Received: 25 May 2021 | Accepted: 10 January 2022

DOI: 10.1111/een.13124

ORIGINAL ARTICLE

Ecological Entomology

Carry-over effects of larval food stress on adult energetics and life history in a nectar-feeding butterfly

Kristjan Niitepõld[1,2,3] | Carol L. Boggs[1,2,3,4]

[1]Department of Biological Sciences, University of South Carolina, Columbia, South Carolina, USA

[2]Department of Biology, Stanford University, Stanford, California, USA

[3]Rocky Mountain Biological Laboratory, Crested Butte, Colorado, USA

[4]School of Earth, Ocean and Environment, University of South Carolina, Columbia, South Carolina, USA

Correspondence
Kristjan Niitepõld, The Finnish Science Centre Heureka, Tiedepuisto 1, 01300 Vantaa, Finland.
Email: kristjan@niitepold.net

Present address
Kristjan Niitepõld, The Finnish Science Centre Heureka, Vantaa, Finland

Funding information
Division of Integrative Organismal Systems, Grant/Award Numbers: 0923411, 134367

Associate Editor: Toomas Tammaru

Abstract

1. Stressful juvenile developmental conditions can affect performance and fitness later in life. In holometabolous insects such as butterflies, development under stressful conditions may lead to smaller adult size, lower reproductive output, and shorter lifespan. However, how larval developmental stress affects energy intake and expenditure in adult individuals is poorly understood.
2. We subjected last-instar larvae of *Speyeria mormonia* Edwards (Lepidoptera: Nymphalidae) to periodic dietary restriction (DR) to examine the allocation of energy and nutrients among different life history processes. We measured adult food intake, resting metabolic rate (RMR), metabolic flight capacity, lifespan, and reproductive output. Consistent with pressure to disperse from a poor environment while maintaining offspring number, we predicted that stressed individuals would have increased adult food intake and higher flight capacity.
3. Adult body size was strongly reduced. Contrary to predictions, we found no compensatory adult feeding. Mass-adjusted flight metabolic rate was reduced, suggesting poor dispersal capacity. Larval DR did not affect adult lifespan, nor did the rate of metabolic senescence change. Larval DR did affect RMR, as stressed females had a steeper slope between RMR and body mass, which may reflect differences in physiological activity due to condition.
4. Fecundity decreased less than predicted based on body mass. Instead of investing in flight capacity, females increased relative allocation to reproduction, which may partly buffer against poor environmental conditions.
5. Understanding the interplay of energy acquisition and allocation to life history traits across the life cycle is vital for predicting responses to environmental change.

KEYWORDS
dietary restriction, flight, growth, insect, metabolic rate, metabolic senescence

INTRODUCTION

Natural populations are affected by changes in environmental conditions such as short-term variation in temperature and precipitation (Coulson et al., 2001; Pearce-Higgins et al., 2015), and large-scale processes such as climate change (Parmesan, 2006) and anthropogenic habitat loss and fragmentation (Hanski & Ovaskainen, 2000). Recent reports on declining animal populations in a number of ecosystems (Didham et al., 2020; Dirzo et al., 2014; Pimm et al., 2014) highlight the urgency of understanding how environmentally induced stress translates into population dynamics. To understand population dynamics, three individual-level processes are of paramount importance: reproductive success, lifespan, and dispersal. Stressful conditions that occur during development are of particular interest as they

 © 2022 Royal Entomological Society.

can affect future phenotype, performance, and fitness (Boggs, 2009; Dussutour et al., 2016; Grangeteau et al., 2018; Krittika & Yadav, 2019; Monaghan, 2008), often in ways that are specific to a particular life history strategy (Nestel et al., 2016). Apart from growth, developmental stress can also affect the storage of resources, which may have implication on fitness later in life (Hahn, 2005).

Typical consequences of poor developmental conditions include increased development time (Esperk et al., 2007; Gibbs et al., 2012) and small body size (Blanckenhorn, 1999; Boggs & Freeman, 2005; Gibbs et al., 2012; Nylin & Gotthard, 1998), often leading to a negative correlation between development time and body size at maturity, a common pattern in many insects (Teder et al., 2014). These negative effects reflect the reverse side of the so-called silver spoon effect, where optimal developmental conditions provide an advantage later in life (Grafen, 1988). However, developmental conditions may also serve as a cue for adaptive responses that increase fitness in a given environment (Douhard et al., 2014; May et al., 2015; Wang et al., 2016). Potential adaptive responses may include changes in energy storage and utilisation (Wang et al., 2016), increased dispersal rate (Angelo & Slanksy, 1984, Bitume et al., 2014), and improved tolerance to locomotion-induced stress (Saastamoinen et al., 2010). Data for flight behaviour are inconsistent across species and studies, however. Larvae of *Lycaena tityrus* Poda (Lepidoptera: Lycaenidae) that experienced a bout of starvation in the last larval instar were less likely to fly in a maze test and had shorter flight durations in a flight performance test (Reim et al., 2019), and workers of *Apis mellifera* Linnaeus (Hymenoptera: Apidae) with reduced food as larvae did not forage or perform waggle dances as effectively as workers that were well-fed as larvae (Scofield & Mattila, 2015). Unfortunately, directly measuring dispersal is often difficult, which has led to the use of many indirect proxies. In insects, wing dimorphism has served as an important trait for developing an understanding of factors affecting dispersal (Zera & Denno, 1997), but the presence or absence of wings is still a crude proxy of dispersal and limited to certain taxa. In wing-monomorphic animals, investigators have focused on continuous morphometric traits such as relative flight muscle mass (Marden, 1987) and thorax/body mass ratio (Berwaerts et al., 2002; Hill et al., 1999), or physiological traits such as flight metabolic rate (Niitepõld et al., 2009).

An open question is, to what extent can animals compensate for poor early-life conditions later in life? Compensatory growth may occur when conditions improve, but our understanding of the consequences of fluctuating environmental conditions on energy use, fitness, and lifespan is still limited (Krittika & Yadav, 2019; Metcalfe & Monaghan, 2001). There are examples of long-lasting energetic costs, as in the case of elevated resting metabolic rate (RMR) in adult zebra finches (*Taeniopygia guttata* Vieillot [Passeriformes: Estrildidae]) that were fed low-quality food as chicks (Criscuolo et al., 2008). Food restriction during the larval stage increased relative thorax size in adult Mormon fritillary (*Speyeria mormonia* Edwards [Lepidoptera: Nymphalidae]) butterflies (Boggs & Niitepõld, 2016), which may indicate increased investment in dispersal. As flight is an energetically expensive trait and investment in flight may reduce the available resource pool for other life history traits, we must consider interactions among traits such as reproduction, storage and survival, and how these are reflected in the energy use of the individual.

Using *S. mormonia*, we subjected last-instar larvae to periodic dietary restriction (DR) and measured its effects on energetics and resource allocation among different life history traits throughout the entire adult stage. Based on previous work (Boggs & Freeman, 2005; Boggs & Niitepõld, 2016), our main hypothesis was that quantitative larval DR would increase flight capacity and reduce longevity. Specifically, we predicted that females subjected to larval DR would have higher mass-independent metabolic flight capacity than control females. We further predicted that the higher investment in flight capacity would come with the cost of accelerated rate of senescence in metabolic flight capacity. Larval DR was predicted to increase mass-independent RMR as a consequence of higher physiological activity of stressed individuals. Reflecting the higher need for energy and resources, we predicted that larval DR would increase food intake in adult butterflies. We predicted that body mass should be reduced due to larval DR, and that absolute fecundity would also be reduced.

MATERIAL AND METHODS

Larval rearing and experimental treatment

The parent generation of *S. mormonia* for this study was captured in the field at Rocky Mountain Biological Laboratory (38°57′N, 106°58′W, 2900 m asl), Gunnison County, Colorado, United States, in August 2011. Females were kept in cylindrical glass cages under controlled temperature and light conditions (see Boggs & Ross, 1993). We collected the eggs, allowed them to hatch, and stored the larvae in 1.85 ml vials at 4°C at Stanford University. We broke diapause for several cohorts of larvae between January and March 2012 and reared them in a greenhouse under a LD 16:8 h and 27:16°C temperature cycle. Larvae were kept in family groups in organdy bags tied over leaves of the potted host plant *Viola soraria* (Violaceae). When last-instar larvae reached a length of 25 mm, we allocated mass-matched siblings to two treatments: control with food available ad libitum, or treatment with food restriction (DR). Individuals in the DR treatment received fresh leaves half as often as controls, resulting in three to four periods of ca. 24 h without food. Up to five siblings were kept in the same bag. Pupae were collected on the day after pupation, weighed, and placed in an individual container in the greenhouse.

Sample size, adult food intake, body mass, and offspring number

Adult butterflies were sexed at emergence. Males were marked, fed a 25% v:v sucrose water solution and placed in a family-specific container. Females were mated with an unrelated virgin male in the afternoon of their first day. Males were not used further in the analyses. The following morning, the female was marked, weighed, and placed

in an individual cylindrical glass cage with a violet leaf to stimulate oviposition, and kept in the greenhouse. The females originated from a total of 13 families; see Table S1. The total number of mated females was 16 in the control treatment and 23 in the larval DR treatment. Note that individuals had been allocated to treatments before they could be sexed, which is why the number of females was not equal among families and treatments.

Females were fed twice daily with 25% v:v sucrose water. A droplet of known volume measured with a Hamilton syringe was placed on a plastic plate. The butterfly's wings were held by smooth forceps, with the feet touching the plate, and the proboscis was manually extended. The remaining volume was again measured with a Hamilton syringe, and the imbibed volume calculated by the difference. Eggs were collected every afternoon. Adult lifespan was calculated as the number of calendar days when the butterfly was recorded to be alive.

Respirometry

On the first full day after emergence and every third day thereafter, we measured the resting and flight metabolic rate of each female. RMR was measured as CO_2 emission rate using flow-through respirometry while the individual was kept in a 1-litre respirometry chamber in darkness (Niitepõld et al., 2014). Individuals were motionless during the measurement and emitted CO_2 at a stable rate (see Figure 1). Using the previously validated methodology, we averaged 1.5 min of stable CO_2 emission for the calculation of RMR. We then initiated the flight measurement by exposing the butterfly to light and stimulated it to fly by shaking the chamber for exactly 7 min. We then covered the chamber, allowed the CO_2 concentration to return to the resting level, and terminated the recording. We examined three different parameters to describe flight performance: peak flight metabolic rate (PMR) reflecting maximal flight capacity; the total volume of CO_2 emitted during the whole 7-min flight measurement (FMR) reflecting overall performance; and the volume of CO_2 emitted during the last 3 min of the flight experiment, reflecting endurance.

Statistical analyses

We used mixed models to analyse the effect of larval DR on pupal mass, initial adult mass, lifespan, and total fecundity. Family and bag ID were included as random factors in all models, and body mass was included as a covariate in the models for lifespan and total fecundity.

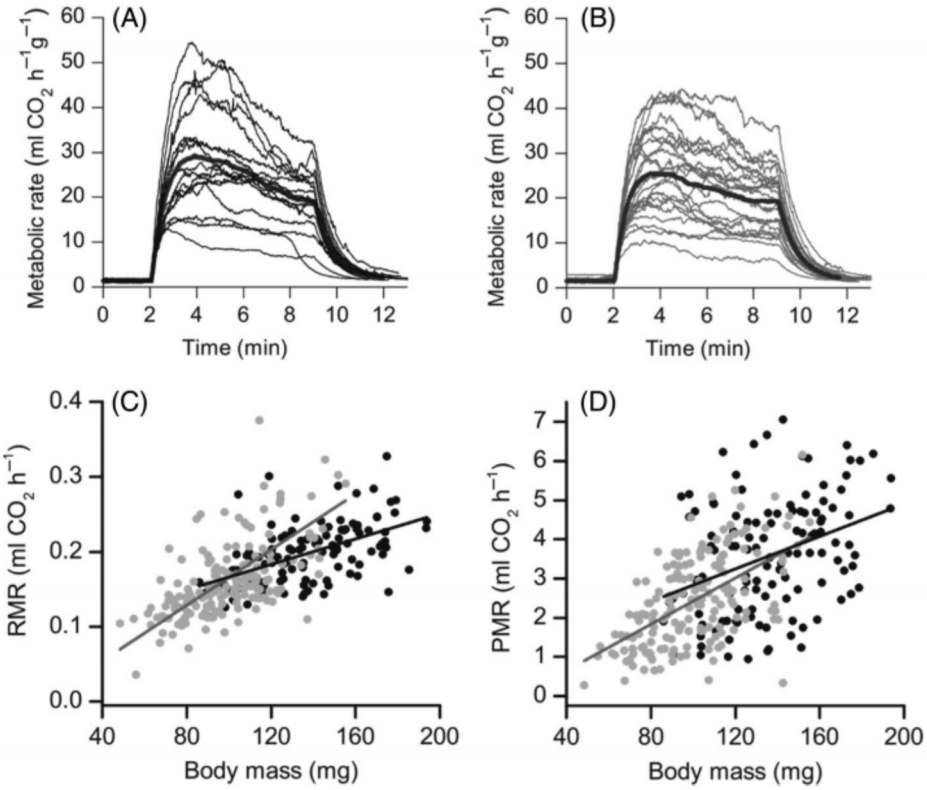

FIGURE 1 CO_2 emission rate in (a) control females and (b) females that had experienced dietary restriction in the last larval instar. The first 2 min represent resting metabolic rate (RMR) and the following 7 min flight metabolic rate. For illustrative purposes, CO_2 emission rate has been divided by body mass. The measurements in the figure were performed at the age of 10 days when flight capacity is at its highest. Panel (c) depicts the relationship between RMR and body mass. Note that each individual was measured up to eight times and the data were analysed with a repeated measures model. There was a significant interaction between mass and treatment. Panel (d) depicts peak metabolic rate (PMR), the highest CO_2 emission rate during flight, plotted against body mass. In the bottom panels, grey circles represent the larval dietary restriction treatment, black circles the control treatment

Two control females that only laid 6 and 11 eggs respectively were removed from the fecundity analyses as they were clear outliers. These females laid fewer eggs during their lifetime than average females laid in 1 day.

Adult body mass, sucrose water intake in the morning feeding, daily fecundity, and the metabolic rates were measured repeatedly throughout the life. We used repeated measures mixed models (Proc Mixed in SAS) and accounted for temporal autocorrelation within a female by using the autoregressive covariance structure (ar(1)), which assumes that the covariance between observations decreases with increasing time. The repeated measures model for body mass contained the random variables family and bag ID, and fixed variables treatment, age, age squared, and after visual inspection of the data, a cubic age term, as well as the treatment by age interaction. The initial sugar water intake model contained the random variables family and bag ID, and fixed variables body mass, treatment, age, age squared, and interactions between treatment and the covariates. The food intake in the afternoon was qualitatively similar to food intake in the morning. Here, we only present the analysis of the latter. Daily fecundity was modelled using family and bag ID as random factors, and fixed factors treatment, age, and age squared. We used the compound symmetric covariance structure in the daily fecundity model due to convergence problems with the autoregressive covariance structure. For metabolic rates, we began with a full model containing the random variables family and bag ID, and fixed variables body mass, treatment, age, time of the day, volume of last sucrose water meal, time since last feeding, measurement temperature, age squared, and interactions between treatment and the covariates. Time since feeding and volume of last sucrose water meal were included as covariates to account for their potential effect on metabolic rates due to the physiological process of specific dynamic action, that is, elevated metabolic rate after feeding. We used backwards elimination and removed non-significant ($p > 0.05$) interaction effects from the models. The age of 22 days was used as a cut-off point for observations included in the models due to the small sample size for older age classes. We used improved Kenward–Roger approximation of denominator degrees of freedom (KENWARDROGER2) in all mixed models. The statistical analyses were performed with SAS 9.2 and SAS Studio. The models and full result outputs are presented in Supplementary material.

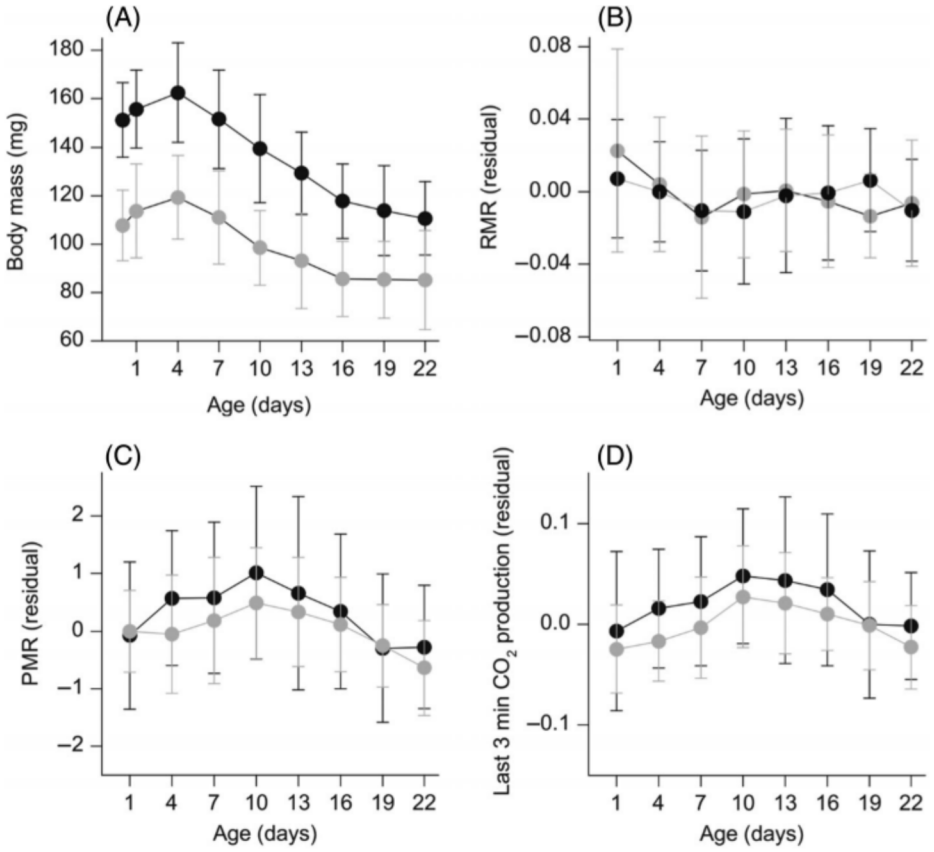

FIGURE 2 (a) Arithmetic means of body mass in females repeatedly measured every third day. The larval dietary restriction (DR) treatment significantly reduced body mass. (b) Mass-independent resting metabolic rate (RMR) plotted against age. (c) Mass-independent maximum CO_2 output rate in flight (PMR). (d) Mass-independent volume of CO_2 emitted during the last 3 min of the flight experiment. The flight metabolic rates were significantly reduced by the larval DR treatment. The mass-independent values were obtained by extracting the residuals of a repeated measures mixed model that contained body mass as a covariate. In all four panels, black circles represent control females and grey circles represent females in the larval DR treatment. The error bars are SDs. PMR, peak metabolic rate

RESULTS

Pupal mass and body mass

Larval DR significantly reduced pupal mass ($F_{1,20} = 24.56$, $p < 0.0001$). Female pupae in the control group weighed on average 350.6 ± 9.5 mg (mean ± SE), while the mean mass of female pupae was 283.3 ± 8.0 mg in the DR treatment. Similarly, the DR treatment reduced the mass of adult females ($F_{1,18.6} = 56.15$, $p < 0.0001$). In the morning of the first full day after emergence, control females weighed on average 150.8 ± 4.4 mg and females in the larval DR treatment weighed 107.7 ± 3.7 mg.

Individuals first gained mass and then began losing mass, after which mass appeared to plateau (Figure 2a). The effect of age on body mass was best explained using a cubic age term (statistics in Table 1). Treatment had a highly significant main effect, and the age by treatment interaction was significant, indicating that the slope between age and mass was steeper in control females.

Resting metabolic rate

RMR was positively affected by body mass ($F_{1,72.7} = 24.74$, $p < 0.0001$) (Figure 1b and Figure S1). The main effect of the DR treatment was significant ($F_{1,94.3} = 5.45$, $p = 0.02$), and there was a significant interaction between body mass and treatment, indicating that the slope between mass and RMR was steeper among individuals in the DR treatment ($F_{1,97.3} = 7.07$, $p = 0.009$). The effect of age on RMR was not significant ($F_{1,123} = 1.26$, $p = 0.26$). The volume of the previous sucrose water meal had a positive effect on RMR ($F_{1,259} = 27.55$, $p < 0.0001$), and time since feeding had a negative effect on RMR ($F_{1,267} = 12.38$, $p = 0.0005$): the longer time the individual had to digest, the lower the RMR.

TABLE 1 Statistical results for body mass

Variable	d.f.	F	p
DR treatment	138.8	56.43	<0.0001
Age	1295	38.83	<0.0001
Age^2	1295	81.40	<0.0001
Age × DR treatment	186.9	9.19	0.003
Age^3	1295	71.91	<0.0001

Note: Body mass was modelled with a repeated measures mixed model where family and bag ID were included as random factors.
Abbreviation: DR, dietary restriction.

Metabolic flight capacity

We examined three response variables: PMR, the total volume of CO_2 emitted during the 7-min flight measurement, and the volume of CO_2 emitted during the last 3 min of the flight measurement. Results were qualitatively almost identical for all three variables (statistics in Table 2a–c). Body mass had a significant positive effect. The larval DR treatment significantly reduced metabolic flight capacity. Age had a non-linear effect such that metabolic flight capacity first increased and then began to decrease at the age of approximately 10 days (Figure 2c, d). Time since feeding had a negative effect on PMR, but feeding did not affect the two other variables describing flight performance.

Sucrose water intake

Food intake in the morning decreased with age ($F_{1,154} = 13.7$, $p = 0.0003$). There was no effect of the larval DR treatment on sucrose water intake ($F_{1,32.8} = 0.75$, $p = 0.39$). The effect of body mass on sucrose water was not significant ($F_{1,66.6} = 0.33$, $p = 0.57$).

Fecundity

We first analysed daily egg production using a repeated measures model. The effect of the larval DR treatment was significant ($F_{1,35.6} = 9.46$, $p = 0.004$). Control females consistently laid more eggs than females in the DR treatment (Figure 3a). Daily egg production followed a pattern where the average number of eggs first increased,

TABLE 2 Statistical results for (a) peak flight metabolic rate, (b) total volume of CO_2 emitted in 7 min, and (c) volume of CO_2 emitted during last 3 min of flight measurement

	(a)			(b)			(c)		
Variable	d.f.	F	p	d.f.	F	p	d.f.	F	p
Body mass	1264	4.56	0.03	1274	6.00	0.01	1255	3.49	0.06
Age	1191	11.66	0.0008	1183	24.29	<0.0001	1173	21.26	<0.0001
DR treatment	124.2	5.17	0.03	119.8	4.36	0.05	121.9	4.84	0.04
Time since feeding	1230	5.16	0.02	1233	2.35	0.13	1245	1.07	0.30
Age^2	1193	28.07	<0.0001	1183	39.24	<0.0001	1166	29.8	<0.0001

Note: The effect of body mass was positive. The effect of time since feeding was negative. The denominator degrees of freedom were calculated using Kenward–Roger 2 approximation. Flight metabolic rates were modelled with repeated measures mixed models where family and bag ID were included as random factors.
Abbreviation: DR, dietary restriction.

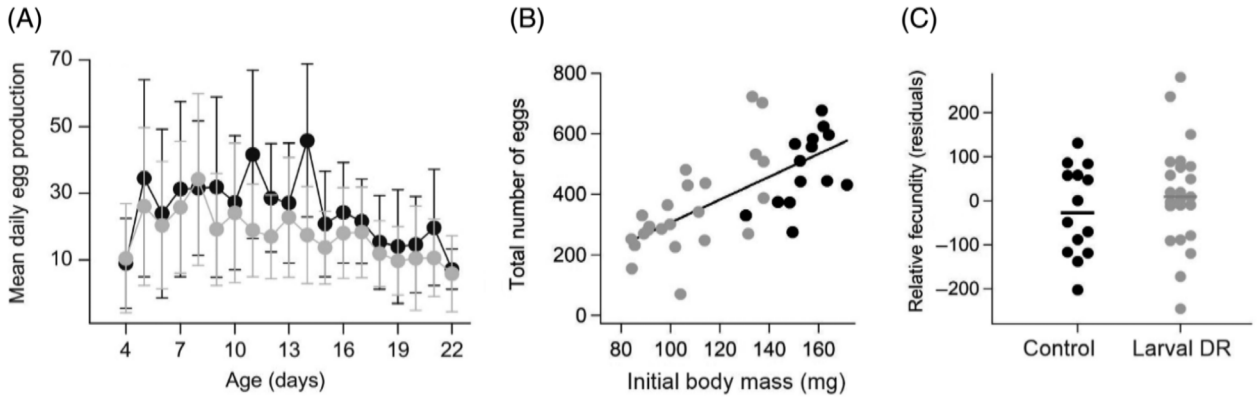

FIGURE 3 (a) Arithmetic means of daily egg production. The error bars represent SD. The larval dietary restriction (DR) treatment significantly reduced egg production. (b) Total sum of eggs laid by each female plotted against body mass on the day of emergence. (c) Relative egg production. The values in the plot are residuals extracted from a mixed model where initial body mass was used as a covariate. Relative egg production was statistically significantly higher in larval DR females compared with control females. In all panels, black circles represent control females, and larval DR females are represented by grey circles

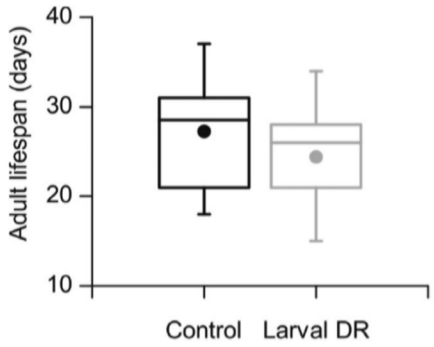

FIGURE 4 Lifespan was not significantly affected by the larval dietary restriction (DR) treatment. The horizontal lines in the box plots depict the median, and the circles indicate the arithmetic mean values

then decreased (age: $F_{1,626} = 18.15$, $p < 0.0001$; age^2: $F_{1,628} = 29.88$, $p < 0.0001$).

We next analysed the lifetime sum of eggs laid by each female. The arithmetic means for lifetime egg production were 484.4 ± 38.8 SE among control females and 353.1 ± 30.3 eggs among females in the larval DR treatment. There was a highly significant, positive effect of body mass on the sum of eggs ($F_{1,31.1} = 41.36$, $p < 0.0001$) (Figure 3b). When the effect of body mass was accounted for, females in the larval DR treatment had higher relative fecundity than control females ($F_{1,27.1} = 5.63$, $p = 0.03$) (Figure 3c). The least square means estimated by the model indicated higher relative egg production among females in the larval DR treatment (458.8 ± 37.8 eggs) compared with control females (321.7 ± 44.7 eggs).

Lifespan

Larval DR did not significantly affect lifespan ($F_{1,36} = 0.66$, $p = 0.42$). The effect of body mass on lifespan was not significant ($F_{1,36} = 0.06$, $p = 0.81$) (Figure 4).

DISCUSSION

DR reduced body size

Quantitative DR in the last larval instar led to reduced pupal and adult mass. The adult mass of DR females was on average 71% of the mass of control females. This finding was strikingly similar to the results of Boggs and Freeman (2005), who also induced DR in the last larval instar of *S. mormonia* and reported a mean mass of 150.5 and 106.8 mg for control and DR females, respectively. In our experiment, mean body mass was 151.3 mg among control females and 107.7 mg among females in the DR treatment. This suggests that adult body mass in *S. mormonia* is a highly plastic trait dependent on food availability during the larval stage.

Effects on metabolic flight capacity

The larval DR treatment had a negative effect on metabolic flight performance. This is the inverse of our prediction that developmental stress would lead to increased investment in flight capacity and hence high flight metabolic rate, resulting in increased dispersal propensity (Niitepõld et al., 2009). The negative effect was unexpected due to earlier findings of higher relative thorax mass in *S. mormonia* that experienced larval DR (Boggs & Niitepõld, 2016), given that potentially dispersing Lepidoptera may differ from residents in relative thorax size (Hill et al., 1999). The result is in line with studies suggesting that larval DR is associated with reduced flight capability in other insect species (Reim et al., 2019; Saastamoinen & Rantala, 2013; Scofield & Mattila, 2015). Combined with our findings of reduced metabolic flight performance in spite of higher relative thorax mass, these studies suggest that morphometric measures of dispersal should be used with caution in DR studies.

Developmental stress did not affect metabolic senescence in *S. mormonia*, as there were no significant differences between the treatments in the relationship between flight capacity and age. This

finding was consistent with there being no effect of larval DR on lifespan.

Effects on maintenance and food intake

We hypothesised that stressful developmental conditions would lead to higher maintenance cost and compensatory food intake at the adult stage. However, this was not the case. We have previously demonstrated that experimental flight treatments elevate RMR in *S. mormonia* (Niitepõld & Boggs, 2015), but the effects of larval DR were not comparable. In other insects, compensatory growth during the final larval stage in response to earlier stress increased RMR in the ladybird *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) at the adult stage (Xie et al., 2015). In contrast, in the butterfly *M. cinxia*, individuals that experienced DR as last-instar larvae emerged as smaller adults with lower RMR, even after mass correction (Saastamoinen & Rantala, 2013). RMR in insects can also show surprising levels of canalisation, such as in the case of wingless and winged morphs of the cricket *Gryllus firmus* Scudder (Orthoptera: Gryllidae), which show no significant differences in their respective RMR (Clark et al., 2015). Our study revealed some curious dynamics in relation to body mass: the slope between RMR and body mass was steeper among females in the larval DR treatment than in control females. As body mass is tied to condition, the observed pattern could reflect higher physiological activity in individuals in good condition compared with individuals in poor condition, but effect is only seen in stressed individuals. Furthermore, we also observed the effects of feeding on RMR, which in itself is a well-known phenomenon, and respirometry studies are ideally carried out on fasted animals (Lighton, 2008), although fasting may have consequences on life history (Niitepõld et al., 2014).

Effects on fecundity

Since fecundity is often found to be tightly linked to female body size (Blanckenhorn, 2000; Kivelä et al., 2012), we expected larval DR to have a negative effect on reproductive output. Indeed, daily egg production and the total number of eggs laid by females in the larval DR treatment were lower compared with control females. This resembles results from *M. cinxia*, where larval DR resulted in lower fecundity (Rosa & Saastamoinen, 2017). However, when examined in relation to body mass, the relative fecundity of DR females was somewhat higher than that of control females. The finding of higher relative fecundity contrasts with the results of Boggs and Freeman (2005), who found no effect of larval DR on mass-adjusted fecundity in *S. mormonia*. Adults in the earlier study were fed honey water; adults here were fed sucrose water. Given that Lepidoptera typically use a mix of larval and adult-derived resources for egg production (O'Brien et al., 2004), the ratios of macronutrients that were available for egg production differed between the two studies, which could impact allocation patterns (Simpson & Raubenheimer, 2012). Nonetheless, it appears that unlimited access to food cannot fully compensate for DR at the larval stage, as egg production decreased in absolute terms in both studies. These findings suggest that limits to egg production can be set by conditions during the larval stage in *S. mormonia*.

Effects on lifespan

The effects of stressful developmental conditions were not carried over the boundary of metamorphosis to affect adult lifespan. We found no effect of larval DR on lifespan, in contrast to previous findings in this species (Boggs & Freeman, 2005) and in other Lepidoptera such as the adult fruit-feeder *Bicyclus anynana* Butler (Lepidoptera: Nymphalidae) (Bauerfeind et al., 2009). Nonetheless, our and earlier results on *S. mormonia* correspond to those from the butterfly *M. cinxia*, for which larval resources are fundamental for reproduction, but the effects of larval DR on lifespan depend on the timing of larval DR in complex ways (Saastamoinen et al., 2013) or can be altogether absent (Saastamoinen & Rantala, 2013). Additionally, adults in the two experiments on *S. mormonia* were fed different food compositions as adults, with sugar water in the present work and honey water in Boggs and Freeman (2005). Since sugar water is less complex nutritionally than honey water, it is possible that any differences between control and treatment groups are masked in our case by the simpler adult food composition. Apart from direct effects on lifespan, developmental stress can affect other processes related to repair and maintenance. In the hawkmoth *Manduca sexta* Linnaeus (Lepidoptera: Sphingidae), stressed larvae showed multiple changes in the immune system (Adamo et al., 2016); honeybees that experienced starvation showed changes in juvenile hormone signalling and glucose levels (Wang et al., 2016); and in *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), larval crowding led to changes in the phospholipid fatty acids, likely affecting cell membrane structure (Moghadam et al., 2015). All are processes that can modulate lifespan, particularly if the individual faces multiple stressors. Nevertheless, lifespan in *S. mormonia* appears to show relatively high levels of stress robustness as lifespan does also not appear always to respond to interventions at the adult stage (Boggs & Ross, 1993; Niitepõld et al., 2014; Niitepõld & Boggs, 2015).

CONCLUSIONS

Stressful conditions during early life can disrupt development or act as signals triggering adaptive responses to the future environment. Early-life stress had a clear effect on adult body size in *S. mormonia*, and mass-independent metabolic flight capacity was also reduced. The reduction in flight metabolic capacity was surprising, as a previous study (Boggs & Niitepõld, 2016) suggested that larval DR would lead to higher investment in thorax size, which has been used as a proxy for flight capacity. The use of a more direct measure of flight capacity

thus brought novel insight into the question of whether stressful developmental conditions affect flight capacity and highlights the importance of measuring functionally important traits. We conclude that *S. mormonia* does not appear to respond to poor developmental conditions by increasing dispersal capacity. We also found no compensatory feeding in the adult stage. Females that were exposed to reduced larval host plant availability grew into small adults that had reduced reproductive output. In relative terms, however, stressed females laid more eggs than their body mass suggested. Our results point to the importance of studying responses to dietary stress in the context of both acquisition and allocation. As diet composition may change during the course of the life of an individual, the role of the availability of critical nutrients such as nitrogen, too, changes among different life history stages (O'Brien et al., 2004). How sensitive species are to environmental variation may thus depend on the characteristics of the species and the life history stage that is affected. Understanding the mechanistic underpinnings of variation in fitness in response to environmental variation has important implications when insect populations around the world show signs of widespread decline (Dirzo et al., 2014; Hallmann et al., 2017). We propose that accurate predictions of population dynamics require knowledge of how species that utilise different life history strategies respond to different environmental changes.

ACKNOWLEDGEMENTS

We thank Gladys Delgadillo, Brad Huang, Mattias Lanas, Clare LeDuff, Morgan McCluskey, Alejandra Mejia, XiaoSong Mu, Anika Naidu, Simon Scarpetta, Charlotte Wayne, and Amy Zuckerwise for assistance during the experiment. We thank Virpi Ahola, Anne Duplouy, Aapo Kahilainen, Elena Rosa, Alex Rubin, and Saskya van Nouhuys for comments on the manuscript. Three anonymous reviewers provided valuable feedback and helped to improve the manuscript. The work was done under USDA APHIS Permit #P526P-12-03928 for interstate transport of Lepidoptera including *Speyeria mormonia*, and CDFA No. 43-07-12 for importation of Lepidoptera into California. The study species is common throughout most of its range and is not under protection. Permissions to work on private land for collecting material are on file at the Rocky Mountain Biological Laboratory. Funding came from the National Science Foundation to Carol L. Boggs (grant numbers IOS 0923411 and IOS 134367).

CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

Carol L. Boggs and Kristjan Niitepõld designed the experiment, Kristjan Niitepõld and Carol L. Boggs carried out the experiment, Kristjan Niitepõld and Carol L. Boggs analysed the data, Kristjan Niitepõld wrote the draft manuscript, and Carol L. Boggs contributed to revisions.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad at https://doi.org/10.5061/dryad.v41ns1rz4.

REFERENCES

Adamo, S.A., Davies, G., Easy, R., Kovalko, I. & Turnbull, K.F. (2016) Reconfiguration of the immune system network during food limitation in the caterpillar *Manduca sexta*. *Journal of Experimental Biology*, 219, 706–718.

Angelo, M.J. & Slanksy, F. (1984) Body building in insects: trade-offs in resource allocation with particular reference to migratory species. *Florida Entomologist*, 67, 22–41.

Bauerfeind, S.S., Perlick, J.E.C. & Fischer, K. (2009) Disentangling environmental effects on adult life span in a butterfly across the metamorphic boundary. *Experimental Gerontology*, 44, 805–811.

Berwaerts, K., Van Dyck, H. & Aerts, P. (2002) Does flight morphology relate to flight performance? An experimental test with the butterfly *Pararge aegeria*. *Functional Ecology*, 16, 484–491.

Bitume, E.V., Bonte, D., Ronce, O., Olivieri, I. & Nieberding, C.M. (2014) Dispersal distance is influenced by parental and grand-parental density. *Proceedings of the Royal Society B: Biological Sciences*, 281, 8.

Blanckenhorn, W.U. (1999) Different growth responses to temperature and resource limitation in three fly species with similar life histories. *Evolutionary Ecology*, 13, 395–409.

Blanckenhorn, W.U. (2000) The evolution of body size: what keeps organisms small? *Quarterly Review of Biology*, 75, 385–407.

Boggs, C.L. (2009) Understanding insect life histories and senescence through a resource allocation lens. *Functional Ecology*, 23, 27–37.

Boggs, C.L. & Freeman, K.D. (2005) Larval food limitation in butterflies: effects on adult resource allocation and fitness. *Oecologia*, 144, 353–361.

Boggs, C.L. & Niitepõld, K. (2016) Effects of larval dietary restriction on adult body morphology, with implications for flight and life history. *Entomologia Experimentalis et Applicata*, 159, 189–196.

Boggs, C.L. & Ross, C.L. (1993) The effect of adult food limitation on life history traits in *Speyeria mormonia* (Lepidoptera: Nymphalidae). *Ecology*, 74, 433–441.

Clark, R.M., Zera, A.J. & Behmer, S.T. (2015) Metabolic rate is canalized in the face of variable life history and nutritional environment. *Functional Ecology*, 30, 922–931.

Coulson, T., Catchpole, E.A., Albon, S.D., Morgan, B.J.T., Pemberton, J.M., Clutton-Brock, T.H. et al. (2001) Age, sex, density, winter weather, and population crashes in Soay sheep. *Science*, 292, 1528–1531.

Criscuolo, F., Monaghan, P., Nasir, L. & Metcalfe, N.B. (2008) Early nutrition and phenotypic development: 'catch-up' growth leads to elevated metabolic rate in adulthood. *Proceedings of the Royal Society B: Biological Sciences*, 275, 1565–1570.

Didham, R.H., Barbero, F., Collins, C.M., Forister, M.L., Hassall, C., Leather, S. R. et al. (2020) Spotlight on insects: trends, threats and conservation challenges. *Insect Conservation and Diversity*, 13, 99–102.

Dirzo, R., Young, H.S., Galetti, M., Ceballos, G., Isaac, N.J.B. & Collen, B. (2014) Defaunation in the Anthropocene. *Science*, 345, 401–406.

Douhard, M., Plard, F., Gaillard, J.-M., Capron, G., Delorme, D., Klein, F. et al. (2014) Fitness consequences of environmental conditions at different life stages in a long-lived vertebrate. *Proceedings of the Royal Society B: Biological Sciences*, 281, 20140276.

Dussutour, A., Poissonnier, L.-A., Buhl, J. & Simpson, S.J. (2016) Resistance to nutritional stress in ants: when being fat is advantageous. *Journal of Experimental Biology*, 219, 824–833.

Esperk, T., Tammaru, T. & Nylin, S. (2007) Intraspecific variability in number of larval instars in insects. *Journal of Economic Entomology*, 100, 627–645.

Gibbs, M., Van Dyck, H. & Breuker, C.J. (2012) Development on drought-stressed host plants affects life history, flight morphology and reproductive output relative to landscape structure. *Evolutionary Applications*, 5, 66–75.

Grafen, A. (1988) On the uses of data on lifetime reproductive success. In: Clutton-Brock, T.H. (Ed.) *Reproductive success*. Chicago, IL: The University of Chicago Press, pp. 454–463.

Grangeteau, C., Yahou, F., Everaerts, C., Dupont, S., Farine, J.-P., Beney, L. et al. (2018) Yeast quality in juvenile diet affects *Drosophila melanogaster* adult life traits. *Scientific Reports*, 8, 13070.

Hahn, D.A. (2005) Larval nutrition affects lipid storage and growth, but not protein or carbohydrate storage in newly eclosed adults of the grasshopper *Schistocerca americana*. *Journal of Insect Physiology*, 51, 1210–1219.

Hallmann, C.A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H. et al. (2017) More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS One*, 12, e0185809.

Hanski, I. & Ovaskainen, O. (2000) The metapopulation capacity of a fragmented landscape. *Nature*, 404, 755–758.

Hill, J.K., Thomas, C.D. & Blakeley, D.S. (1999) Evolution of flight morphology in a butterfly that has recently expanded its geographic range. *Oecologia*, 121, 165–170.

Kivelä, S.M., Välimäki, P., Carrasco, D., Mäenpää, M.I. & Mänttäri, S. (2012) Geographic variation in resource allocation to the abdomen in geometrid moths. *Naturwissenschaften*, 99, 607–616.

Krittika, S. & Yadav, P. (2019) An overview of two decades of diet restriction studies using *Drosophila*. *Biogerontology*, 20, 723–740.

Lighton, J.R.B. (2008) *Measuring metabolic rates: a manual for scientists*. New York, NY: Oxford University Press.

Marden, J.H. (1987) Maximum lift production during takeoff in flying animals. *Journal of Experimental Biology*, 130, 235–258.

May, C.M., Doroszuk, A. & Zwaan, B.J. (2015) The effect of developmental nutrition on life span and fecundity depends on the adult reproductive environment in *Drosophila melanogaster*. *Ecology and Evolution*, 5, 1156–1168.

Metcalfe, N.B. & Monaghan, P. (2001) Compensation for a bad start: grow now, pay later? *Trends in Ecology & Evolution*, 16, 254–260.

Moghadam, N.N.H.M., Manenti, T. & Loeschcke, V. (2015) Phospholipid fatty acid composition linking larval-density to lifespan of adult *Drosophila melanogaster*. *Experimental Gerontology*, 72, 177–183.

Monaghan, P. (2008) Early growth conditions, phenotypic development and environmental change. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, 363, 1635–1645.

Nestel, D., Papadopoulos, N.T., Pascacio-Villafán, C., Righini, N., Altuzar-Molina, A.R. & Aluja, M. (2016) Resource allocation and compensation during development in holometabolous insects. *Journal of Insect Physiology*, 95, 78–88.

Niitepõld, K. & Boggs, C.L. (2015) Effects of increased flight on the energetics and life history of the butterfly *Speyeria mormonia*. *PLoS One*, 10, 16.

Niitepõld, K., Smith, A.D., Osborne, J.L., Reynolds, D.R., Carreck, N.L., Martin, A.P. et al. (2009) Flight metabolic rate and *Pgi* genotype influence butterfly dispersal rate in the field. *Ecology*, 90, 2223–2232.

Niitepõld, K., Perez, A. & Boggs, C.L. (2014) Aging, life span, and energetics under adult dietary restriction in Lepidoptera. *Physiological and Biochemical Zoology*, 87, 684–694.

Nylin, S. & Gotthard, K. (1998) Plasticity in life-history traits. *Annual Review of Entomology*, 43, 63–83.

O'Brien, D.M., Boggs, C.L. & Fogel, M.L. (2004) Making eggs from nectar: the role of life history and dietary carbon turnover in butterfly reproductive resource allocation. *Oikos*, 105, 279–291.

Parmesan, C. (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, 37, 637–669.

Pearce-Higgins, J.W., Ockendon, N., Baker, D.J., Carr, J., White, E.C., Almond, R.E.A. et al. (2015) Geographical variation in species' population responses to changes in temperature and precipitation. *Proceedings of the Royal Society B: Biological Sciences*, 282, 20151561.

Pimm, S.L., Jenkins, C.N., Abell, R., Brooks, T.M., Gittleman, J.L., Joppa, L. N. et al. (2014) The biodiversity of species and their rates of extinction, distribution, and protection. *Science*, 344, 1246752.

Reim, E., Eichhorn, D., Roy, J.D., Steinhoff, P.O.M. & Fischer, K. (2019) Nutritional stress reduces flight performance and exploratory behavior in a butterfly. *Insect Sci.*, 26, 897–910.

Rosa, E. & Saastamoinen, M. (2017) Sex-dependent effects of larval food stress on adult performance under semi-natural conditions: only a matter of size? *Oecologia*, 184, 633–642.

Saastamoinen, M. & Rantala, M.J. (2013) Influence of developmental conditions on immune function and dispersal-related traits in the Glanville fritillary (*Melitaea cinxia*) butterfly. *PLoS One*, 8, e81289.

Saastamoinen, M., van der Sterren, D., Vastenhout, N., Zwaan, B.J. & Brakefield, P.M. (2010) Predictive adaptive responses: condition-dependent impact of adult nutrition and flight in the tropical butterfly *Bicyclus anynana*. *The American Naturalist*, 176, 686–698.

Saastamoinen, M., Hirai, N. & van Nouhuys, S. (2013) Direct and transgenerational responses to food deprivation during development in the Glanville fritillary butterfly. *Oecologia*, 171, 93–104.

Scofield, H.N. & Mattila, H.R. (2015) Honey bee workers that are pollen stressed as larvae become poor foragers and waggle dancers as adults. *PLoS One*, 10, e0121731. https://doi.org/10.1371/journal.pone.0121731

Simpson, S.J. & Raubenheimer, D. (2012) *The nature of nutrition: a unifying framework from animal adaptation to human obesity*. Princeton: Princeton University Press, p. 256.

Teder, T., Vellau, H. & Tammaru, T. (2014) Age and size at maturity: a quantitative review of diet-induced reaction norms in insects. *Evolution*, 68, 3217–3228.

Wang, Y., Kaftanoglu, O., Brent, C.S., Page, R.E. & Amdam, G.V. (2016) Starvation stress during larval development facilitates an adaptive response in adult worker honey bees (*Apis mellifera* L.). *Journal of Experimental Biology*, 219, 949–959.

Xie, J., De Clercq, P., Pan, C., Li, H., Zhang, Y. & Pang, H. (2015) Physiological effects of compensatory growth during the larval stage of the ladybird, *Cryptolaemus montrouzieri*. *Journal of Insect Physiology*, 83, 37–42.

Zera, A.J. & Denno, R.F. (1997) Physiology and ecology of dispersal polymorphism in insects. *Annual Review of Entomology*, 42, 207–230.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Data S1. Supporting information.

How to cite this article: Niitepõld, K. & Boggs, C.L. (2022) Carry-over effects of larval food stress on adult energetics and life history in a nectar-feeding butterfly. *Ecological Entomology*, 47(3), 391–399. Available from: https://doi.org/10.1111/een.13124