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RESEARCH ARTICLE

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Interacting with change: Diet mediates how larvae respond to their thermal environment

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Abstract

1. Temperature and nutrition are amongst the most common environmental challenges faced by organisms and will become increasingly so with ongoing climate change. While we have learnt a great deal about how temperature and nutrition affect life-history traits on their own, we know very little about their combined effect on animal performance. Given that animals in the wild are likely to experience changes in both their thermal and nutritional conditions, we need to understand how interactions between these conditions shape an animal's response if we hope to mitigate the effects of environmental change.
2. In the present research, we investigated the combined effects of nutrition and temperature on key life-history traits in *Drosophila melanogaster*. Using nutritional geometry, developing larvae were exposed to a range of diets varying in their protein and carbohydrate content and to one of two developmental temperature regimes (25°C and 28°C). We then examined key life-history traits: development time, viability, and two estimates of body size—wing and femur size.
3. We found that developmental temperature significantly changed the response to nutrition for all traits. Increased temperature led to more restricted trait optima for all traits and exacerbated the negative effects of carbohydrate-rich diets, resulting in harsher trade-offs between life-history traits. For example, at 25°C there were more diets that led to high viability, fast development and large body size than at 28°C. However, for the diets that produced the best outcomes for each trait, temperature had less of an effect.
4. These findings highlight the importance of studying the effects of combined stressors when assessing animals' responses to changing environmental conditions.

KEYWORDS
development, life-history traits, nutrition, nutritional geometry, plasticity, temperature

1 | INTRODUCTION

Rapid ongoing climate change will impact species' fitness by altering multiple aspects of their environment (Dawson, Jackson, House, Prentice, & Mace, 2011; Foden et al., 2013; Thomas et al., 2004). As a consequence of this, researchers have placed considerable effort into predicting how species will respond to changing environmental conditions. For the most part, studies have focussed on how species respond to thermal extremes, as this is expected to be the main driver of how species respond to climate change (Bush et al.,

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2016; Deutsch et al., 2008; Somero, 2010, 2011). However, rising temperatures will affect other features of a species habitat, such as timing and abundance of food resources. Further, ongoing changes in temperature, CO_2 levels and water availability will also affect the macronutrient composition of plant organs upon which animals feed (Rosenblatt & Schmitz, 2016). Because food availability and quality are amongst the most common stressors faced by animals in nature (Cross, Hood, Benstead, Huryn, & Nelson, 2015; Raubenheimer, Simpson, & Tait, 2012), the ability of species to respond to simultaneous changes in nutrition and temperature will be key for their persistence.

In addition to focussing on responses to single stressors, current assessments of vulnerability to climate change have mostly focused on the response of adult traits to environmental change (Bush et al., 2016; Deutsch, Ferrel, Seibel, Portner, & Huey, 2015; Kellermann, van Heerwaarden, Sgro, & Hoffmann, 2009). However, changing the environmental conditions of juvenile animals often has significant effects on an animal's fitness, altering the ability for the animal to survive to adulthood as well as affecting numerous fitness-related traits like body size and reproductive capacity (Aguila, Hoshizaki, & Gibbs, 2012; Van Heerwaarden & Sgrò, 2011; Rodrigues et al., 2015; Sørensen & Loeschcke, 2001; Tu & Tatar, 2003).

We understand the most about how changing environmental conditions affect juvenile stages from studies in insects (Clissold & Simpson, 2015; Flatt, 2005; Mirth & Shingleton, 2012). Insects are the most abundant class of animal on the planet (Erwin, 1983), fulfilling countless ecosystem functions, and serving as the main food source for many animals (Hallmann et al., 2017). As ectotherms, insects are particularly vulnerable to changes in temperature. This is especially true in the larval stage where growth and survival are highly dependent on the thermal and nutritional conditions experienced (Kingsolver & Huey, 2008; Mirth & Shingleton, 2012), and where behavioural avoidance of thermal stress is limited (Medina-Muñoz & Godoy-Herrera, 2005). Because many fitness-related traits, such as body size, reproductive capacity, and survival to adulthood, are established in the larval stage of insects, it is imperative to understand both pre-adult and adult responses to changing environmental conditions.

The individual effects of either diet or temperature on growth and life-history traits have been studied extensively. For instance, higher temperatures result in shorter development time (Atkinson, 1994; Miller, Clissold, Mayntz, & Simpson, 2009), increased larval growth rates (Atkinson & Sibly, 1997), lower larval viability (Angilletta, 2004; Kozlowski, Czarnoleski, & Danko, 2004) and smaller adult sizes (Angilletta & Dunham, 2003; Atkinson, 1994), which reduce adult reproductive success and fitness. Nutrition itself affects key life-history traits, and many studies have shown that caloric content, macronutrient composition and the relative ratios between macronutrients all have significant effects. For instance, low protein to carbohydrate (P:C) ratios generally result in lower pre-adult viability and longer development time and smaller body sizes in a range of insects, including caterpillars (Roeder & Behmer, 2014; Simpson, Sibly, Lee, Behmer, & Raubenheimer, 2004) and Drosophilid fruit flies (Bakker, 1959; Bradshaw & Holzapfel, 2008; Gray, Simpson, & Polak, 2018; Kristensen et al., 2016; Matavelli, Carvalho, Martins, & Mirth, 2015; Rodrigues et al., 2015; Silva-Soares, Nogueira-Alves, Beldade, & Mirth, 2017). Further, nutritional optima vary depending on the trait and species under study (Gray et al., 2018; Matavelli et al., 2015; Rodrigues et al., 2015).

The few studies that have explored the combined effects of temperature and nutrition on key life-history traits have found that the thermal environment changes the way animals respond to nutrition. In caterpillars, growth rate increased with increasing protein to carbohydrate (P:C) ratio or increasing temperature; however, temperature and nutrition interacted such that the increase in growth rate with temperature was more pronounced as P:C ratio increased (Lee & Roh, 2010; Lemoine & Shantz, 2016). Similar interactions between macronutrient ratio and temperature were found for viability and pupal mass of the caterpillar *Spodoptera litura* (Lee, Jang, Ravzanaadii, & Rho, 2015). These findings indicate that temperature can alter the response of at least some traits to nutrition, making predictions of climate change risk based on single stressors inaccurate.

While the above studies suggest that interactions between temperature and nutrition may generally affect animal responses to changing environmental conditions, their interpretations are restricted to a small region of nutrient space because they used a limited number of diets. Because trait optima can change considerably across nutrient space (e.g. Rodrigues et al., 2015; Silva-Soares et al., 2017), investigating the effects of temperature on life-history traits across a broader range of diets will provide more accurate insight into the conditions under which temperature and nutrition will interact to affect fitness. This is especially important given the uncertainty associated with predictions of nutritional changes under climate change (Rosenblatt & Schmitz, 2016).

Here, we examine the combined effect of thermal and nutritional conditions during larval development in *Drosophila melanogaster*. We assess four traits known to depend on larval rearing conditions: egg-to-adult development time, viability, and two adult size traits, wing centroid size and femur length. To account for effects of macronutrient balance as well as calorie content of diet, we used nutritional geometry (Simpson & Raubenheimer, 1993, 1999, 2012; Simpson et al., 2004) to generate a nutrient space composed of 36 diets of varying P:C ratio and calorie content. We explicitly tested for an interaction between nutrition and temperature during development by rearing all flies on these 36 diets at either 25 or 28°C. We found that temperature modified the response to nutrition for all traits examined. Our study emphasizes why studying environmental stressors in combination, rather than independently, will provide more insight into the complex nature of animal responses to climate change.

2 | MATERIALS AND METHODS

2.1 | Fly stocks

We used an outbred population of *D. melanogaster* from Ballina, Australia, initiated from wild-caught females collected in April 2016

(Lasne, Hangartner, Connallon, & Sgrò, 2018). Flies were maintained at 25°C and 12-hr light (L):12-hr dark (D) photoperiod on yeast–dextrose–potato medium (potato flakes 20 g/L; dextrose 30 g/L; Brewer's yeast 40 g/L; agar 7 g/L; nipagen 6 ml/L; and propionic acid 2.5 ml/L). They were maintained as a mass-bred population at a census size of approximately 2,000 individuals for approximately 60 generations before the experiments described below.

2.2 | Nutritional geometry

We created 36 experimental diets varying in their protein to carbohydrate (P:C) ratios and calorie concentrations. We produced these diets following a protocol similar to Rodrigues et al. (2015). Briefly, six different P:C ratios (1:16, 1:8, 1:4, 1:3, 2:3, and 3:2) were created by varying the quantities of inactive yeast, dextrose and potato flakes. Each ratio was prepared at a concentration of about 620 g of dry nutrient mass per litre and was then diluted sequentially by 50% in each step to make six different concentrations per ratio, giving a total of 36 diets. The low viability of individuals on the lowest P:C ratio and lowest calorie concentration meant that the sample sizes for body size measures would be highly imbalanced, and therefore, these diets were not included in the size analysis (wing centroid size and femur length), meaning that adult body size was measured across 25 diets rather than 36. Importantly, the chosen nutrient space extends across the range of P:C ratios and caloric concentrations found in rotting fruit (Matavelli et al., 2015; Silva-Soares et al., 2017), ensuring that our results are relevant to the feeding ecology of the fruit feeding *D. melanogaster* in the wild.

To obtain focal flies for the experiments described below, parental flies were placed in egg-laying chambers and left to oviposit overnight on food medium made from the same ingredients as our standard food but with the addition of blue food colourant and with twice the amount of agar (14 g/L). We transferred 20 eggs into vials containing 7 ml of treatment food, with ten vials per diet. Five of the diet replicate vials were placed at 25°C, and the other five were placed at 28°C in controlled-temperature cabinets with a 12-hr L:12-hr D photoperiod. Vials were relocated within the cabinet twice a day to avoid temperature and light gradient biases. Developing larvae were left to feed ad libitum until adult eclosion.

2.3 | Developmental temperature

Experimental flies were reared at each of two constant rearing temperatures: 25°C and 28°C. These temperatures were chosen because they represent the average summer temperate currently experienced in south-eastern Australia (25°C treatment) and the 3°C increase in temperature (28°C treatment) projected under climate change for the same region (www.bom.gov.au).

2.4 | Egg-to-adult development time and viability

To assess egg-to-adult development time, vials were checked twice a day at 9 a.m. and at 6 p.m. for eclosing adults. Once counted, eclosed adults were removed from their vial. For each vial, the assay ended when four consecutive time periods yielded no flies, at which point vials were discarded. Each fly received a development time score counted as the hours to eclosion from the mid-point of egg laying. Egg-to-adult viability was measured as number of adults eclosed relative to the 20 eggs transferred into each vial.

2.5 | Adult body size

To account for differences in scaling of different body parts in response to temperature and nutrition (Shingleton et al., 2009), body size was estimated using wing centroid size and femur length. Wings and frontal legs of 30 females per treatment were collected from adults that eclosed in the development time vials because previous studies show that such a sample size is sufficient to detect treatment effects (Lasne et al., 2018), and it allowed us to ensure a balanced design. Although size is a sexually dimorphic trait, understanding sex-specific responses to the combined effects of nutrition and temperature was not the focus of the present study, and so, only females were used. The left wing and left front leg of females were placed on a microscope slide and photographed (Leica M80 stereo microscope; Leica). Wing centroid size was calculated following Clemson, Sgrò, and Telonis-Scott, (2016). Briefly, eight wing vein landmark positions were obtained and their *x* and *y* coordinates determined using the software TPSDIG (Rohlf, 2008) version 2.17; wing area was then measured as centroid size (the square root of the sum of the squared distance from each landmark to the centroid) using COORDGEN8 (Sheets, 2003). Femur length was measured in IMAGEJ (Schindelin et al., 2012).

2.6 | Statistical analysis

The effect of protein and carbohydrate content of the food on all traits studied was analysed following the methods described by Lee et al. (2008). For viability, a generalized linear model (GLM) was fit to the data using a binomial family. Development time was rank-transformed to fit a Gaussian distribution, since transformation did not improve the fit of the data to any family of distributions. Then, a linear mixed-effects model was fit, including replicate vial as a random effect. Wing centroid size and femur length were analysed using linear models (LM). Model fit was validated by visual inspection of the residuals.

The full models included the linear and quadratic components of protein and carbohydrate and their cross-product, temperature and its cross-product with both the linear and quadratic components of carbohydrate and protein, and the three-way interaction between temperature and the linear components of protein and carbohydrate (similar to methods in Lee et al., 2008). This model allowed us to identify which, if any, macronutrient(s) drove the differences in response to temperature.

Additionally, we used response surface comparison analysis following Silva-Soares et al. (2017) to compare response shapes between temperatures for each trait. Specifically, we compared the full

model described above to a model that retained all terms except for the interactions between temperature, carbohydrate and protein. These two models were compared using partial *F* tests.

If significant interactions between protein and/or carbohydrate and temperature were found for a trait, the data were subset by temperature. Within each subset, the linear and quadratic effects of protein, carbohydrate and their cross-product were examined using the models described above but excluding temperature.

The response of each trait to the nutrient space at both temperatures was visualized using nonparametric thin-plate splines (TPS) (Blows & Brooks, 2003) using the fields package in R (version 3.4.1, R Development Core Team, 2017, https://www.r-project.org/). To better understand how the response surface differed between thermal regimes, we visualized how the increase in temperature affected the trait at each point of the nutrient space. To do so, temperature effect plots were generated by subtracting the TPS predictions for the 28°C subset from the TPS predictions for the 25°C subset. This new dataset was visualized in the same way as the trait response surfaces, using nonparametric TPS. All statistical analyses were done in R (version 3.4.1, R Development Core Team, 2017, https://www.r-project.org/).

3 | RESULTS

Our primary aim was to determine whether temperature changed the effects of larval nutrition on key life-history traits. To address this, we measured egg-to-adult viability, development time, wing centroid size and femur length of *D. melanogaster* on an array of diets (36 for viability and development time and 25 for the size traits) varying in protein, carbohydrate and caloric content at two temperatures (25°C and 28°C).

3.1 | Egg-to-adult viability

Our results revealed a significant interaction between temperature and both macronutrients (Table 1), as well as an overall difference in the shape of the nutrient response surfaces between temperatures (Table 2, Figure 1a-c). This means that temperature changed how egg-to-adult viability responded to nutrition.

To further probe how temperature alters how viability responds to diet, we analysed the viability across diets for each temperature individually. We found that the linear and quadratic components of both carbohydrate and protein, as well as their interaction, had a significant effect on egg-to-adult viability at both temperatures (Table 3). The conditions that generated the highest viability were similar across temperatures and occurred in the diets with the highest protein and calorie concentrations (Figure 1a-c). Viability decreased with decreasing protein and calorie concentrations in both thermal conditions (Figure 1a-c).

Although temperature did not change which diets generated the highest viability, it did alter the slope of the response of viability to macronutrient concentration. This leads to a higher viability at 28°C

TABLE 1 Effects of carbohydrate (C), protein (P), temperature (T) and their squares and products in the larval diet on four life-history characters: egg-to-adult viability, development time, wing centroid size and femur size

Life-history trait	T	P	C	P^2	C^2	P × C	T × P	T × C	T × P^2	T × C^2	T × P × C	Adjusted R^2
Viability												
β	0.0761	**0.006**	**0.00870**	**−0.29E−06**	**−1.79E−05**	**1.56E−05**	**−0.009**	0.00123	3.06E−06	**−5.75E−06**	**3.52E−05**	–
z value	0.784	**2.687****	**8.266*****	**−4.508*****	**−10.248*****	**2.544***	**−2.845****	0.828	0.322	**−2.296****	**3.774*****	
Development time												
β	**−4.85E−01**	**−2.45E−02**	**7.88E−04**	**5.48E−05**	**4.64E−06**	**1.61E−05**	**−1.11E−02**	4.78E−04	**1.85E−05**	−9.59E−07	**1.40E−05**	.630
t value	**−14.505*****	**−37.775*****	**2.678*****	**31.109*****	**8.387*****	**9.151*****	**−11.794*****	1.133	**7.357*****	−1.171	**5.412*****	
Wing centroid size												
β	**−0.086**	**4.04E−04**	**2.97E−04**	**−1.45E−06**	**−7.03E−07**	3.60E−07	**2.98E−04**	8.81E−05	−3.64E−07	−1.74E−07	−7.41E−07	.402
t value	**−12.723*****	**3.867*****	**5.103*****	**−5.203*****	**−6.820*****	1.196	**2.012***	1.070	−0.919	−1.189	−1.735	
Femur size												
β	**−0.0150**	**1.84E−04**	**1.26E−04**	**−6.74E−07**	**−3.12E−07**	1.93E−07	**1.65E−04**	−5.73E−08	−2.27E−07	−2.72E−08	−3.32E−07	.238
t value	**−5.371*****	**4.244*****	**5.222*****	**−5.807*****	**−7.275*****	1.541	**2.674****	−0.002	−1.376	−0.447	−1.864	

Notes: For all traits with the exception of viability, linear models were fitted to the data. Viability was analysed with a generalized linear model with binomial family and a logit link. Development time was rank normalized to meet the assumptions with a mean of 0 and a standard deviation of 1. Significant coefficients are in bold. Significance level codes: $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$.

TABLE 2 Comparisons between the response surfaces of flies reared at 25°C and flies reared at 28°C for the four measured traits

Trait	Degrees of freedom	*F*-value/deviance	*p*-value
Development time	5	34.897	<.001***
Viability	3	14.402	.00241**
Wing centroid size	5	3.565	.00330**
Femur length	5	5.320	<.001***

Notes: All response surfaces were compared using partial *F* tests, except for viability for which, due to its binomial distribution, response surfaces were compared using a chi-square test. Significance level codes: $^{**}p$ <.01, $^{***}p$ < .001.

wcompared to 25°C for intermediate P:C ratios (between 1:4 and 2:3 P:C), but up to a 15% decrease in survival at 28°C compared to 25°C on either high calorie and low P:C ratio diets, or on intermediate calorie and high P:C ratio diets (Figure 1c). Thus, higher temperature can have a positive effect on viability on some diets but a negative effect on others.

3.2 | Egg-to-adult development time

We found a significant difference in how development time responded to nutrition in larvae reared at 25°C when compared to larvae reared at 28°C, as shown by the significant interaction between temperature and both the linear and quadratic components of protein and the three-way interaction between temperature, protein and carbohydrate (Table 1). Further, when we compared response surfaces between temperatures we also found they were significantly different in shape (Table 2, Figure 1d-f). This indicates that the response of development time to the macronutrient composition of the diet depends on the temperature experienced throughout development.

At both 25°C and 28°C, we found that the linear and quadratic components of both carbohydrate and protein as well as their interaction had significant effects on egg-to-adult development time (Table 3). At both rearing temperatures, flies developed fastest at intermediate calorie concentrations (160–320 g/L). However, at 25°C flies developed fastest at intermediate P:C ratios (1:3), while at 28°C flies developed fastest at the highest P:C ratio (3:2; Figure 1d,e). Thus, temperature shifted the dietary conditions that result in the fastest development times.

Temperature also affected the severity of the delay caused by diet (Table 2, Figure 1c): Development time was delayed as P:C ratio decreased, but this delay was more marked at 25°C compared to 28°C (e.g. we found that the highest carbohydrate and lowest protein diet delayed development by 15 days at 25°C but just by 9 days at 28°C). The difference in development time due to temperature was minimized at the intermediate caloric concentrations and intermediate P:C ratios (1:3 and 1:4; Figure 1f). Taken together, temperature affected the way development time responded to diet by shifting the diets that gave rise to the fastest development time and by exacerbating the effects of the diets that produced the longest development time.

3.3 | Female wing centroid size and femur length at eclosion

We used two different proxies to account for differences in body size, wing and femur size, since size traits have been shown to differ in their response to nutrition in females (Shingleton, Masandika, Thorsen, Zhu, & Mirth, 2017). For both wing and femur size, we found that temperature significantly affected the way trait size responded to diet (Tables 1 and 2, Figure 2). The interaction was driven by protein, as we found no significant interaction between temperature and any component of carbohydrate (Table 1).

At both temperatures, trait size (be it wing or femur size) was significantly affected by the linear and quadratic components of both carbohydrate and protein (Table 3), but in different ways. For individuals reared at 25°C, the response surfaces of trait size for both wing and femur showed a maximum size at intermediate-high P:C ratios (1:3–2:3) and intermediate-high caloric concentrations, with size decreasing towards the extremes (Figure 2a,d). For individuals reared at 28°C, the maximum size (both for wing and femur) was shifted towards lower caloric values and higher P:C ratios (Figure 2b,e) leading to a significant difference in the response surface between the 25°C and the 28°C rearing temperature for both traits (Table 2, Figure 2c,f).

Diets of lowest P:C ratio and highest calorie concentration generated the smallest flies, and the magnitude of this response was further mediated by temperature, resulting in more extreme effects on body size at 28°C than at 25°C. On the other hand, the effects of high temperature on size were reduced at intermediate to high (1:3 and 2:3) P:C ratios at intermediate calorie concentrations (160–320 g/L). Additionally, our data suggest that size across diets varies more at 28°C than at 25°C. This was true for both size traits, but more markedly so for femur length (Figure 2), which showed a decrease in size of about 3% between the largest and smallest flies at 25°C but a decrease of about 8% at 28°C. Thus, we observed that temperature changed the way size traits responded to the dietary composition both by shifting the peaks that generate the largest trait sizes and by modulating the magnitude of the effects of diets that lead to the smallest trait sizes.

4 | DISCUSSION

Most risk assessments for climate change have focused solely on the effects of thermal stress (Bush et al., 2016; Kellermann et al., 2009; Sinervo et al., 2010). However, we know that ongoing environmental change will lead to changes in the nutrient composition of the primary producers upon which herbivores feed (Rosenblatt & Schmitz, 2016), which in turn have the potential to alter an animal's vulnerability to a changing thermal environment. It is therefore likely that temperature

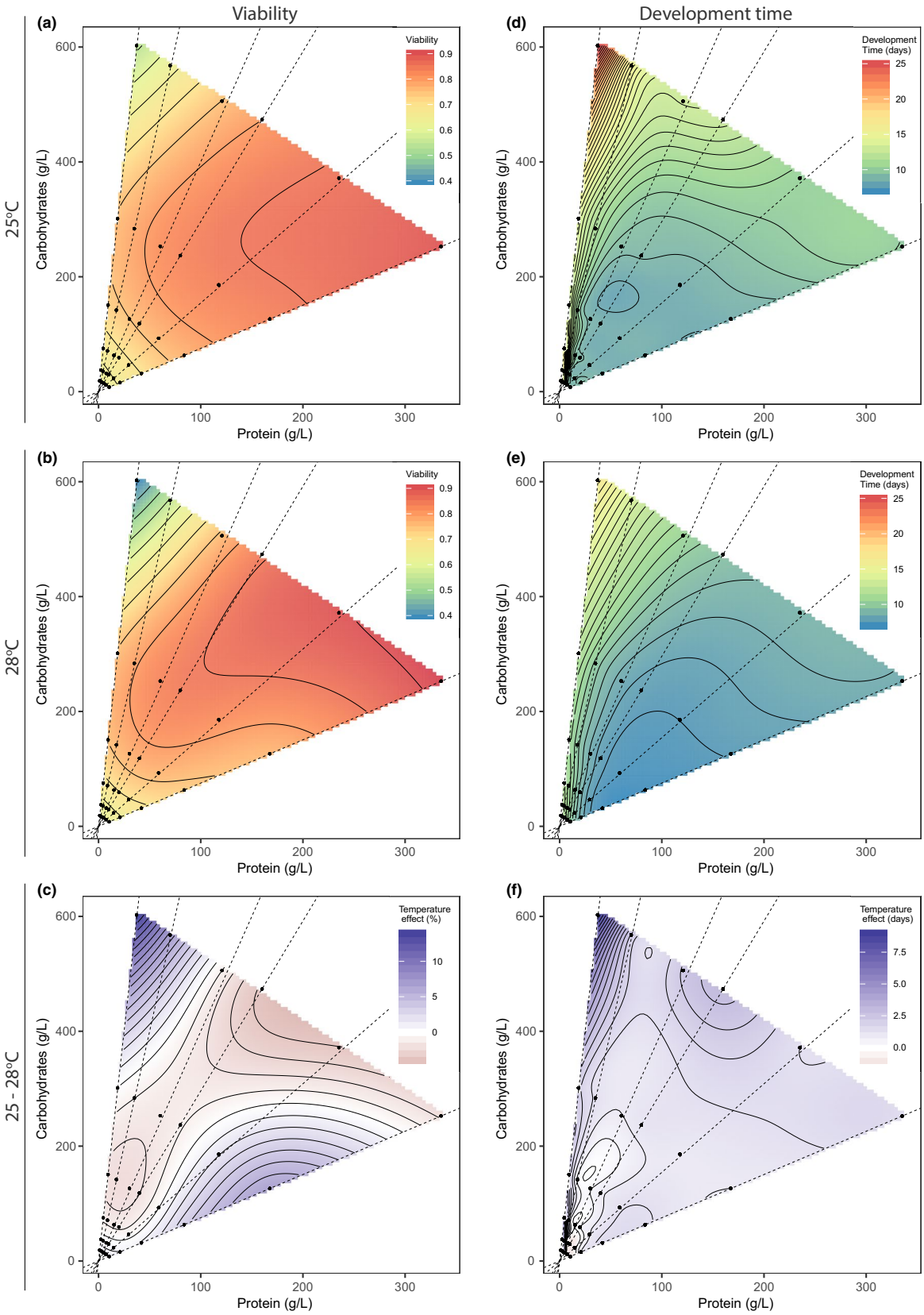

FIGURE 1 The effects of protein and carbohydrate content of the larval diet, represented as the fitted response surfaces of the effects of 36 different diets varying in protein and carbohydrate content. *Dashed black lines* represent P:C ratios. *Filled black circles* represent the respective nutritional coordinates of each of the 36 diets used. *First column (a–c)*: Egg-to-adult viability; *second column (d–f)*: Egg-to-adult development time. *Fist row*: 25°C rearing temperature; *second row*: 28°C rearing temperature; *third row*: the difference in the response between flies reared at 25°C and flies reared at 28°C

TABLE 3 Effects of carbohydrate (C), protein (P) and their squares and interaction terms in the larval diet on four life-history traits: egg-to-adult development time and viability, wing centroid size and femur size for flies reared at 25°C and flies reared at 28°C

Rearing temp.	P	C	P^2	C^2	P × C	Adjusted R^2
Viability						
25°C						
β	**0.006**	**0.009**	**−2.93E−05**	**−1.79E−05**	**1.56E−05**	—
z value	**2.687****	**8.266*****	**−4.508*****	**−10.248*****	**2.544***	
28°C						
β	−0.003	**0.010**	**−2.63E−05**	**−2.36E−05**	**5.08E−05**	—
z value	−1.292	**9.449*****	**−3.811*****	**−13.154*****	**7.238*****	
Development time						
25°C						
β	**−0.025**	**7.88E−04**	**5.48E−05**	**4.64E−06**	**1.61E−05**	0.575
t value	**−42.579*****	**3.018****	**35.064*****	**9.453*****	**10.315*****	
28°C						
β	**−0.036**	**0.001**	**7.33E−05**	**3.68E−06**	**3.02E−05**	0.570
t value	**−47.164*****	**3.807*****	**36.911*****	**5.531*****	**14.442*****	
Wing size						
25°C						
β	**4.04E−04**	**2.97E−04**	**−1.45E−06**	**−7.03E−07**	3.60E−07	0.114
t value	**3.924*****	**5.179*****	**−5.281*****	**−6.921*****	1.214	
28°C						
β	**7.01E−04**	**3.85E−04**	**−1.82E−06**	**−8.77E−07**	−3.81E−07	0.203
t value	**6.597*****	**6.506*****	**−6.390*****	**−8.330*****	−1.240	
Femur length						
25°C						
β	**1.84E−04**	**1.26E−04**	**−6.74E−07**	**−3.12E−07**	1.93E−07	0.130
t value	**4.280*****	**5.266*****	**−5.856*****	**−7.337*****	1.554	
28°C						
β	**3.48E−04**	**1.26E−04**	**−9.01E−07**	**−3.39E−07**	−1.39E−07	0.232
t value	**7.901*****	**5.16*****	**−7.651*****	**−7.786*****	−1.087	

Notes: For all traits with the exception of viability, linear models were fitted to the data. Viability was analysed with a generalized linear model with binomial family and a logit link. Development time was rank normalized to meet the assumptions with a mean of 0 and a standard deviation of 1. Significant coefficients are in bold. Significance level codes: *p <.05, **p <.01, ***p <.001.

and nutrition will interact to shape the response of animals to climate change. To these ends, the aim of this study was to understand how the thermal and nutritional environment experienced during development interacts to shape three key life-history traits in *D. melanogaster*. Our results reveal that temperature alters the response of all traits to dietary conditions, and further highlight that this approach has the potential to greatly improve our predictions of the conditions in which species will be most vulnerable to climate change.

4.1 | Larval traits differ in their response to the macronutrient composition of the diet

Our results for how life-history traits respond to nutrition at 25°C are generally in agreement with previous work in *D. melanogaster* (Gray et al., 2018; Rodrigues et al., 2015). Development time was shortest at intermediate (1:3) P:C ratios and intermediate caloric content, and female wing size and femur length at eclosion were largest at high P:C ratio (2:3 and 3:2) and intermediate/high calorie concentrations.

Similarly, our findings for viability at 25°C agreed overall with those from Rodrigues et al. (2015) and Gray et al. (2018). In all cases, low P:C ratios in combination with high caloric content always led to reduced viability. However, there are also discrepancies in the nutritional response surfaces for viability between studies: we found highest viability at the highest P:C ratio (3:2 P:C) and calorie concentration (640 g/L), whereas Rodrigues et al. (2015) found highest viability at 3:2 P:C but intermediate calorie concentration (240 g/L), and Gray et al. (2018) showed two local maxima one at 1:16 P:C and

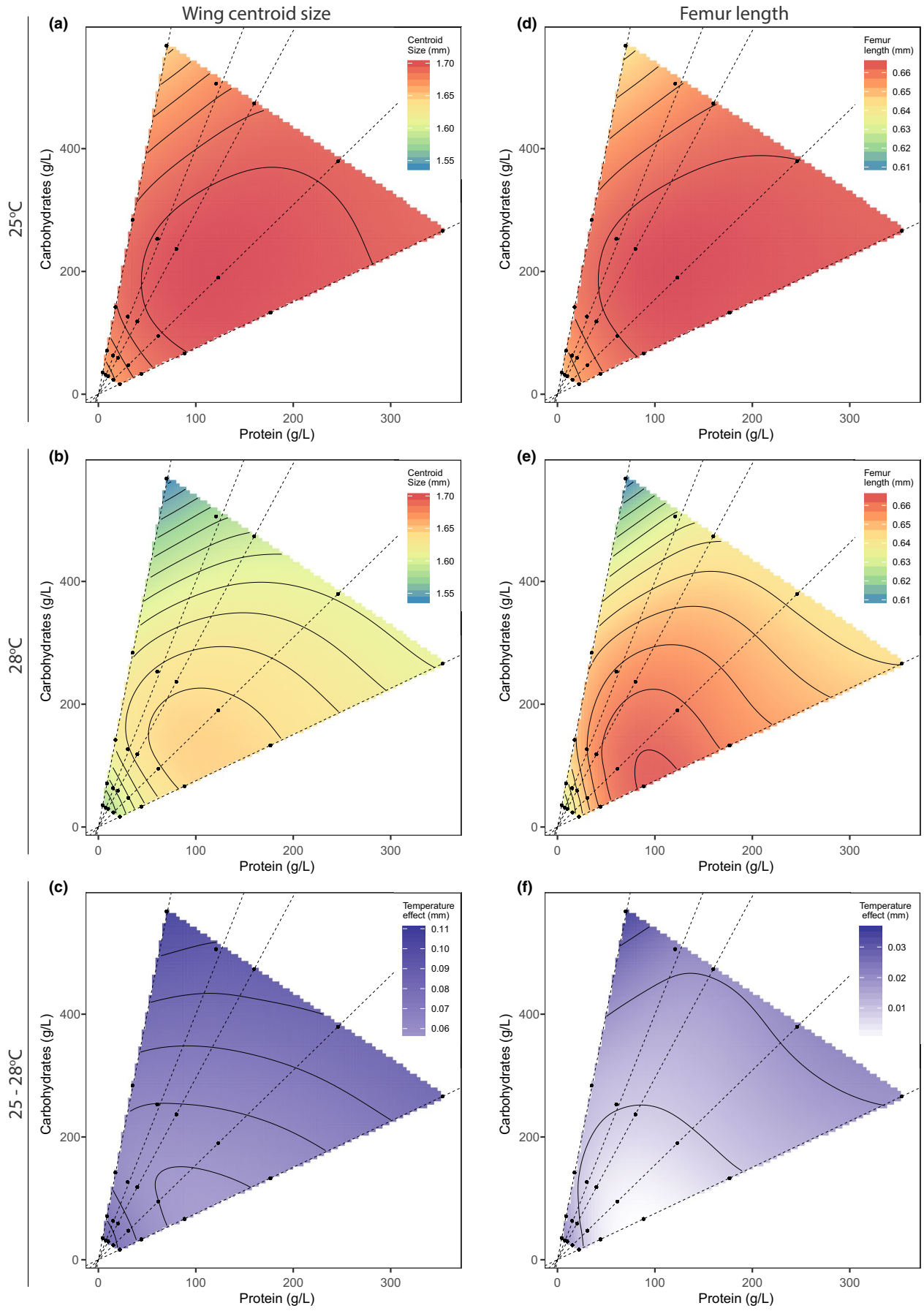
Wing centroid size
Femur length
25°C
28°C
25 - 28°C
(a)
(b)
(c)
(d)
(e)
(f)
Carbohydrates (g/L)
Protein (g/L)
Centroid Size (mm)
1.70
1.65
1.60
1.55
Femur length (mm)
0.66
0.65
0.64
0.63
0.62
0.61
Temperature effect (mm)
0.11
0.10
0.09
0.08
0.07
0.06
0.03
0.02
0.01
0
100
200
300
400

FIGURE 2 The effects of protein and carbohydrate content of the larval diet, represented as the fitted response surfaces of the effects of 25 different diets varying in protein and carbohydrate content. *Dashed black lines* represent P:C ratios. *Filled black circles* represent the respective nutritional coordinates of each of the 25 diets used. *First column (a–c)*: Female wing centroid size; *second column (d–f)*: Female femur length. *Fist row*: 25°C rearing temperature; *second row*: 28°C rearing temperature; *third row*: the difference in the response between flies reared at 25°C and flies reared at 28°C

one at 2:1 P:C. This is could be due to different origins of the fly populations used or perhaps because of differences in the ingredients used to create the diets.

Consistent with (Rodrigues et al., 2015), our data suggested the potential for a trade-off such that diets that led to highest viability did not result in the fastest development nor largest body size. However, there was a wide range of diets (i.e. intermediate to high calorie concentrations of intermediate to high P:C ratios) at 25°C that resulted in high overall performance.

4.2 | Increasing temperature modifies the way larval traits respond to diet

When temperature is increased by three degrees, we found that the number of diets that generated optimal traits values was reduced. Fastest development shifted towards more protein-rich diets, and viability on diets that optimized size and development time was lower at 28°C than it was at 25°C. The combination of fewer diets that resulted in optimal trait values and shifts in the diets that produce trait optima has the potential to result in harsher trade-offs in hotter environments.

These differences in response to nutrition due to temperature increase also mean that larvae have temperature-specific nutritional optima, presumably to accommodate metabolic differences caused by the thermal environment. Differences in nutritional optima have been shown previously between species that share food resources to reflect differences in ecological niche (Matavelli et al., 2015; Silva-Soares et al., 2017). Here we show that nutritional optima can also change for a given species due to temperature.

While the number of diets that produce the best outcomes for traits is reduced at the hotter temperature, the effects of a hotter environment on larvae within this more narrow nutritional space are minimized. For example, development time was fastest on intermediate P:C ratios and calorie concentrations at both 25 and 28°C, and on those diets our models showed virtually no difference in development time between temperatures. It seems intuitive that foods that optimize key developmental traits would help buffer animals against other types of environmental stress; however, more research is necessary to see if this finding can be generalized across traits.

On the diets that produced the poorest trait outcomes, higher temperatures made these outcomes even worse. A similar interaction between temperature and diet was found in the caterpillar *S. litura* (Lee et al., 2015) which underwent a more acute decrease in size with carbohydrate-rich food (1:5 P:C) when this diet was paired with higher temperatures, consistent with the current study.

In other studies, temperature has its most pronounced effects in the diets that produced the best values for traits (Lee & Roh, 2010; Lemoine & Shantz, 2016): caterpillar growth rate increased with increasing temperature for all diets with the largest difference in growth rate at the nutritional optimum (1:1 P:C). This contrasts with the trend we found for development time, size and viability in *D. melanogaster* larvae where the impact of temperature was less marked at the nutritional optimum. Therefore, the way temperature and diet interact is difficult to predict and the response of one trait cannot be used to predict the behaviour of another.

Given the diverse array of effects generated by interactions between the thermal and nutritional environments, our results highlight the importance of investigating a broad range of environmental contexts when making predictions about population responses to environmental change.

4.3 | Relating laboratory-based nutritional geometry to nutrient availability in the wild

Our results clearly show that the nutritional environment of animals can influence life-history responses to increased temperatures. Thus, understanding how the nutritional environment of an animal will change with climate change is an important next step. In nature, Drosophilid larvae obtain most of their protein from yeast communities growing on rotting fruit (Buser, Newcomb, Gaskett, & Goddard, 2014; Starmer & Fogleman, 1986). Yeast communities on fruit show a species-specific pattern of succession in their colonization of decaying fruits (Fogleman, Starmer, & Heed, 1981; Morais, Martins, Klaczko, Mendonça-Hagler, & Hagler, 1995) which in turn affects the macronutrient composition of the fruit (Matavelli et al., 2015). How climate change will affect the growth and composition of yeast communities growing on rotting fruit is at present unknown.

Further, the combined changes in temperature and nutrition may be met by a change in the feeding behaviour of larvae. Even though larvae are constrained to forage on a small area (Medina-Muñoz & Godoy-Herrera, 2005; Stamps, Buechner, Alexander, Davis, & Zuniga, 2005), different parts of the rotting fruit may vary in their P:C content due to the nature of the rotting process. We know that *D. melanogaster* larvae switch between foods of different macronutrient composition to achieve their P:C target (Rodrigues et al., 2015), while other species like *Drosophila suzukii* and *Drosophila biarmipes* control their nutrient intake primarily by regulating their feeding rate (Silva-Soares et al., 2017). It has also been shown that caterpillars and beetles can change their macronutrient target with increasing temperature (Lee et al., 2015; Rho & Lee, 2017). Our study suggests that *D. melanogaster* will face a more restricted nutritional space for optimal performance under increased temperature. The question of whether *D. melanogaster* will modify its feeding behaviour with

increasing temperature to match the new nutritional optimum remains open.

Finally, the current study used constant temperatures, while in nature larvae experience daily temperature fluctuations, and it is possible that the interactions between temperature and diet reported here may differ under fluctuating temperature regimes. It is also possible that, in nature, microclimatic variation and larval behaviour could play a role in mediating responses to the combined effects of thermal stress and nutrition. For example, it has been shown that locust nymphs can detect the deficiency of a specific nutrient in their food and change their preferred temperature to improve the absorption of that nutrient (Clissold, Coggan, & Simpson, 2013). *Drosophila melanogaster* larvae could potentially display a similar behaviour despite their limited mobility. Further studies would clarify the extent to which fluctuating temperatures and microclimatic variation play a role in the way larvae respond to diet and temperature. Finally, while we have focussed on pre-adult traits in the current study, it would be interesting to determine whether adult life-history-traits such, as fecundity or lifespan, are also affected by temperature and nutrition interactions, and whether such responses are sex-specific.

5 | CONCLUSION

Our study is the first to assess how animals will respond to the combined effects of temperature and nutrition across a broad nutrient space. We show that diet modulates the effects of temperature on key life-history traits in complex ways, a complexity only revealed because of the broad nutritional space examined. Importantly, we show that increasing developmental temperatures can change trait optima, generating trade-offs between life-history traits. Finally, we also show that when fly larvae feed on their optimal diets, the impact of increased temperature (i.e. smaller size, faster development and lower viability) is minimized. Our work highlights that the combined effects of both temperature and nutrition should be considered when assessing species responses to environmental change, as failure to do so may result in inaccurate predictions of risk.

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AUTHORS' CONTRIBUTIONS

T.C.K. collected all experimental data. All authors contributed to the experimental design, data analysis and final manuscript preparation.

DATA AVAILABILITY STATEMENT

All data and scripts are available on Figshare (https://doi.org/10.26180/5cfe1ddaaafac) and all materials are available upon request.

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