analysis All statistical analysis was carried out using R version 3.5.1 and R Studio (The R Foundation, 2019). Nutrient consumption was analysed by comparing the data from each larva between each swap as to analyse how protein and carbohydrate intake changes throughout the duration of development under different temperatures. Using data from the water loss test group (described above) rate of water loss was calculated and used to adjust all consumption data whereupon the actual nutrient content was calculated with reference to the known nutrient percentages in the diets. Any larvae that died before receiving the initial diet swap were removed from subsequent analysis.

To visualise larval growth in relation to the amount of protein and carbohydrate consumed over the duration of their development period, I produced response surfaces using splines. To maximise sample sizes and therefore statistical power, I amalgamated data from consecutive swaps where possible, i.e. swaps where the pattern of growth in relation to protein and carbohydrate eaten was similar. I used Generalised Additive Mixed Effect Models (GAMMs) before creating the splines to determine whether swap numbers should be amalgamated, using the lme4 (Bates et al., 2018), gamm4 (Wood, 2017), and mgcv (Wood, 2019) packages to do so. GAMMs were fitted using weight change per swap as the response variable and swap number as a predictor, with a bivariate smooth fitted to protein and carbohydrate for each level of swap number. I began by fitting a GAMM with none of the swap numbers amalgamated and then fitted GAMMs where swap numbers were merged, working backwards starting with the final swaps, and comparing results. I selected the best fitting models based on the AIC of each GAMM (the lower the AIC the more significant it was, and I regarded models within 2 AIC points as equivalent). After this, I produced non-parametric thin-plate splines as the response surfaces based on the GAMM amalgamation. Splines were fitted using protein and carbohydrate intake data as predictors and the larval growth data as the response surface.

Survival over the larval period (up to pupation) was conducted by fitting survival regression models using the survreg function (Therneau, 2015) in the survival package in R. Time was measured as the number of swaps that larvae received. Predictor variables included in the analyses were the diet they were provided and the temperature of the environment.

RESULTS

Consumption

The ambient temperature at which a larvae developed affected most measured aspects of their development. The temperature larvae were kept in during the test period directly affected how much protein and carbohydrate was consumed at each swap (Fig. 1 & 2) evidenced by a clear 3-way interaction between temperature, ratio and concentration (linear model containing temperature, diet ratio, concentration and their interactions as predictors. Carbohydrates; $F_{2,217}$=9.78, p=<0.001, and Protein; $F_{2,217}$=14.14, p=1.695e-06). The greatest effect of temperature on nutrient consumption was seen in the diets at 47.5% concentration (Fig. 1). The amount of carbohydrates consumed peaked in 20°C but was the lowest in 25°C, unlike the amount of protein consumed which was highest in 25°C and lowest in 20°C (Fig. 1).

Larvae underwent an unexpected change in behaviour when developing at 25°C (Fig. 1 & 2). At 15°C and 20°C, larvae controlled how much carbohydrate they were ingesting while letting protein intake vary approximately with the protein:carbohydrate ratio of the diet, whereas at 25°C their behaviour shifted so the opposite was true (Fig. 1a,b). Similar results were seen for weight gain (see below).

The 2-way interaction between diet concentration and dietary P:C ratio had a significant effect on the amount of carbohydrate consumed (linear model containing diet ratio, concentration, and their interactions as predictors. Carbohydrate; $F_{2,217}$=3.4, p=0.03598), unlike protein (Protein; $F_{2,217}$=1.5, p=0.22272). The amount of carbohydrate consumed increased as diets became more carbohydrate biased (i.e. typically highest when fed E diets), and this effect was enhanced on more concentrated diets (i.e. A1, C1, E1 which were 47.5% diet) (Fig. 1).

Larval Weight Gain

There was no significant statistical effect of temperature on larval weight gain (linear model using temperature, diet ratio, and concentration as predictors; $F_{1,224}$=0.04, p= 0.845). In contrast, larval weight gain was influenced greatly by the effects of dietary P:C ratio and concentration independently of each other (linear model of total larval weight gain with diet ratio, concentration, and their interactions as predictors. Ratio; $F_{2,225}$=6.8, p=0.0013. Concentration; $F_{1,225}$=18, p=3.45e-05). Diets with a higher amount of protein resulted in higher growth rates on average (i.e. diets A1 & A2 produced the largest weight gain and diets E1 & E2 produced the smallest weight gain) (Fig. 3 & 4). Similarly, this pattern remained true across the two concentrations however, larval weight gain was much higher on diets at 47.55% diet concentration (Fig. 4).

In addition to looking at the macronutrient ratios and concentrations in the diets presented to larvae, I also examined a model of weight gain with temperature and the actual consumption of carbohydrate protein as predictors. The effect of the 3-way-interaction was not statistically significant, however the interaction between temperature and amount of carbohydrate consumed had a significant effect on larval weight gain (linear model of total larval weight gain with protein consumed, carbohydrate consumed, temperature, and their interactions as predictors. $F_{1,221}$=4.5, p=0.035). While consuming the same amount of carbohydrate, larval weight gain decreased with an increase in temperature, larvae in 15°C being significantly larger on average (Fig. 3). In contrast, larval weight gain was influenced greatly by the effects of protein consumed and carbohydrate consumed independently of each other (Protein consumed; $F_{1,221}$=69, p=1.023e-14. Carbohydrate consumed; $F_{1,221}$=15, p=0.0001404). However, protein clearly had a greater effect, with larvae growing much larger when consuming a higher amount of protein than carbohydrate (Fig. 3).

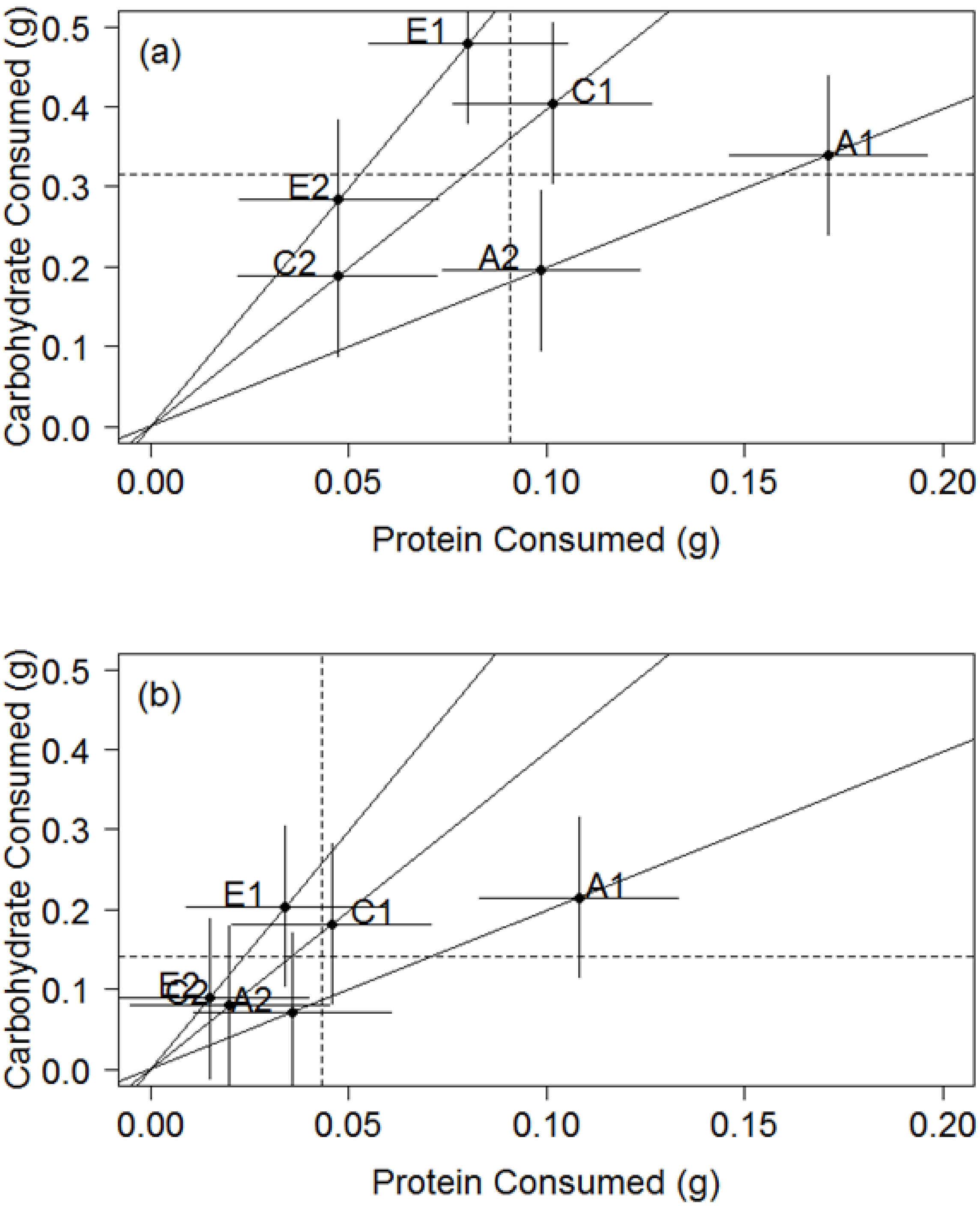
Survival/Time To Death

The experiment yielded a high mortality rate, with only a handful of larvae reaching the cocoon spinning stage. This was the case across all diet and temperature groups (Fig. 4 & 5), and so results must necessarily be treated with caution and interpreted relatively loosely.

Time to death was not affected by the 3-way interaction between temperature, dietary ratio and dietary concentration and instead was influenced greatly by the effects of dietary concentration and temperature independently of each other (Concentration; X^2_2=19.4, p=1.06e-05, Temperature; X^2_2=15, p=1.01e-04) (Fig. 4 & 5). Time to death was similar for all diet ratios at 20°C and 25°C, but at 15°C, the E diets (the most carbohydrate biased) lived longest (Fig. 4 & 5).

Time to death was longest in an environment of 15°C, with all but one diet group reaching ~60 days before all larvae in that group either spun a cocoon or died. Conversely, time to death was shortest in 25°C with larvae dying before 40 days (except one group that lasted for 60 days) however, time to death in 20°C was similar with most groups finishing on and after 40 days plus one group reaching 70 days. Across all diet and temperature groups, time to death was longest on the diets with the highest concentration of diet (Fig. 4 & 5).

I then looked at nutrients actually consumed, rather than simply their concentrations in the diets presented to the larvae. To do this, I examined a model of time to death with temperature, the amount of carbohydrate consumed and the amount of protein consumed as predictors. The effect of this 3-way-interaction was not statistically significant, however time to death was significantly affected by a 2-way interaction between temperature and amount of protein consumed (Protein:Temperature; X^2_1= 4, p=4.5e-02). The effect of protein consumption upon time to death was generally positive, but was greatest at high temperatures, such that in an environment of 25°C time to death was maximised when consuming high protein:low carb, followed by high protein:medium carb (Fig. 4 & 5). Similarly, the 2-way-interaction between the amount of protein and the amount of carbohydrate consumed significantly affected time to death (Protein:Carbohydrate; X^2_1= 8.6, p=3.3e-03). Generally, time to death was improved with a high consumption of either nutrient but protein consumption was more beneficial, with a ratio of high protein:low carbohydrate consumed or high protein:medium carbohydrate consumed resulting in some of the longest time to death across all temperature groups (Fig. 4 & 5). No other interactions significantly affected time to death.


(a)
E1
C1
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0.0
0.1
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Carbohydrate Consumed (g)
0.00
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0.10
0.15
0.20
Protein Consumed (g)
(b)
E1
C1
A1
E2
C2
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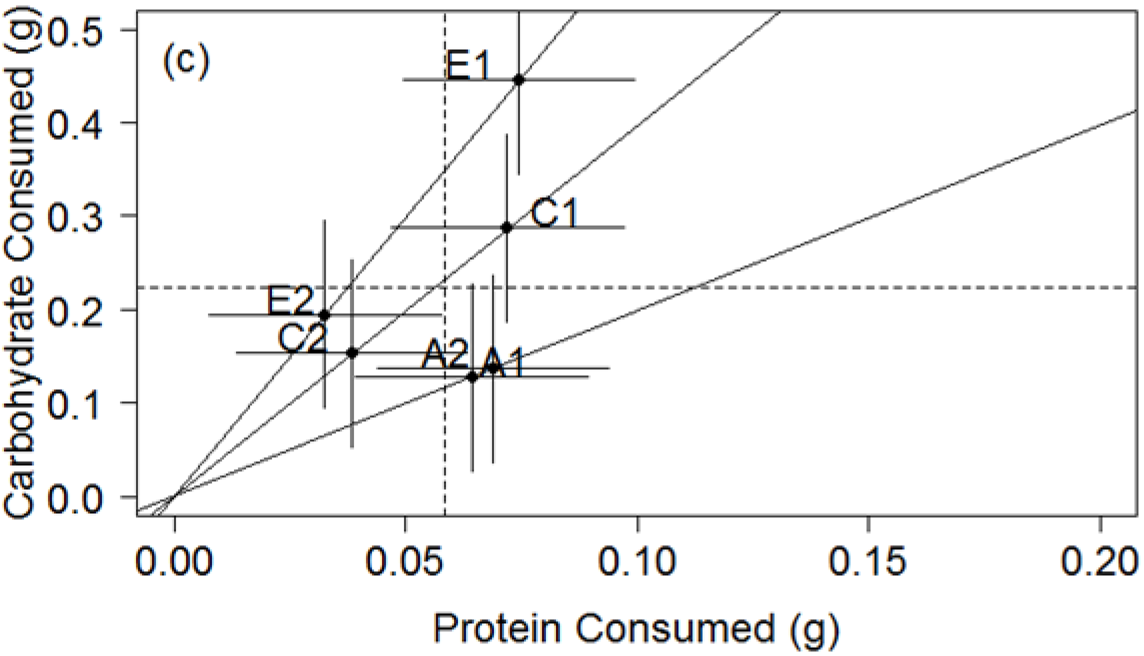


Fig. 1. Average amount of Protein and Carbohydrate consumed, in grams, by larvae on each diet in each temperature. (a) 15C, (b) 20C, (c) 25C. Letters and solid lines represent the three diet P:C ratios (A = 1:2, C = 1:4, E = 1:6). Numbers following the letters represent diet concentration (1 = 47.5%, 2 = 40%). Dotted lines represent average nutrient consumption of all larvae (horizontal = Carbohydrate, vertical = Protein).

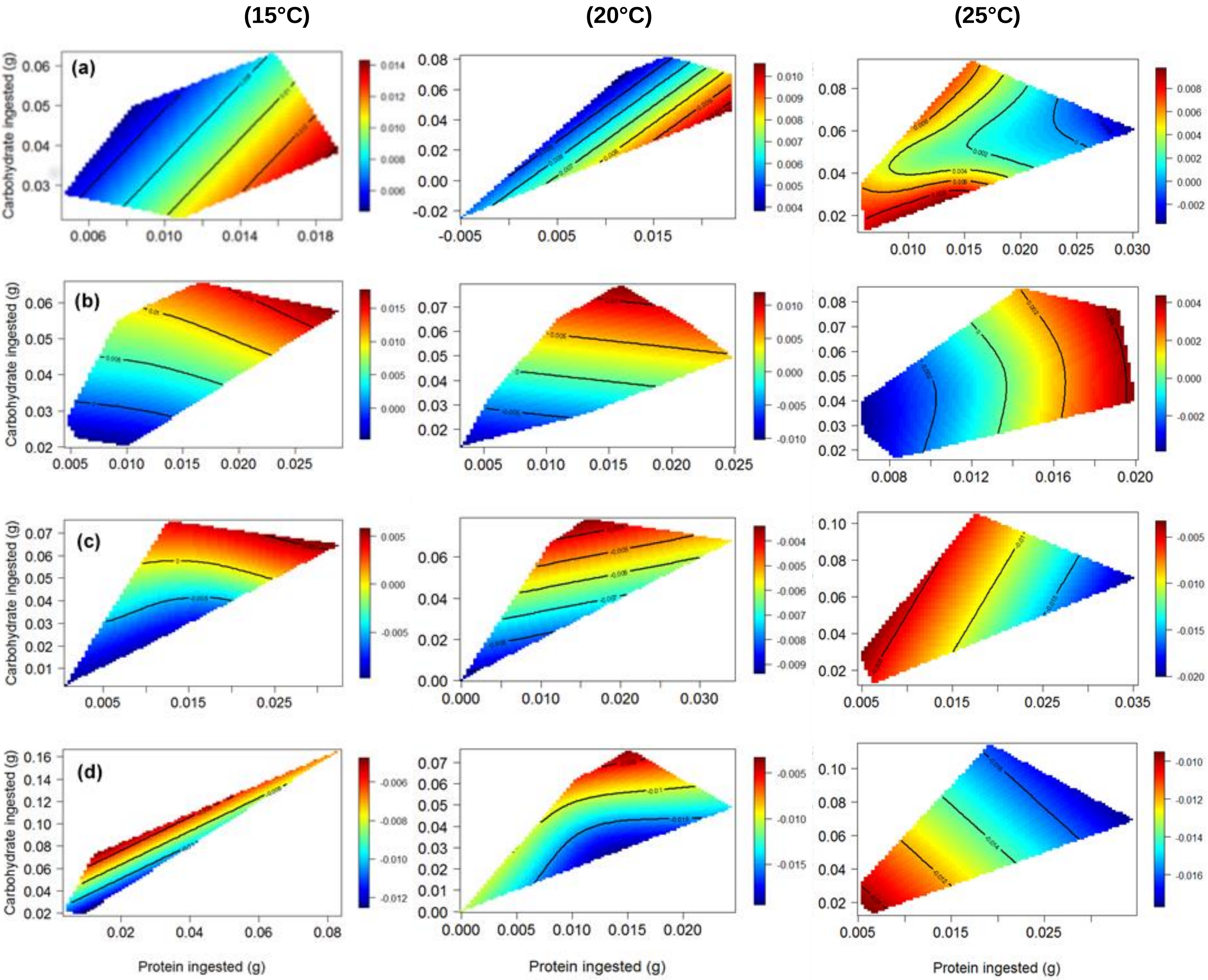


Fig. 2. Surface plots showing weight gain of larvae ((g), colour scale) plotted against protein and carbohydrate ingestion over the entire course of manipulation for larvae at 15°C, 20°C, and 25°C. Red shows the most growth (or least loss of mass) and blue shows the least growth. The four individual splines per temperature group show growth rate at different stages of the experiment; **(a)** shows growth rate at swap 1, **(b)** at swap 2, **(c)** amalgamated results for swaps 3 to 5, and **(d)** amalgamated results for swaps 6 to 16.

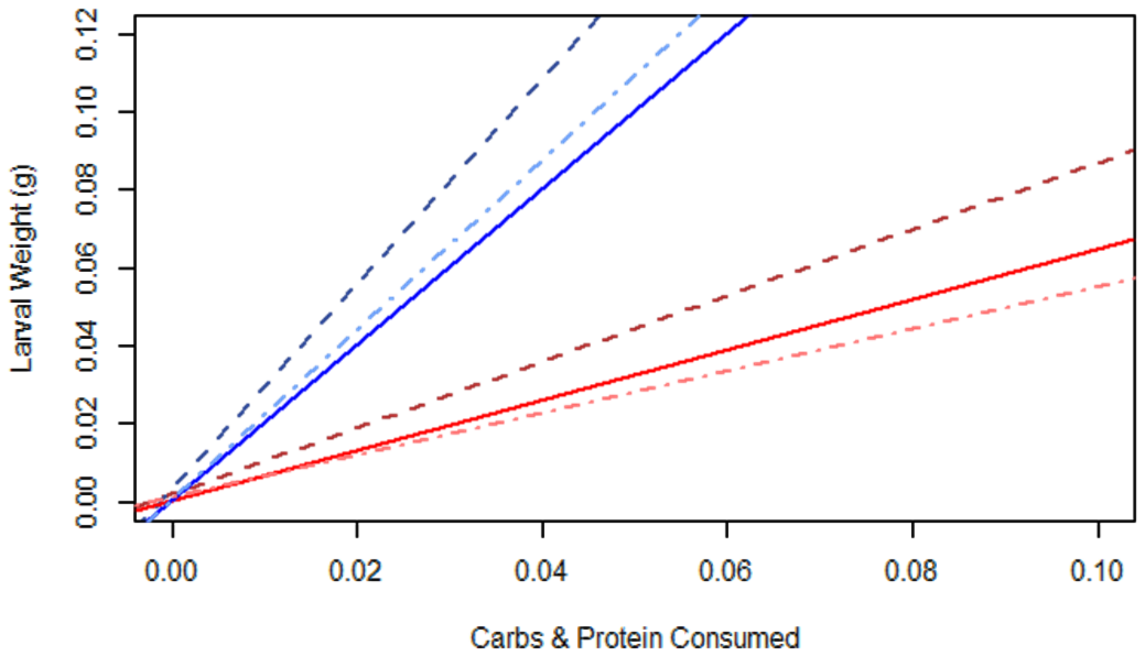


Fig. 3. Estimated larval weight gain (per swap) against the amount of protein or carbohydrate consumed in each temperature group. Lines represent the estimated best fit lines from the linear model (see text for details). Key to groups: Blue=Protein, Red=Carbohydrate; Dark shade/Dashed line=15°C, Regular shade/Solid line=20°C, Light shade/Dot Dashed line=25°C.

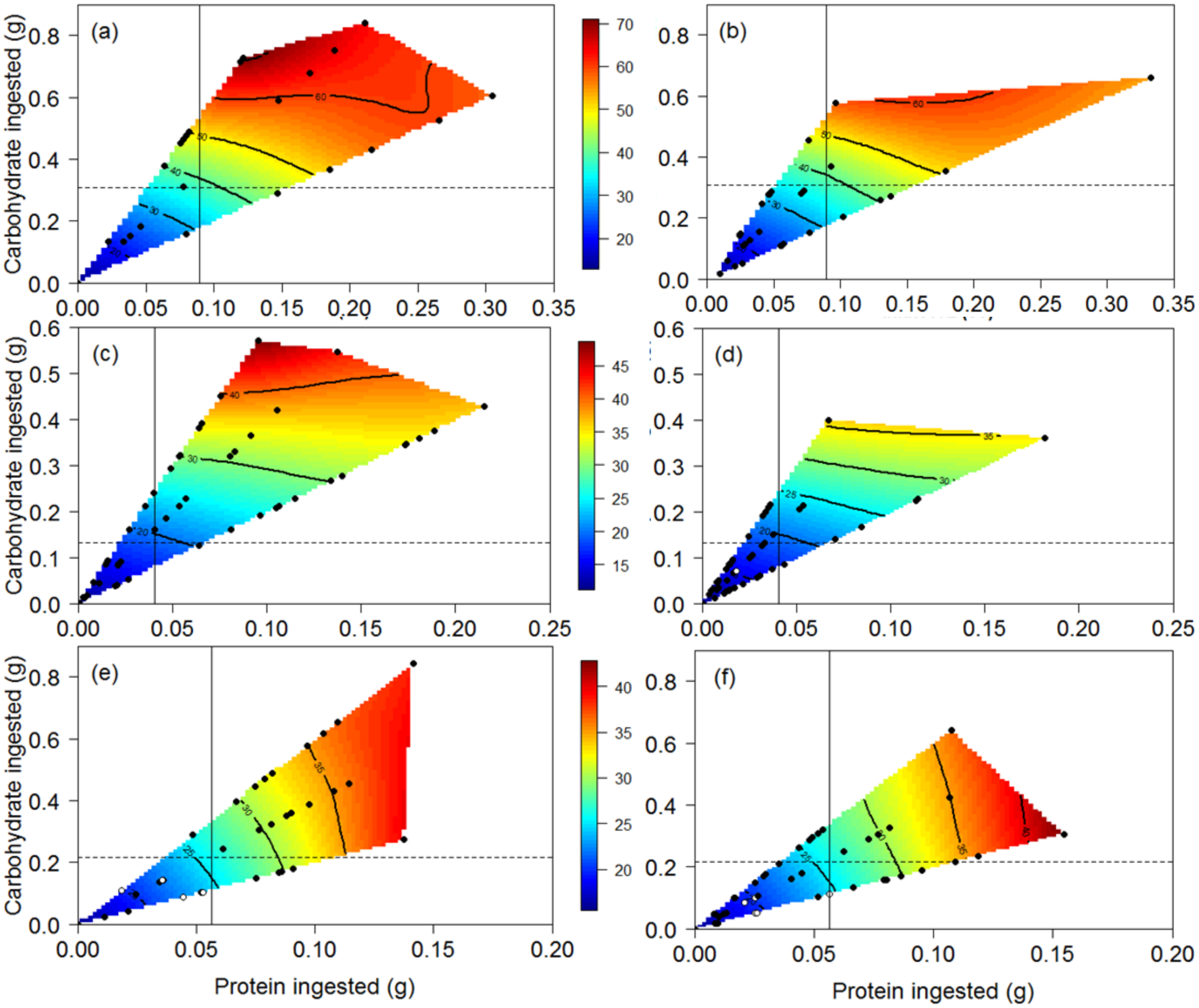
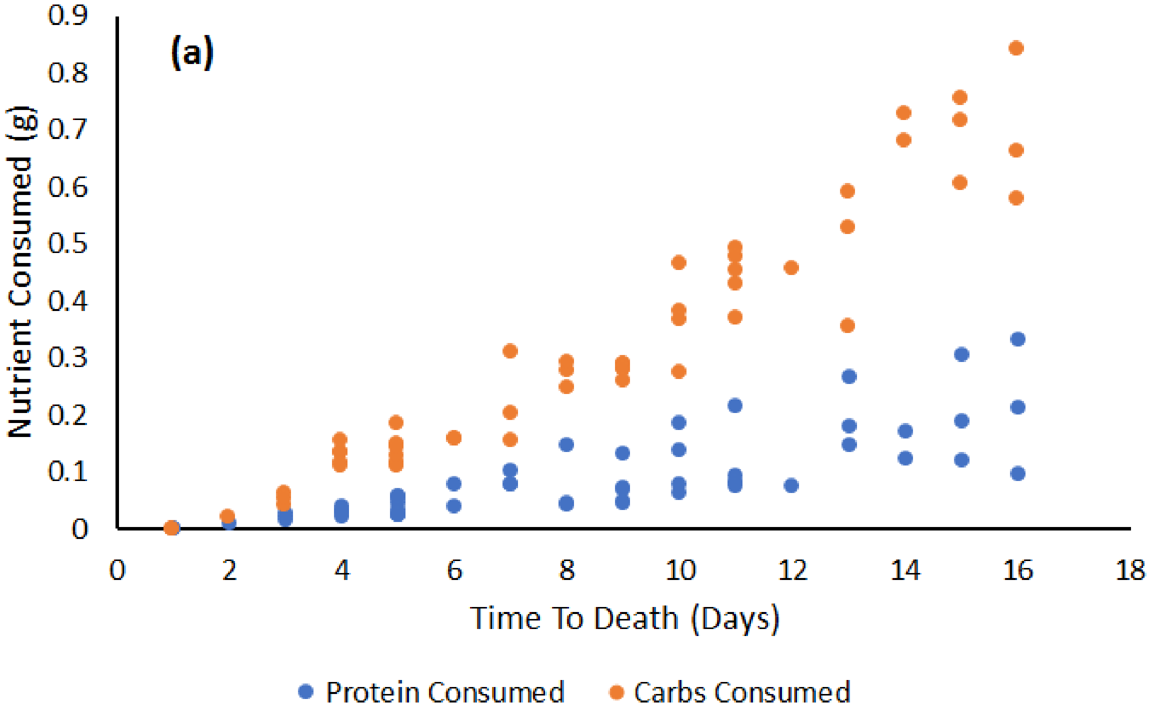
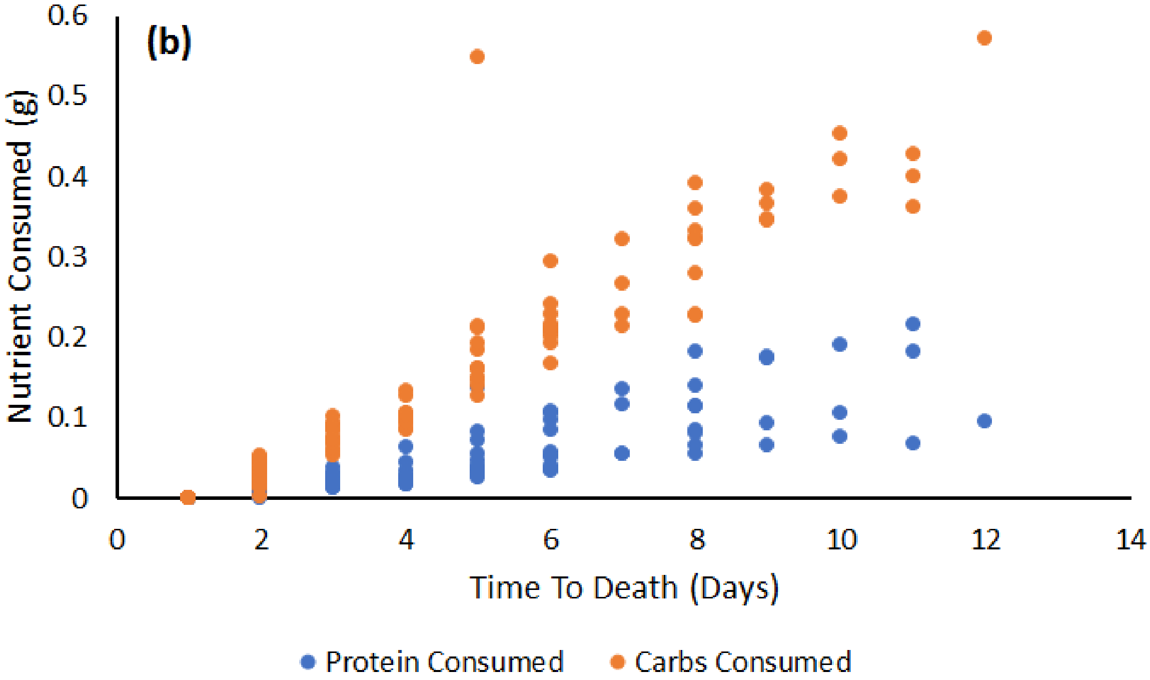


Fig. 4. Surface plots showing body mass of larvae ((g), colour scale) plotted against protein and carbohydrate ingestion (g) for larvae fed on diets of two different concentrations and at 15°C, 20°C, and 25°C. (a & b) represent larval growth at 15C, (c & d) at 20C, and (e & f) at 25C. (a, c & e) represent larval growth on 47.5% concentrated diets, and (b, d & f) on 40%. The horizontal dotted line depicts the average carbohydrate consumption, and the vertical solid line depicts the average protein consumption. Dots represent individual larvae, black dots show larvae that died and white show larvae that survived.


(a)
Nutrient Consumed (g)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
14
16
18
Time To Death (Days)
● Protein Consumed
● Carbs Consumed


(b)
Nutrient Consumed (g)
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
14
Time To Death (Days)
● Protein Consumed
● Carbs Consumed

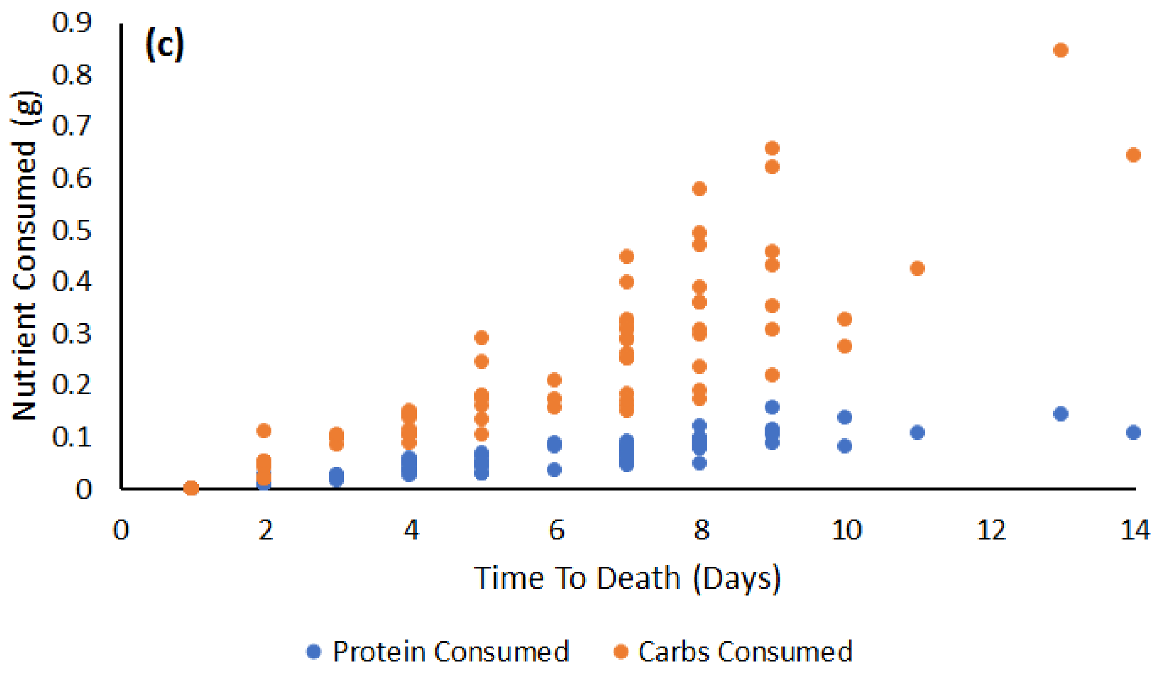


Fig.5 Larvae time to death (in days), from across all temperatures, in response to the amount of carbohydrate and protein consumed. **(a)** represents survival data from 15°C **(b)** from 20°C, and **(c)** from 25°C. Key to groups: Blue points = Protein consumed, Orange points = Carbs consumed.

DISCUSSION

Temperature is one of the most important environmental factors affecting the development of ectotherms. The temperature experienced by an insect during development affects many of its important life history traits including their body size, development time, and fecundity (Radmacher, 2011). Ambient temperatures in temperate regions vary greatly, meaning insects experience significant variations in temperature while undergoing development. Effects on development here will be reflected in the insect its whole life (Roeder and Behmer, 2014), so it is important that we understand our impact as we continue to influence global temperatures. Some eusocial insects, like the honeybee, are able to maintain a constant average temperature for their brood to develop in and negate the effects of fluctuating temperatures (which is not to say that changing environmental temperatures have no effect on honeybees, Radmacher 2011). However, most bee species are solitary who cannot thermoregulate for their brood, leaving them exposed to fluctuating temperatures. Without the ability to use thermoregulation, we might expect solitary bee species to have developed strategies or mechanisms of dealing with fluctuating temperatures during development.

In this thesis, I investigated whether *Osmia bicornis* larvae demonstrated any adaptive mechanisms when exposed to different ambient temperatures during the prepupal stage of their development by feeding groups of larvae on different diets of known protein:carbohydrate ratios under one of three ambient temperatures. Although I did not clearly identify an optimal intake amount for either nutrient, it is clear from my results that larval *Osmia* *bicornis* prioritised consuming an optimal amount of carbohydrates while developing, at least at lower temperatures (Fig. 1, 2 & 4). Carbohydrates are necessary for optimum growth, development, reproductive activity, and survival; but in *Osmia's* case also diapause. Diapause is a period of developmental rest where larvae spin cocoons and let metabolic processes slow down to survive the winter. The body size of *Osmia* is related to the size of their nutrient stores and likely reflects the size of their fat body, the area of an insect's body where carbohydrate-derived fat is stored (Bosch et al., 2010). A large fat body is crucial for surviving diapause therefore it is necessary for larvae to receive a high dietary carbohydrate concentration to meet the chemical and physical demands of diapause which helps to explain the larvae's prioritization of carbohydrates (Fig. 1, 2 & 4), selecting for the nutrient that would best improve their fitness when they emerge as an adult. However, insect larvae differ in amounts of dietary carbohydrates required for their normal growth and development (Satyasri Ch, 2017); Figure 1, 2, 3 & 4 shows that carbohydrate-derived growth in *O. bicornis* is highest at low temperatures. A colder summer may predict a colder winter, in which it would be important to lay down enough C for diapause, and larvae appear to detect and respond to this by prioritising meeting an optimal carbohydrate intake target while developing in the lower temperature groups (Fig. 1, 2 & 3).

The body size of an insect is directly related to their fitness in many ways (Honěk, 1993). In the case of solitary bees, being larger provides advantages to foraging efficiency, fecundity, nest usurpation, and overwintering survival (Radmacher & Strohm, 2009). Across the different diets used in this study, larval weight was positively associated with the percentage of protein in the diet (Fig. 2 & 4). Diets A, C, and E had a protein:carbohydrate ratio of 1:2, 1:4, and 1:6 respectively and, as established by Figures 1, 2 & 4, larvae were eating to reach a carbohydrate target with apparently no regard for the amount of protein they were consuming (Simpson & Raubenheimer, 1993. See their figure 2). To meet the carbohydrate intake target, larvae fed on diets A1 & A2 were consuming the most protein while consuming similar amounts of food as groups C and E (Fig. 1) as that diet had the greatest protein content (P:C 1:2) which resulted in greater weight gain. Diets with carbohydrates at high concentrations have been known to inhibit some biological processes however (Satyasri Ch, 2017). For example, dietary pentoses, arabinose, ribose, and xylose inhibit larval growth of southwestern corn borer, *Diatraea grandiosella*, and yellow mealworm, *Tenebrio molitor* (Satyasri Ch, 2017) so maintaining a balance must be important. Where choice is limited to only a single diet, we would expect larvae to have evolved a rule of compromise that maximises their fitness while avoiding the inhibiting effects of consuming too much of one nutrient. “Nutrient-generalist” species (like most solitary bee species) who find nourishment from a wide range of diet compositions are able to tolerate swings in availability of the nutrient which is least limiting (Simpson and Raubenheimer, 2012). *O.bicornis* larvae on fed on pollen provisions which are among the most protein rich sources of plant nutrition (Mattson, 1980), with protein content ranging between 2-60% unlike carbohydrate often being the limiting nutrient at 0-22% (Roulston and Cane, 2000a). Therefore, given the abundance of protein in their natural food source, *O.bicornis* larvae are more likely to find carbohydrate limiting than protein, and so theoretically should aim to meet a carbohydrate intake target. Furthermore, tolerance to variation in dietary protein like that seen in this study is generally linked to predators (Kohl et al., 2015; Raubenheimer et al., 2007). Although bees are considered herbivorous and typically follow protein-based decision rules (e.g. adult bumblebees, Vaudo et al., 2016), bees have a common ancestor with ants, almost all predators and which favour a carbohydrate target wherever studied (Peters et al., 2011; Dussutour and Simpson, 2009). As stated above, body size is directly related to fitness, however body size may be a lower priority to *O.bicornis* larvae than meeting their carbohydrate intake target given their tolerance to excess protein.

Diapause is one reason why carbohydrate intake may be a priority in *O.bicornis.* The species breeds once a year, diapausing over the winter period before hatching out in the Spring (March/April) (Falk 2015). Diapause is a state of developmental rest where preparatory biochemical adjustments occur, such as the accumulation of lipid and carbohydrate reserves, which enables the insect to survive otherwise adverse environmental conditions (Satyasri Ch, 2017). Most diapausing insects rely upon reserves of carbohydrates like glycogen and trehalose, as well as small amounts of proteins and amino acids, to support their metabolism. These reserves mean the insect can supply glucose to meet the limited energy demands and begin the synthesis of polyhydric alcohols. Polyols protect the insects by collecting in the tissues and contribute to increased cold hardiness of the insects, enhancing the already present supercooling abilities in those species where freezing would prove fatal and minimising damage in the freezing tolerant insects (Satyasri Ch, 2017). *Osmia* would therefore require a reserve of carbohydrates which they are attempting to build up by selecting for carbohydrates in the experimental diets (Fig.1, 2 & 4).

As an adult, bees are able to sensibly regulate their own nutrition. However, bees also represent an extreme example of parental care by providing exclusively for their offspring who have unique nutritional requirements. Adult *Osmia* supply their young with pollen from the environment in a single provision that should last their important growth phase so the young rely on the nutritional choices made on their behalf by their parents (Stabler et al., 2015, Vaudo et al., 2016). Pollen varies markedly in nutritional quality, from 2% to 60% protein for example (Rouston, Cane & Buchmann, 2000), and intensive agriculture is altering the nutritional landscape of the environment which may mean for some bees it will be harder to provide their offspring with the correct balanced diet. As a result, this could reduce the fitness of their young unless during their prepupal phase bee larvae can detect the quality of the pollen ball and respond accordingly. My experiment suggests larvae were able to register the protein:carbohydrate ratio of the diets I provided and adjust their intakes accordingly. Each group of larvae were provided with a diet that varied in protein:carbohydrate ratio, with the diets becoming more carbohydrate biased each time (P:C 1:2, 1:4, 1:6). However, while all larvae were consuming similar amounts of food they controlled their intake of carbohydrate to similar levels across all diets, at least at 15°C and 20°C (Fig. 1, 2 & 4). If a larva had no way of regulating nutrients ingested, we would expect larvae to have consumed markedly more carbohydrates as diets became more carbohydrate rich while consuming the same amount of food, given the only choice available to larvae in this experiment was how much food they should eat not how much of a specific nutrient. Since the larvae in my study appeared to regulate carbohydrate, they may depend on their parents to regulate how much protein they are provided with. Some studies have found that bee parents may be able to detect the macronutrient composition of pollen because of the naturally large variation of protein content in pollen and therefore control the composition of the pollen provisions they provide their offspring with (Muth et al., 2016; Ruedenauer et al., 2016). However, many other studies have shown otherwise (Konzmann and Lunau, 2014; Roulston and Cane, 2002; Roulston and Cane, 2000b). Instead, adults may collect pollen for their young without thought for its composition, relying on the larva’s tolerance to varying protein content (as shown in this study) and the variation in pollen quality naturally balancing out because of a history of rich floral diversity in the environment (Bukovinszky et al., 2017). The current agricultural landscape removes floral diversity for expanses of monocultures perhaps making this strategy harmful which means the larvae’s ability to deal with varying amounts of protein is crucial. Future research should now concentrate on post-ingestive processing of macronutrients by larvae.

The surface plots (Fig. 2) reveal how early larvae start making decisions about their nutrition. The surface plots show how the body mass gain of larvae (colour scale) changes depending on both protein and carbohydrate ingestion after certain stages of the manipulation. Larvae kept at 15°C and 20°C clearly gained the most mass when eating more protein for the first few days of development (swap 1), but for the rest of their development larval growth was associated with high carbohydrate consumption. The development of *Osmia bicornis* can be separated into several growth stages. In the spring the embryonic and larval development stages (which are further subdivided into five growth stages) take place, and then in the summer the prepupal and pupal stages occur (Giejdasz & Fliszkiewicz, 2016). A major aspect of these early larval stages of development are preparing for the reproductive diapause where larvae spin cocoons to survive the winter. It may be that the early protein consumption characterises a period of initial somatic growth, but that a switch to carbohydrate consumption characterises the preparatory diapause step, developing a large carbohydrate store to be tapped for resources over winter. Evidence of adaptive behaviours such as this might inform how we protect solitary pollinator species. For example, by sowing plants that will provide pollen with a high protein content (e.g. a member of the Brassicaceae family; Liolis et al., 2015) early in the season to compliment their developmental requirements.

Observed behavioural patterns changed at 25°C. The amount of food eaten at each swap was relatively consistent between temperature groups, but increased slightly in 25°C heat (Fig. 1). Remarkably, larvae demonstrated a complete reversal of their rules of compromise while developing under 25°C heat, once prioritising carbohydrates (in 15°C & 20°C heat) to then shift to a protein-based rule of compromise (Fig. 1, 2 & 4). Carbohydrate is essential for insect development and is directly related to successful diapause, however climatic conditions of 25°C heat during summer may indicate this may no longer be the priority. Giejdasz and

Fliszkiewicz (2016) investigated the effect temperature had on the development of *Osmia bicornis*, moving newly occupied *Osmia* nests to laboratory incubators, monitoring their development at three constant temperatures, 20˚C, 25˚C, and 30˚C. They revealed a clear linear relationship between temperature and development time, with the egg stage of development ending after an average of 3 days at 20˚C and 25˚C but one day shorter when the temperature was raised to 30˚C (Giejdasz & Fliszkiewicz, 2016). It is well known that temperature accelerates many physiological processes, including metabolism and oxygen consumption (Giejdasz & Fliszkiewicz, 2016), but importantly it has been shown to control the onset of diapause. In the case of *Osmia bicornis*, the beginning of diapause is signalled by depletion of fat body resources and the inhibition of ovary development but it is possible to manipulate this with high or low temperatures (Giejdasz & Fliszkiewicz, 2016). The shifting of prioritizations from carbohydrates to protein in this study may relate to the larvae responding to the temperature by delaying diapause and as a result having to cater to different requirements. Furthermore, I observed that at high temperatures time to death was the longest on heavily carbohydrate-biased diets (Fig. 5) however within that, time to death for larvae that consumed the most protein was longest in high temperatures (Fig. 4 & 5). There is a clear shift in intake rules towards regulating protein under 25˚C (Fig. 1, 2 & 4) but the only choice the larvae could make to meet this intake target is to control how much diet they eat in total. Larvae provided with the heavily carbohydrate-biased diets would have to digest a high amount of carbohydrates along with the minimal amounts of protein to reach their protein intake target. Most insects convert excess carbohydrates to lipids and store it. Deposition of lipids has physiological costs however, sometimes requiring up 20-25% of the energy content from the food they have just digested (Le Gall & Behmer, 2014), and a study which involved rearing *Heliothis virescens (Fabricus)* caterpillars on diets ranging in protein and carbohydrate levels showed that eclosion success for males was lower when they consumed an excess of carbohydrates (Roeder & Behmer, 2014). In this study, the high ambient temperatures would have generated a high metabolism in the larvae which the excess carbohydrate consumed could have gone towards fueling and perhaps negate some of the processing costs otherwise seen. Keeping *Osmia* larvae in constant, controlled temperature while it develops affects its ‘activation time’, so rearing larvae like this could be a good way to manage commercial populations and synchronise adult emergence with selected crop bloom periods (Giejdasz & Fliszkiewicz, 2016).

In nature, an animal’s fitness can be negatively impacted by stress, which can be applied by several factors ranging from changes in the environment to predation (Archer et al. 2014; Buchanan, 2000). By increasing the ambient temperature at which the larvae were developing, I applied a degree of stress the larvae had to combat. Even moderate stress exposure elicits responses that require resources and although 25˚C heat proved beneficial to growth rate it can negatively impact survival. Giejdasz and Fliszkiewicz’s (2016) study for example showed an increase in the mortality rate of adult insects developing at 25℃ or 30℃ during the wintering period. Mortality was similar across all temperature groups. However, time to death got shorter as temperature increased (Fig. 5). The only larvae to last 16 days before perishing were in the 15℃ group (Fig. 5).. Organisms develop physiological and behavioural responses to counter the negative impacts of stress and increase their survival, but resources are often required in order to maintain or produce these responses (Archer et al. 2014). Key resources generally include energy and/or specific nutrients such as carbohydrates (used to fuel thermoregulation) and protein (used to synthesise detoxification enzymes) (Archer et al. 2014). As a result, stress resistance scales with the amount or ratio of nutrients the individual consumes (Archer et al. 2014). In the cases where an organism’s stress resistance depends upon a specific nutrient, the stressed individual should prioritise consuming more of the limiting nutrient in order to improve their fitness as a result of trade-offs with other factors (Archer et al. 2014). *Spodoptera littoralis* (Egyptian cotton leafworm) immune responses, for example, rely on their protein intake and so immune responses are stronger for individuals who consume protein-rich diets (Archer et al. 2014). Similarly, rats that were exposed to low temperatures chose to increase their consumption of carbohydrate, and maintain how much protein is consumed in order to meet the energy demands of thermoregulation (Musten, Peace & Anderson, 1974). Like the rats of Musten et al’s study (1974), *Osmia* maintained a constant protein intake rate across all diets in 25℃ while carbohydrate consumption fluctuated, generally similar to the other temperature groups but increasing with the more carbohydrate rich diets (Fig. 1, 2 & 4). It is possible this switch of regulation from carbohydrates to proteins the *Osmia* demonstrated in this study was to combat the energetic demands applied by the significant increase in temperature.

Larval weight was positively associated with protein consumed across the different diets used in this study (Fig. 2, 3 & 4). Body size is directly related to fitness in many insects (Honěk, 1993) and in solitary bees, studies have shown that having a larger body size provides individuals with advantages in foraging efficiency, fecundity, nest usurpation, and overwintering survival (Radmacher & Strohm, 2009). Therefore, factors affecting offspring body size (like temperature) in solitary bees could play an important role in reproductive success and population dynamics (Radmacher & Strohm, 2009). For an estimation of potential consequences of climate change on bees, fundamental knowledge about their thermal requirements and restrictions would be required, but we know little about how climate change will affect bees, and in particular how bees will deal with changed nutritional landscapes in an uncertain future.

Despite the generally high mortality, it was reassuring that I observed the larvae in this experiment are shown to be following the temperature size rule, which states that ectotherms will develop more slowly and develop a larger adult body size while developing under lower temperatures (see Atkinson, 1994 for examples) (Radmacher, 2009). On average larval weight gain increased as temperature decreased (Fig. 3) and the most cocoons were spun in 25°C heat (the hottest temperature group) as larvae there reached pupation before the rest. Of the 14 larvae that spun a cocoon, 11 of them developed in 25°C heat and began spinning a cocoon several weeks before the other temperature groups. Note that development rate could not be modeled, as mortality in this experiment was high meaning few larvae reached the pupal stage. Furthermore, larvae kept in an environment of 15°C (the coldest temperature group) developed the slowest, receiving more swaps on average (Fig. 5). We know that in nature the rate of larval development depends on air temperature that fluctuates throughout the day. Depending on the stage of development, for *Osmia* air temperature in spring reaches a physiological zero between 9.5 to 14.2, the temperature below which there is no embryonic development (Maeta, 1978).

As mentioned above, solitary bees are a useful group of pollinators to rear commercially when the activation time of diapause time can be controlled (see Bosch & Kemp, 2002 for examples). Solitary bees make-up ~225 species out of 270 in the UK; and individually outperform social bees as pollinators (Garibaldi et al., 2013) so provide a large population of potentially marketable pollinators but are under threat (Biesmeijer et al., 2006). Pollinators provide a lot for the natural environment. Ecosystem stability and global food security depend upon healthy populations of bees, the collective business worth hundreds of billions of dollars annually relying on bees to pollinate their crops. Pollination underpins 78% of global flowering plant reproduction and enhances production in 75% of globally important crops (Breeze et al., 2016). Our dependency on pollinators will only become more intense as agriculture intensifies worldwide in response to growing human populations and as global temperatures continue to rise (Garibaldi et.al., 2013). Many *Osmia* species are managed because of their superiority as fruit tree pollinators (Bosch & Kemp, 2000; Bosch et al., 2000; Sedivy & Dorn, 2014). For example, female O. lignaria prefer foraging from fruit trees flowers to collect their pollen and nectar, proving to be excellent pollinators of almonds, cherries, plums, pears, and apples (Bosch & Kemp, 2000; Bosch et al., 2000; Sedivy & Dorn, 2014). Typical management strategies involve taking overwintering adults and placing them in selected orchards with shelters and a supply of nesting materials just before bloom occurs (Bosch & Kemp, 2000; Bosch et al., 2000; Sedivy & Dorn, 2014). Once their nesting period is complete, after about 1-2 months, the female *Osmia* will die and have left their offspring in capped nests (Bosch & Kemp, 2000; Bosch et al., 2000; Sedivy & Dorn, 2014). The offspring are then retrieved and stored at 18C over the spring-summer period to complete development (Bosch & Kemp, 2000; Bosch et al., 2000; Sedivy & Dorn, 2014). Larvae reach adulthood by late summer and spin cocoons to remain dormant in over the wintering period before later emerging in spring. This wintering period is necessary for development but to synchronise bee emergence with the bloom of flowering crops wintering bees are kept at 3-4˚C to delay emergence until bloom (Bosch & Kemp, 2000; Bosch et al., 2000; Sedivy & Dorn, 2014). If bees are required to pollinate much earlier flowering crops, bees are forced to emerge early by raising the temperature (Bosch & Kemp, 2000; Bosch et al., 2000; Sedivy & Dorn, 2014). This management strategy is widely practiced across Europe as it ensures reliable synchronised emergence with the bloom of the chosen crop, however some drawbacks include increased dispersal of pre-nesting females which limits the options of recovering a population from the same batch as large as the one released so managed orchards struggle to be self-reliant (Bosch & Kemp, 2000; Bosch et al., 2000; Sedivy & Dorn, 2014).

Studies such as this that investigate the relationship between temperature and solitary bee development may help to improve existing management strategies, moving towards improved food security and aid struggling pollinator populations.

CONCLUSION

By completing this study I aimed to develop an understanding of how solitary bee larvae detect and respond to changes in the nutritional composition of their food, if at all, and if a changing climate affects this response. Larvae clearly controlled carbohydrate consumption to meet an intake target regardless of protein consumption (Fig. 1, 2 & 4), however, carbohydrate consumption was significantly influenced by temperature. While developing in an ambient temperature of 15°C and 20°C larvae controlled the amount of carbohydrate they consumed with no thought for protein but the reverse is true at 25°C (Fig. 1, 2 & 4). *Osmia* overwinter in a cocoon which requires a large source of carbohydrates to survive, however a dramatic increase in temperature may make beginning diapause at the natural time unfavourable which larvae must respond to by consuming protein instead of building up carbohydrate reserves, delaying diapause. The existing nutritional landscape may be unable to provide the relevant resources to accommodate for behavioural changes like this if global temperatures continue to rise. Further investigation to confirm this shifting behaviour is consistent in high temperatures and that the nutritional landscape could support it in the future is imperative in order to protect pollinators from their already steep decline.

Pollinator numbers are already under decline as a result of increasing global temperatures and an ever changing nutritional landscape. In 2017, Radboud University used 1500 samples from across 63 sites to discover the biomass of insect life in Germany had declined by 75% over the last 25 years (Damian Carrington Environment, 2017). Dave Goulson, of Sussex

University, participated in the Radboud study, suggesting humans are imposing an “ecological Armageddon” by making large areas of land uninhabitable for wildlife, adding that "if we lose the insects then everything is going to collapse" (Damian Carrington Environment, 2017). The estimated rate of decline in flying insect biomass by the University of East Anglia is at roughly 6% a year (Damian Carrington Environment, 2017).

Global awareness of the importance and decline of bee populations has developed rapidly in recent years. The European Commision (2019) stated that 80% of crop and wild flowers in the EU depend on insect pollination, equating to roughly €15 billion of the EU’s agricultural output (The European Commission, 2019) so in response to an increase in decline in pollinator populations the EU formed the Pollinators Initiative in June 2018. The EU Pollinators Initiative outlines strategic objectives to be met by EU member states in order to; improve knowledge regarding pollinator decline (including the state of their most important habitats and the impact of pesticides), attempt to deal with the causes of decline, and raise awareness by getting citizens engaged and promoting collaboration (through education and providing incentives where necessary) (The European Commission, 2019). The scale of this initiative sets some of the long-term goals to be met by 2030, with other short-term actions to be undertaken by 2020 (The European Commission, 2019). At that point, the commission will review the progress/success of the initiative and implement any changes or further action if necessary (The European Commission, 2019). After completing this study, I would hope a significant portion of the preparatory stages set out by this initiative focuses on the effects and expected projections of climate change. Temperature affects an insect’s crucial life history traits, in the case of *Osmia bicornis* temperature changes can delay diapause, so although management of bee populations and the surrounding area is the basis for the provision of pollination temperature heavily informs the success of management.

This study suffered from an unexpectedly high mortality rate which lead to complications with the statistical analysis. I theorised this is a result of suspending the diet in an agar solution. Suspending the diet in agar allowed it to be frozen ahead of time and enabled me to process more larvae than I otherwise would have been able to if I made fresh diet every time it was required. However, over the course of the experiment, I did not take into consideration the high water content of the diet cubes I was providing the larvae with. While designing the experiment, I planned to use diets with a concentration of either 95 or 80% but failed to consider by suspending the diets in 50:50 agar the concentration would actually be 47.5 and 40%. The agar must have severely diluted the diet cubes so larvae were not receiving enough nutrients and perished. This experiment was conducted concurrently with Austin & Gilbert’s (2018) so unfortunately we realised this issue together. I believe the results generated are valuable however. While writing this thesis I helped Fiona Tainsh and Shannon Woodmansey (Tainsh et al., 2020) construct a methodology for their experiment to investigate potential alternative dietary dilution agents for creating diets for nutritional studies. Like this study, we reared *Osmia bicornis* larvae on pollen diluted with sporopollenin (pollen exines) and agar, alongside undiluted controls (Tainsh et al., 2020). Unlike this study however, diets were diluted 70:30 with agar not 50:50 and ~90% of larvae survived to pupation (Tainsh et al., 2020). This shows the larvae are capable of dealing with diluted food but the balance of diet and dilutant was not suitable.

The consumption and weight gain models could really be mixed models -- LMMs -- with "swap" as a random effect however thanks to issues with the statistics this could not be done.

I would like to see this work continued in the future with these diet issues resolved to allow a reliable analysis of larvae survival. Furthermore, it would be great to see future studies take this work and expand on it. For example, using the methods of this paper but with a non-deleterious diet would help to find an optimum intake rate for protein and carbohydrates, and increasing the temperature rails to upwards of 30°C to reflect the peaks of recent summers in Europe.

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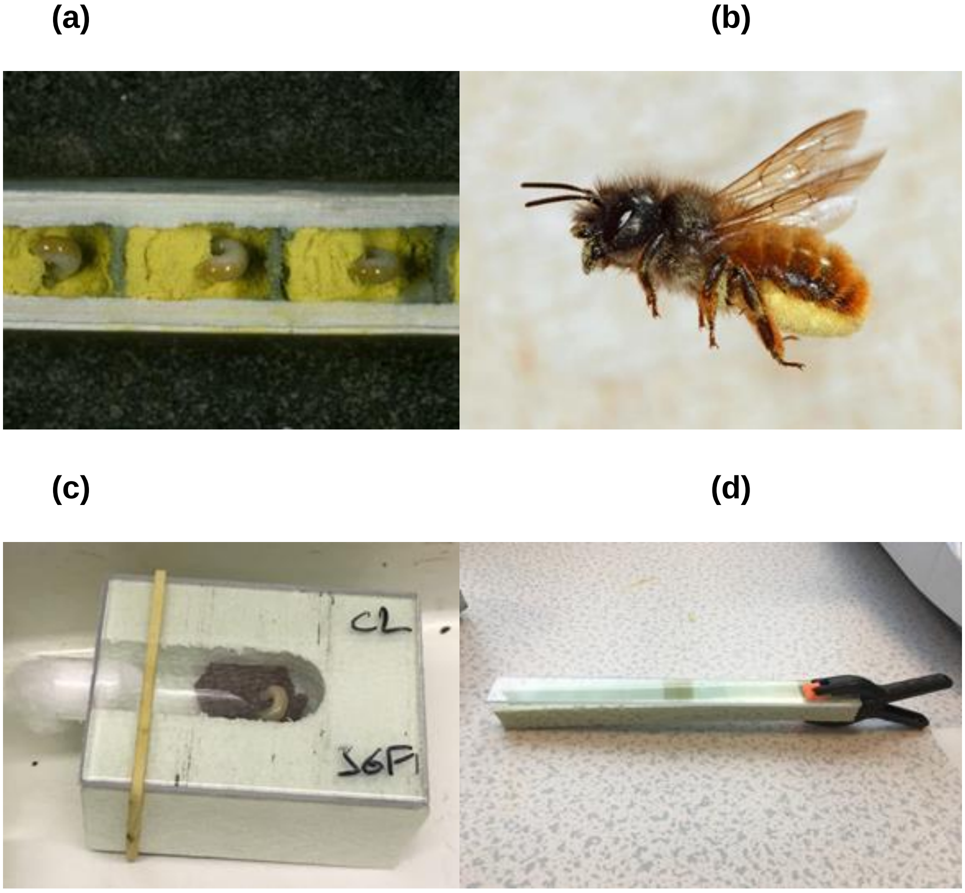
APPENDIX



(a) *Osmia bicornis* female in flight (photographed by Jeremy Early, 2012)

(b) *Osmia bicornis* brood cells with larvae on pollen balls (photographed by Albert Krebs, 2011)

(c) An empty nest box that would have been used in the artificial hives (photographed myself)

(d) Individual nest block with larvae on a fixed diet (photographed myself)