Behavioural Processes 175 (2020) 104122

Contents lists available at ScienceDirect

Behavioural Processes

journal homepage: www.elsevier.com/locate/behavproc

Long-term reproductive success is predicted by sexual behavior and impaired by temporary nutritional stress during sexual maturation

Daniela Wilner*, E.V(Ginny) Greenway, Lauren A. Cirino, Christine W. Miller

Entomology and Nematology Department, University of Florida, 1881 Natural Area Drive, Bldg. 970, PO Box 110620, Gainesville, FL, USA

ARTICLE INFO

Keywords:
Diet
Fertility
Food deprivation
Nutrition
Reproduction
Stress

ABSTRACT

Nutritional stress during the earliest stages of an animal's life can have long-term effects on its behavior and reproductive performance, but the effects of brief periods of nutritional stress later in life are less well-studied. We manipulated female diet in *Narnia femorata* (Hemiptera: Coreidae) and investigated to what extent nutritional stress during sexual maturation affects subsequent sexual behavior and long-term offspring production. We show that nutritional stress at this key point during early adulthood can have lasting effects on reproduction, impairing long-term offspring production despite the subsequent return of good nutrition. These results demonstrate that nutritional availability during late stages of development, in young adults, can be crucial to future fitness. We found no effect of temporary nutritional stress on female receptivity to mating or attractiveness to males; although females that were less receptive also produced fewer offspring in the next month. Overall, we demonstrate that even brief periods of nutritional deprivation late in development can have drastic long-term effects, apparently beyond compensation, and despite a good early nutritional environment.

1. Introduction

Most organisms experience episodes of nutritional stress during their lifetimes, the consequences of which can be varied and severe (Boggs, 2009; Chan et al., 2015; Lindström, 1999; Monaghan, 2008). Many have evolved some ability to recover from poor nutrition, but compensation is not always possible or complete; many life history traits may be impacted (Metcalfe and Monaghan, 2001). While we know much about the effects of early-life nutrition, we know little about the impacts of poor nutrition during the process of sexual maturation. Poor nutrition during this time period may be common because resource availability is dynamic in nature. Thus, it is crucial to investigate the potential impacts of nutritional stress during sexual maturation, especially on traits important to biological fitness.

Our goal was to investigate the extent to which temporary nutritional stress during the transition to adulthood impacts sexual behavior and long-term reproductive output in those that survive. We used the leaf-footed cactus bug, *Narnia femorata* (Hemiptera: Coreidae), to investigate this question (Fig. 1). Like many arthropods, *N. femorata* juveniles grow in discrete stages and have determinate growth. They achieve their final external dimensions at the adult molt, before maturing sexually (Allen et al., 2018). We were thus able to study the effects of nutritional stress in animals that had achieved their adult size and form but were still maturing sexually. These insects are also very amenable to sexual behavior observations and long-term fertility tracking. They readily mate in captivity and have highly stereotyped mating behavior, where females visibly accept or reject males' mating attempts. Females can begin laying eggs at sexual maturity (typically 12–14 days after the final molt), regardless of whether they have mated or not, and they can continue to lay multiple clutches for several weeks in the laboratory, with production peaking around week six after adult eclosion and decreasing thereafter (Allen et al., 2018).

Leaf-footed cactus bugs feed on the same host plants (prickly-pear cactus, *Opuntia mesacantha*) throughout their entire lives, and they routinely experience fluctuations in food availability due to cactus flowering and fruiting phenology as well as competition for fruit from other herbivores (Cirino and Miller, 2017; Gillespie et al., 2014; Sasson et al., 2016). These bugs grow faster, have lower mortality, and achieve larger gonads and adult body size if they have access to ripe fruit rather than cactus pads alone (Gillespie et al., 2014; Nageon de Lestang and Miller, 2009; Sasson et al., 2016), but ripe fruit is only available for a few months each year (Gillespie et al., 2014; Sasson et al., 2016). Despite the ephemeral nature of their optimal food source, these bugs lay egg clutches through most of the year in Florida (Cirino and Miller,

* Corresponding author at: Entomology and Nematology Department, 1881 Natural Area Drive, Bldg. 970, PO Box 110620, University of Florida, Gainesville, FL 32611-0620 USA.

E-mail addresses: danielawilner@gmail.com, danielawilner@ufl.edu (D. Wilner), egreenway@ufl.edu (E.V. Greenway), lacirino@ufl.edu (L.A. Cirino), cwmiller@ufl.edu (C.W. Miller).

https://doi.org/10.1016/j.beproc.2020.104122
Received 11 December 2019; Received in revised form 24 March 2020; Accepted 28 March 2020
Available online 04 April 2020
0376-6357/

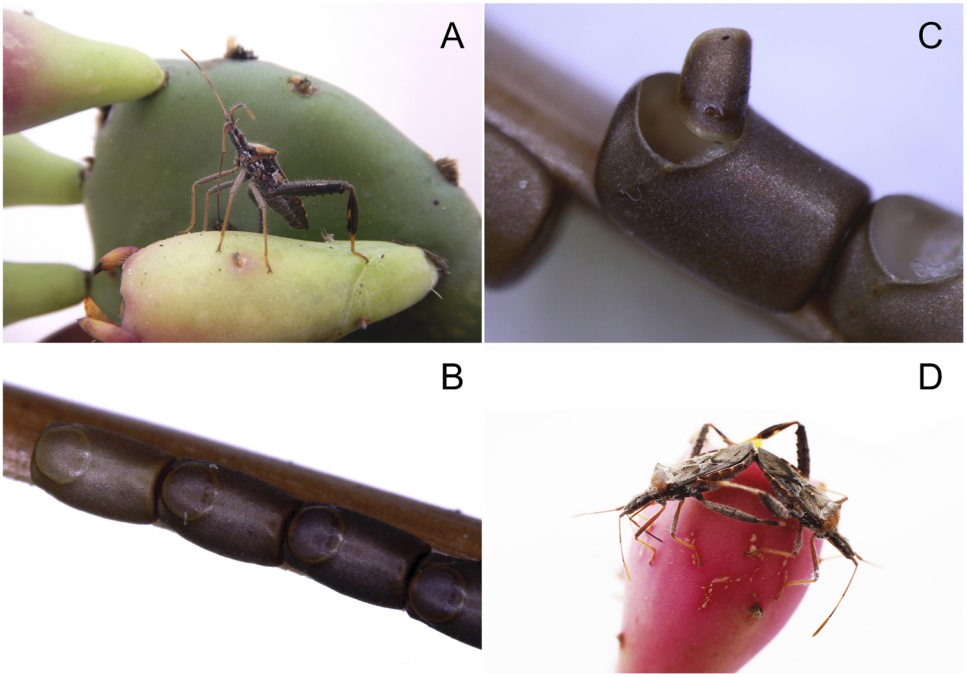

Fig. 1. *Narnia femorata* adults, eggs, and host plant. A) Adult probing ripening cactus fruit with mouthparts. B) Unhatched eggs on pine needle. C) Hatched eggs on pine needle. D) Mating pair on ripe cactus fruit. Photos A-C by Daniela Wilner; photo D by Christine Miller.

2017). Individuals in different cohorts can thus experience periods of nutritional stress at different life stages, depending on the timing and location of hatching. We were therefore able to implement ecologically relevant diet manipulations in the laboratory by controlling access to cactus fruit.

We tested whether temporary nutritional stress affected sexual behavior and long-term offspring production in these insects by manipulating diet during sexual maturation in previously well-fed females. We then returned them to good nutrition and allowed them to mate (Fig. 2). We expected this nutritional stress during ovarian maturation to impair females' long-term ability to produce eggs. We predicted that temporarily deprived females would go on to produce fewer offspring than their consistently well-fed counterparts, without recovering despite the return of excellent nutrition. We also hypothesized that females would exhibit nutrition-dependent mating receptivity. We predicted that, compared to well-fed females, deprived females would initially exhibit decreased receptivity to mating because of their poor nutritional state.

Our alternative hypothesis was that adaptations to life in variable conditions would enable *N. femorata* females to withstand or recover from brief periods of nutritional stress without compromising reproductive output. In this case, we would expect previously nutritionally deprived females to either maintain similar levels of offspring production to their well-fed counterparts, or if offspring production was initially compromised, to compensate with a higher rate of offspring production later. Under this alternative hypothesis, we predicted no difference in receptivity between temporarily deprived and well-fed females, and either no difference in offspring production, or an initial reduction relative to well-fed females followed by an increase.

2. Material and methods

2.1. Insect rearing

The insects used in this experiment were reared in the lab and

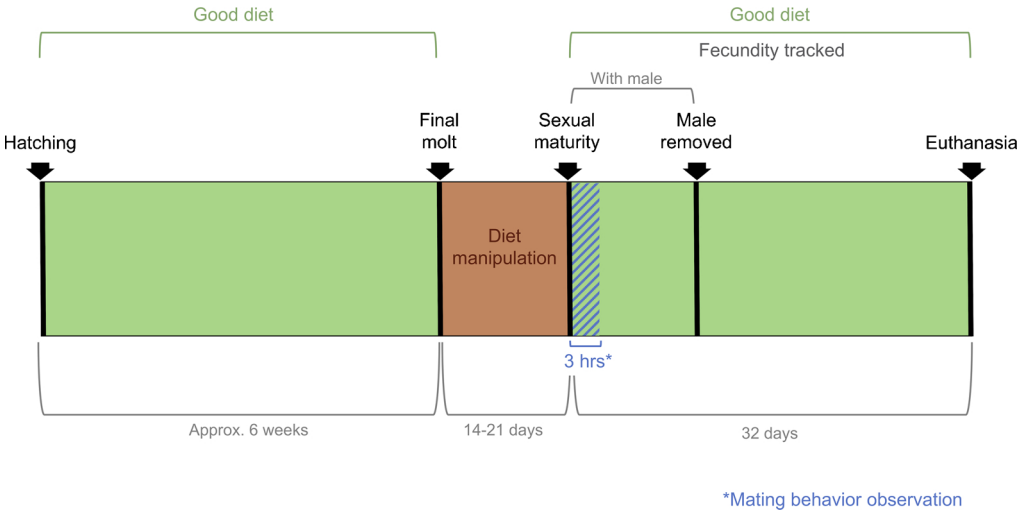

Fig. 2. Experimental design diagram representing timeline of an experimental female, from hatching to euthanasia. During the diet manipulation period, well-fed females received the optimum diet (cactus with fruit), and temporarily deprived females received a poor diet (cactus without fruit). All females received the optimum diet outside the diet manipulation period.

descended from wild *Narnia femorata* caught during late summer and early fall 2017 in North Central Florida. They had a mixture of wild-caught parents and first-generation lab-reared parents. We kept all insects in clear plastic, 32-oz deli cups with soil and an *Opuntia mesacantha* cactus pad. They also had late-season (ripening) cactus fruit, except for the temporarily deprived females during the treatment period (see Fig. 2). Cacti and fruit were monitored for quality, replacing fruits as necessary and moving insects to a new cactus cup if their cactus began to wilt or deteriorate. All insects were treated as humanely as possible.

Because *N. femorata* nymphs aggregate in the wild (Vessels et al., 2013), we reared nymphs in sibling groups until the fourth (penultimate) instar, each clutch in one cup. However, to limit the potential detrimental effects of high and low densities (Allen Monge, 2017), we split clutches that were larger than 13, and combined sibling clutches that were smaller than 5, so the range of nymph group sizes was 5–13 individuals per cup. Rearing temperatures were variable during the insects' first two instars of development (around 28 °C, occasionally rising over 32 °C) because they were distributed among greenhouse space and indoor rearing spaces, but all insects were assigned treatments randomly so that treatments were distributed across all rearing spaces. All insects experienced a 14:10 photoperiod. All juveniles were kept at approximately 28 °C during their third instar, and upon reaching the fourth instar, each juvenile was assigned a unique identification number, transferred to its own individual cup, and then kept in the greenhouse for the rest of its development. Insects that died at any point during the experiment were removed and not considered further; the focus of this study was on the survivors.

2.2. Treatments

Newly molted adult females were randomly assigned to one of two diet treatments: well-fed (n = 85) or temporarily deprived (n = 76). The former group had continual access to a cactus pad with late-season fruit; while the latter received a cactus pad *without* fruit during the diet manipulation period of 14–21 days (Fig. 2), which ended just after the time of sexual maturity (approximately day 13 post-eclosion, Allen et al., 2018). Females remained alone, on their respective diet treatments, until 14–21 days post adult molt. Then, each female was allowed fruit again, and 24 h later, she was paired with a randomly selected, unrelated virgin male of approximately the same age (we used the one-week range of ages to facilitate pairing with unrelated males that were also sexually mature but still young at the time of pairing).

2.3. Mating behavior observations

Pairs were placed in a deli cup with soil and a cactus pad with fruit, and observed (indoors, at approximately 28 °C, with fluorescent lights and an oscillating fan) for the first three hours they were together. We tracked male mating attempts and female receptivity to mating over these three hours. We considered a male to be attempting to mate if we observed him mounting (if he placed at least three legs on top of the female and paused, but not if he simply walked over her without pausing). Female receptivity was estimated by whether or not she accepted a mating attempt during the observation period (i.e. if a mounting resulted in genital contact, and this contact was maintained as the male and female positioned themselves at an angle of 90° to 180° to each other, see Fig. 1 D).

After the three-hour observation period, we provided each pair with pine needles as an egg-laying substrate (as in Allen et al., 2018). We then kept each pair together in its mating cup for 2–16 days, before removing males for use in a companion study (Greenway et al., 2020). *N. femorata* females can store sperm and continue to produce viable eggs for months after having mated, and the amount of time spent with a male has not previously been found to affect long-term egg production (Allen et al., 2018).

2.4. Fertility and fecundity monitoring

We kept each female for a total of 32 days after initial pairing with a male and monitored egg laying, dividing this period in two halves to observe patterns over time. We thus quantified female fertility and fecundity until approximately 7 weeks after adult eclosion. This is a relevant time period because the large month-to-month fluctuations in population numbers found in local wild populations (Cirino and Miller, 2017) suggest that these insects rarely live longer than a month as adults in the field, though they can live much longer in the laboratory (D Wilner, pers. obs).

Each female spent the first 16 days after pairing in the greenhouse. On Day 16 after the initial pairing, we moved each female to a temperature- and daylight-controlled room (approximately 28 °C, and 14:10 photoperiod); if there were already eggs or hatchlings in the female's cup, we also transferred the female to a new cup at this point. We performed weekly egg checks, and on day 32 from pairing, each female was cold-euthanized and stored at -18 °C for eventual photographing and morphometrics.

We monitored each egg clutch for at least 14 days after discovery because *N. femorata* eggs typically hatch seven to fourteen days after being laid (D Wilner, pers. obs.; Vessels et al., 2013). At that time, we counted the number of hatched and unhatched eggs per clutch to estimate the number of live offspring produced. Hatched *N. femorata* eggs have a very distinctive appearance, easily distinguished from unhatched eggs (and from those that are merely destroyed by predators or other causes): when the hatchling emerges, the egg's round pseudoperculum opens, like a small door, so that eggs with open and closed pseudopercula are easily distinguishable (Fig. 1 B and C).

2.5. Morphometrics

We photographed euthanized females next to a micro ruler, using a digital camera (Canon EOS 50D) connected to a dissecting scope, and we then used the program ImageJ, v.1.46 (Schneider et al., 2012) to obtain external body measurements: pronotum width; head length; fore femur length; hind femur length, width, and area; hind tibia area. We chose these seven measurements because, when used in a principal component analysis, they have been found to encompass most of the variation in female body size and predict egg production in this species (Miller et al., 2013). External dimensions become fixed at the final molt (before our diet manipulation period) and thus remained unchanged throughout our behavior and egg-monitoring period; we therefore only needed to photograph and measure each adult female once.

Males were cold-euthanized immediately after being removed from their mates' cups and dissected for a companion experiment (Greenway et al., 2020), then dried for 72 h at approximately 60 °C and weighed using a Mettler Toledo XP6 microbalance. We used male body mass measurements as a metric of male body size to include as a covariate in our analyses. We did so because we suspected a link between male body size and female offspring production, potentially because larger males may produce more sperm (Greenway et al., 2020)

2.6. Statistical analyses

We performed all statistical analyses using IBM SPSS Statistics v.25. We first ran a Principal Component Analysis (PCA) with a correlation matrix to distill the most representative component of female body size variation from our seven morphological measurements (n = 156, after removing 5 females with missing data). These seven traits were highly correlated (Table A1). We used a minimum eigenvalue of 1 as the extraction criterion and extracted a single component (PC1) that had an eigenvalue of 5.953 and accounted for 85.036 % of the variation (Table A2, Table A3Table A2); the results thus required no rotation. We then used this single variable (PC1) as a metric of female body size in subsequent analyses.

To test our hypothesis of the effect of diet on reproductive output, we first analyzed the effect of nutritional deprivation during sexual maturation on whether or not females produced any eggs over time. We built two separate generalized linear models (GLMs) to see if early and late egg production were affected differently by our treatments (e.g. if egg production in deprived females was initially compromised but increased later), one GLM for the first half of our egg-monitoring period (days 1 − 16 after pairing), and one for the second half (days 17 − 32). The response variable in each case was whether eggs were produced (yes or no) in that time period, with a binomial distribution and logit link function. The explanatory variables included female diet treatment (well-fed or temporarily deprived) as the main effect, and both female body size (PC1 from the PCA) and male body mass as continuous covariates. Females that were missing female and/or male body size data were excluded, leaving a sample size of 75 well-fed females and 69 temporarily deprived females. We initially included all two-way interactions in these models; we then sequentially removed the least statistically significant interaction (up to a threshold of $p = 0.05$) to arrive at the final models (no fixed effects were removed, only interactions). We only found interactions with $p < 0.05$ for egg production for the first half of the monitoring period.

We then focused in on the females that produced at least one egg to evaluate the effect of diet treatment on the magnitude of reproductive output over time. We again built one GLM for each half of the total egg-monitoring period. In each model, we excluded those females that had not produced any eggs in that half, and we used the number of live offspring (hatched eggs) produced in that time as the response variable. Resulting sample sizes consisted of 69 well-fed females and 50 temporarily deprived females in the first half, and 61 well-fed and 40 temporarily deprived females in the second half of the egg-monitoring period. We used a negative binomial distribution and log link function because the count data were overdispersed. Explanatory variables again included female diet treatment as the main factor, with female body size and male mass as covariates. We again initially included all two-way interactions between fixed effects, but the interactions were statistically non-significant ($p > 0.05$) and therefore not included in the final models.

Finally, we examined whether initial male mounting behavior and female receptivity (during the first three hours of encountering each other) predicted the number of live offspring produced over the next 32 days (our metric of long-term reproductive output). During our observation period, 88.8 % of males mounted their female partners, and 90.9 % of those pairs mated. We built two generalized linear models with the number of live offspring (ranging 0–253) produced in the 32 days of monitoring as a response variable. We used a negative binomial distribution and log link function to account for overdispersion. First, we asked whether male mounting behavior was predictive of live offspring production, and we used male mounting (did the male attempt to mate by mounting, yes or no) as the only explanatory variable ($n = 161$). Then, we asked whether female receptivity to mating was predictive of live offspring production; we excluded the 18 females that had not been mounted during the observation period and ran the same model as before but with female receptivity (did the female accept a mating attempt during the three-hour observation period, yes or no) as the explanatory variable ($n = 143$).

3. Results

The number of eggs laid by each female during the 32 days of monitoring varied from 0 to 278, and the number of live offspring per female varied from 0 to 253; Table 1 shows mean and maximum reproductive output in each half of the monitoring period. Most females in both diet treatments exhibited a reduction in live offspring production between the first and second halves of the monitoring period. Visual inspection of the relationship between the total number of offspring produced by a female and the amount of time she had spent with a male (2–16 days) suggested no evidence of an effect.

Table 1
Reproductive output (number of eggs laid and live offspring produced) per female ($n = 161$) in days 1 through 16 and 17 through 32 after initial pairing with a male.

	Eggs		Live offspring	
	Mean	Maximum	Mean	Maximum
Days 1 − 16	38.73	150	37.05	148
Days 17 − 32	25.14	141	23.92	124

Twenty-nine females did not lay eggs in the first 16 days (4 of these did lay eggs later); while 50 females did not lay eggs in the second 16 days (25 of these had laid at least one egg before). Twenty-five (15.5 %) of the 161 females never laid any eggs at all (6 well-fed and 19 temporarily deprived females), and 3 females laid at least one egg during the 32 days but still had no hatching success (2.2 % of the females that laid eggs). However, overall hatching success was quite high: on average, 92.6 % of a female's eggs hatched, and in females that produced at least one hatched egg (97.8 % of the females that laid), hatching rates averaged 94.7 %. On average, females that had been nutritionally stressed during sexual maturation produced 38.6 % fewer eggs and 38.4 % fewer live offspring than well-fed females (Fig. 3).

The first two GLMs supported the patterns revealed by the descriptive statistics, showing that temporary nutritional stress during sexual maturation had a strong negative effect on whether females produced any eggs or not, both in the first 16 days after initial pairing with a male, and in the following 16 days (Table 2). Females that had experienced nutritional stress during maturation were less likely to produce an egg during either time period than well-fed females. In the first 16 days, 26.3 % of temporarily deprived females failed to produce any eggs, versus 10.6 % of well-fed females, and in the second 16 days, 40.8 % of temporarily deprived females failed to produce any eggs, versus 22.4 % of well-fed females.

Larger females were more likely to produce at least one egg in the first 16 days, but female body size was not associated with whether or not females produced an egg in the second 16 days (Table 2). The body mass of males correlated with the reproductive success of their mates in both time periods: females paired with larger males were more likely to produce at least one egg (Table 2). We also uncovered an interaction between male mass and female body size (Table 2): females who had been paired with above average males almost always produced an egg in the first sixteen days, regardless of their own size (Fig. 4 A). Finally, we found a marginally statistically significant interaction between male mass and female diet treatment (Table 2): Egg production was especially unlikely when males were small and females had experienced food deprivation (Fig. 4 B).

We next examined the magnitude of reproductive output for those females that produced at least one egg in each time period. We found no detectable effects of female diet or female size, either in the first or last 16 days after pairing with a male (Table 3). Only male mass had a detectable effect on the magnitude of offspring production. Females that had been paired with larger males produced more offspring in the first 16 days after pairing, but we found no statistically significant effects of any of the explanatory variables in the last 16 days, nor of any interactions in either time period (Table 3).

We found that 80.7 % of the 161 pairs mated within the first three hours of encountering each other, with no apparent effect of female diet treatment (Table 4) or male mass (Fig. A1). The low number of unreceptive females precluded statistical analyses using receptivity as a response variable. Initial female receptivity to mating was predictive of later offspring production, but male mounting behavior was not (Fig. 5). Females that were mounted during the first three hours showed a trend towards higher live offspring production than those that were not mounted in this initial timeframe, but this trend was not statistically

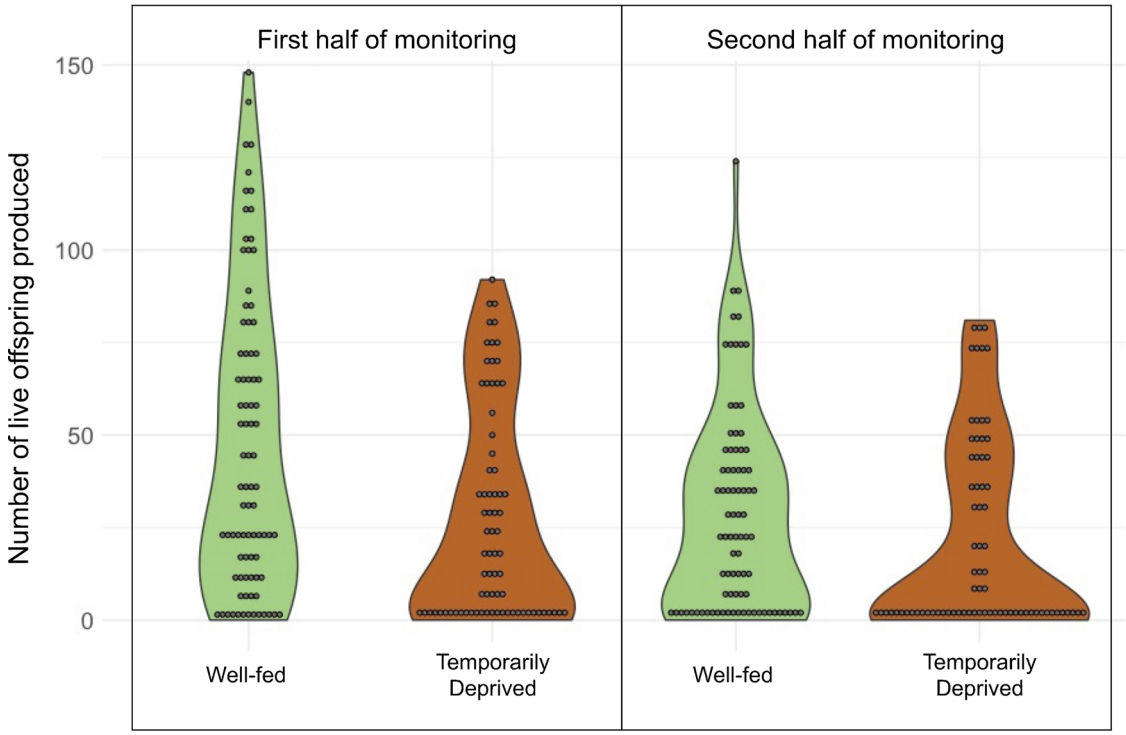

Fig. 3. Temporary nutritional deprivation affects offspring production over time. Number of live offspring produced by *Narnia femorata* females in the first (left) and second (right) halves of the 32-day monitoring period, immediately after first encountering a male, in consistently well-fed females (green) compared to females that experienced nutritional deprivation during sexual maturation (orange). Raw data plotted over violin plot, binned by increments of 5.

Table 2
Results of two GLMs evaluating the effect of diet during female sexual maturation on future reproductive success (whether eggs were laid or not) in the 32 days after initial pairing with a male.

		Eggs laid in days 1 – 16 (yes or no)		Eggs laid in days 17 – 32 (yes or no)	
Source	df	Wald X^2	p	Wald X^2	p
Female diet treatment	1	7.392	0.007	6.165	0.013
Female body size (PC1)	1	10.027	0.002	0.088	0.767
Male mass	1	12.304	< 0.001	22.870	< 0.001
Diet * Male mass	1	3.995	0.046	–	–
PC1 * Male mass	1	8.135	0.004	–	–

significant (Wald $\chi^2 = 0.860$; df = 1; p = 0.354). On the other hand, the positive correlation between female receptivity and live offspring production was statistically significant (Wald $\chi^2 = 10.312$; df = 1; p = 0.001). Nonetheless, sixteen (12.3 %) of those immediately receptive females still produced no hatched eggs at all in the next 32 days.

4. Discussion

Our results demonstrate that temporary nutritional stress during sexual maturation can have lasting, negative effects on female reproductive success, reducing the likelihood of producing even a single egg by 19 %. We did not find an effect of female diet on male mating attempts or female receptivity to mating, but initial female receptivity was predictive of future reproductive output, with initially unreceptive females producing fewer offspring in the long term.

Female *Narnia femorata* that temporarily experienced a poor diet immediately after adult eclosion were less likely to produce eggs over the next month, regardless of body size and despite having access to excellent nutrition before and after deprivation. This strong effect shows that even limited periods of nutritional stress in the last stages of development can be crucial to fitness. It suggests that these episodes can produce irreversible harm and could even eclipse the often-considered critical effects of the early environment. It is possible that nutritional deprivation during sexual maturation permanently stunts or greatly delays ovarian development, but more work is required to determine the mechanisms behind the patterns seen in this study. In those females that did produce at least one egg, we found no effect of diet on the magnitude of offspring production, suggesting an all or nothing phenomenon.

We also did not find evidence of temporarily deprived females increasing offspring production in later weeks, suggesting they are unable to recover. In fact, most females in both of our treatments produced fewer offspring in the last half of our monitoring period, and females that had been nutritionally deprived were especially likely to produce no eggs at all in that half. This pattern suggests that females in this species generally begin to experience decreased offspring production just a few weeks after sexual maturity, consistent with previous findings (Allen et al., 2018). Temporarily deprived females that do not reproduce in those first few weeks can miss their chance entirely. It is important to note that we did not track mortality in this study; future experiments should investigate the potential role of longevity-reproduction trade-offs in the response to temporary nutritional stress.

Nutritional stress often has negative impacts on reproduction when it is experienced early in life, consistently throughout development, or at the time of reproduction (Boggs, 2009; Chan et al., 2015; Leather, 1995; Lindström, 1999; Metcalfe and Monaghan, 2001; Monaghan, 2008; Wheeler, 1996), but few studies have assessed the more long-term impacts of temporary nutritional stress when it occurs in young adults before reproduction. Those that have studied nutritional stress during this period have generally found negative effects on fitness, but which specific traits are affected and in what direction varies (e.g. Hopwood et al., 2013; Kunz and Uhl, 2015; Barrett et al., 2009a, 2009b; Wittmeyer et al., 2001). Our findings highlight the need to further investigate the long-term impacts of nutritional fluctuations at different life stages, and to assess the effects over multiple episodes of

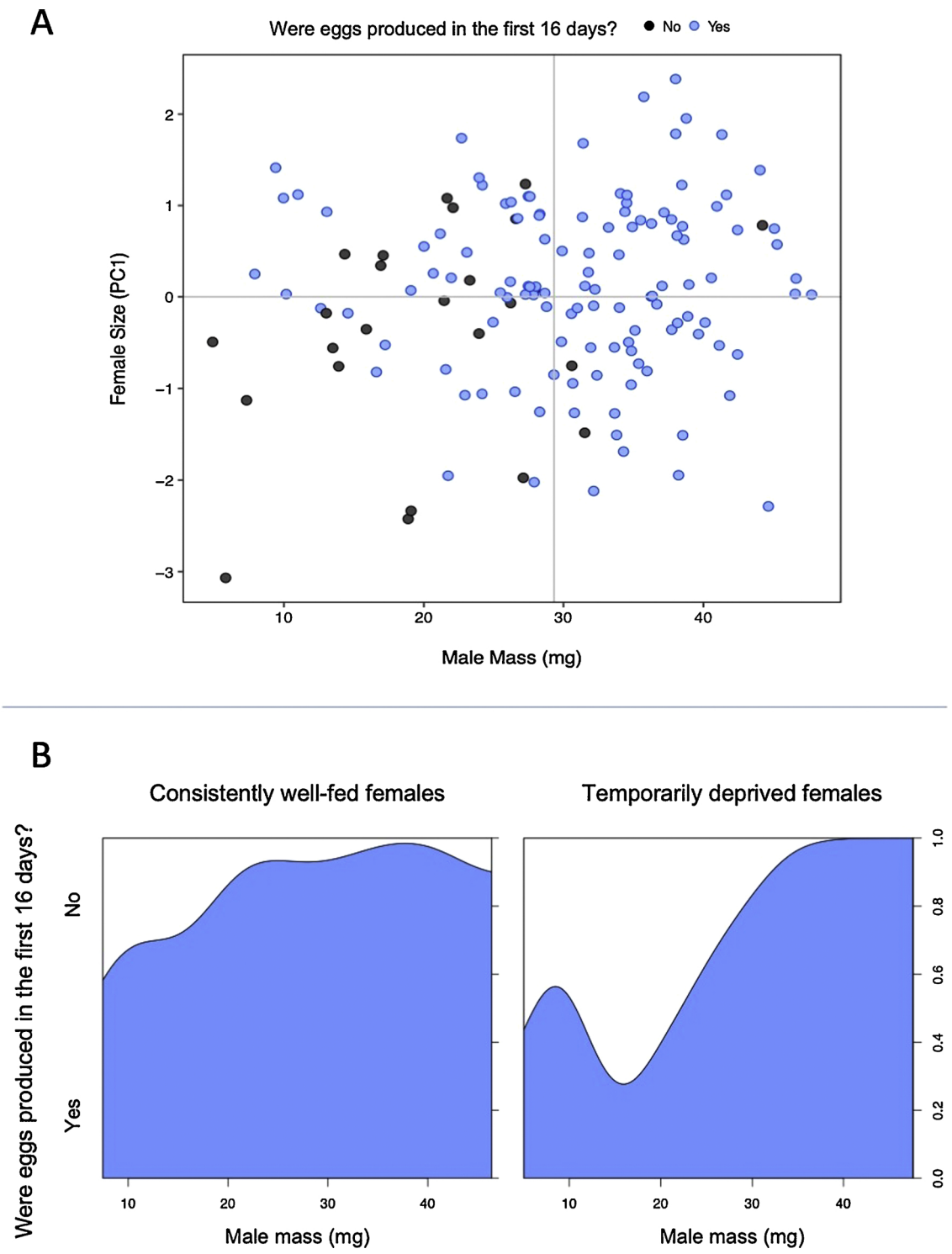

Fig. 4. A) Reproductive success in the first 16 days is related to the interaction between male mass and female size. Females who produced at least one egg in the first 16 days are represented by light blue dots, and those who did not by black dots. Female size (Principal Component 1) is on the y-axis, and the mass of the males they were paired with is on the x-axis. B) Females are more likely to produce eggs in the first 16 days if they were paired with larger males, and this relationship is stronger if the female had been nutritionally deprived. Conditional density plots of reproductive success (whether or not eggs were produced in this time period) in relation to male mass, comparing consistently well-fed females (left) to females who had been temporarily deprived before pairing (right).

reproduction. Manipulating the length of nutritional deprivation also represents an avenue for further investigation.

We uncovered intriguing relationships between male mass and female offspring production. Both reproductive success and the magnitude of offspring production were higher in females that had been paired with larger males. This effect may be driven by male testes size, which correlates with male mass (Greenway et al., 2020). We also found that smaller females generally were less likely to produce eggs in the first 16 days after pairing with a male. This effect was less pronounced in those that were paired with large males. Also, females who had been nutritionally deprived seemed to benefit disproportionately from mating with larger males. We can only speculate about the

Table 3
Results of two GLMs evaluating the effect of diet during female sexual maturation on future reproductive output (number of live offspring produced) in days 1-16 and 17-32 after initial pairing with a male, excluding those females that had not produced eggs in each time period.

		Number of live offspring produced in days 1 – 16		Number of live offspring produced in days 17 – 32	
Source	df	Wald χ^2	p	Wald χ^2	p
Female diet treatment	1	3.600	0.058	0.044	0.833
Female body size (PC1)	1	1.765	0.184	0.186	0.667
Male mass	1	20.802	< 0.001	1.880	0.170

Table 4
We found no relationship between sexual behavior and female diet. Number of well-fed and deprived females that were and were not mounted by their assigned male in the first three hours of pairing, and number of well-fed and deprived females that were and were not receptive to mating when mounted in the first three hours of pairing.

	Well-fed females	Deprived females
Mounted	76	67
Not mounted	9	9
Receptive	70	60
Unreceptive	6	7

mechanisms behind these intriguing patterns; perhaps coercion or direct benefits from male seminal proteins (Avila et al., 2011) and cryptic female choice (Eberhard, 2009) are at play.

Surprisingly, female nutritional history did not have detectable effects on initial sexual behavior (male mating attempts and female receptivity), despite affecting reproductive output, nor did it appear to relate to male mass. We observed very high percentages (near 90 % for both deprived and well-fed females) of both male mating attempts and female receptivity in the first three hours of pairs encountering each other. The extremely high rates of male mating attempts suggest that males may not outwardly assess or respond to the recent nutritional history of females. These results are consistent with those of Gillespie et al. (2014), where female nutritional stress did not influence male mating attempts in *N. femorata*. Unlike Gillespie et al. (2014) we did not find evidence that males discriminated based on female size; however, our current study did not include females as small as those present in Gillespie et al. (2014). The pronotum width of the smallest female in this current study was 77 % larger than the average pronotum width of the smallest females (which drove the observed male mate choice pattern) in the study by Gillespie et al. (CW Miller, pers. comm.).

Not only were males seemingly unresponsive to female nutritional state, the high rates of female receptivity we observed suggest that females do not adjust their sexual behavior in response to their own nutritional state, at least upon first encountering a male. Female nutrition and condition have been found to alter female choosiness and receptivity in other species (Aubret et al., 2002; Richardson and Smiseth, 2019), and mate-choice is considered a condition-dependent trait (Cotton et al., 2006; Jennions and Petrie, 1997), but results vary widely, even within arthropods (e.g. Barrett et al., 2009b; Richardson and Smiseth, 2019). *N. femorata* females, like many other insects, are capable of storing sperm for months (Allen et al., 2018). Mating regardless of current condition may thus be adaptive (especially when mate availability is low and adequate food is currently available) because these females can store the sperm until they are ready to oviposit.

While most females accepted mating attempts within the first three hours of encountering a male, those that did not accept initial mating attempts (13 out of 143) produced fewer live offspring in the following month. These few females that avoided mating may have been those in the very worst physical condition or affected by a factor not measured in this study. For example, other studies have found correlations between sexual receptivity and ovary or oocyte development (Obata, 1988). Our prediction that nutritionally deprived females would be less likely to mate upon first encountering a male was not supported, but this could still be a case of more subtle condition-dependent mating behavior. More research is needed on condition-dependent receptivity to mating and its relationship to diet, measuring different intrinsic factors (e.g. body mass, fat stores, number of oocytes in the

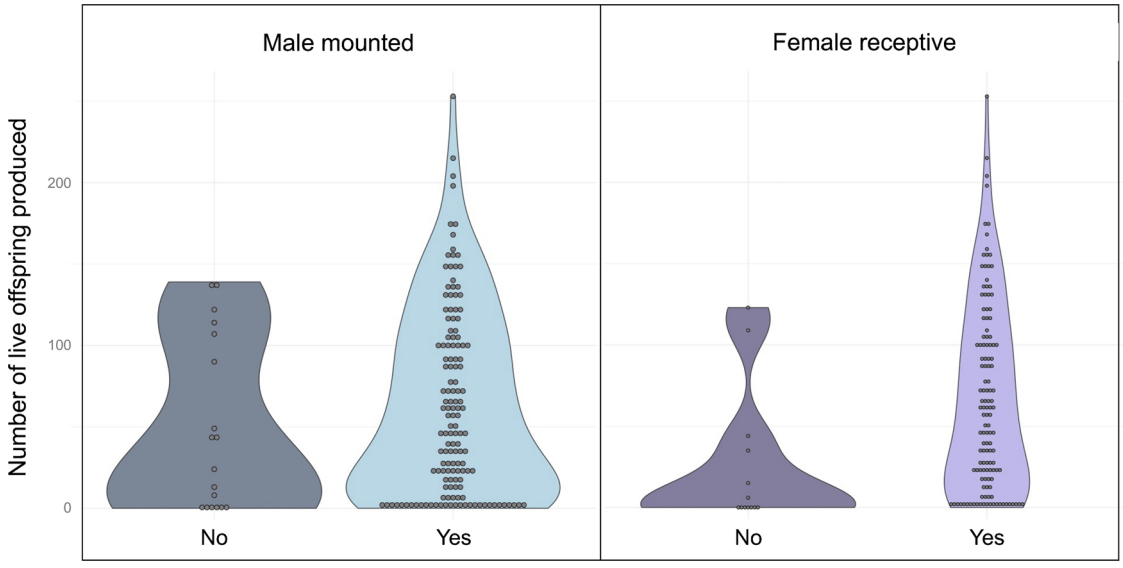

Fig. 5. Female receptivity, but not male mounting, predicts reproductive output. Number of live offspring produced in 32 days after pairing with a male, in *Narnia femorata* females that were not mounted within the first three hours of pairing (blue-grey) compared to those that were (light blue), and in *N. femorata* females that were not receptive to mating attempts within the first three hours of pairing (violet-grey) compared to those that were (lilac). Raw data plotted over violin plots, binned by increments of 5.

reproductive tract) that may affect female mating decisions.

In conclusion, our results support the hypothesis that brief nutritional stress during late stages of development can severely damage lifetime fitness by increasing the chances of complete reproductive failure. The benefits of a good early diet may be overshadowed, and a subsequent return of good nutrition can still be insufficient to reverse the damage done by deprivation during sexual maturation. We also show that this effect is not driven by nutrition-dependent variation in initial receptivity or attractiveness to males. The hypothesis that female receptivity should be nutrition-dependent was not supported, but the fact that initial female receptivity was nonetheless predictive of later offspring production is intriguing. These seemingly contradicting results raise questions about the interplay of nutrition, receptivity, and reproductive output, and about female self-assessment in the context of mating. Our findings may also have broad implications for population biology and ecology. We show that temporary changes in nutrition can affect future reproduction and have lasting effects, not just when experienced in the earliest stages of development, but also in young adults. A single, brief fluctuation in resource availability could thus have lasting effects on multiple cohorts (not just the season's young or the current breeding adults), reducing reproductive success across a population.

CRediT authorship contribution statement

Daniela Wilner: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing - original draft, Writing - review & editing. **E.V(Ginny) Greenway:** Conceptualization, Methodology, Investigation, Writing - review & editing. **Lauren A. Cirino:** Conceptualization, Methodology, Investigation, Writing - review & editing. **Christine W. Miller:** Conceptualization, Methodology, Formal analysis, Funding acquisition, Resources, Supervision, Writing - review & editing.

Declaration of Competing Interest

None.

Acknowledgements

We are very thankful to Dr. Rebecca Kimball and two anonymous reviewers for critical feedback that greatly improved this manuscript. We also are extremely thankful to the undergraduate researchers that contributed their time and effort to this project: Lauren Amodie; Daniela Gomez; Christian Guier; Haley Lenga; Amun Majeed; Elizette Rodriguez; Megan Rogers; Kayli Sieber; Ebony Taylor; Kathleen Wang; and the students of the UF ENY 2890 CURE class of Spring 2018. We also wish to thank the other members of the Miller Lab for their feedback and advice. Funding: This work was supported by the National Science Foundation [grant number IOS-1553100], the University of Florida Agricultural Experiment Station, and the National Institute of Food and Agriculture, U.S. Department of Agriculture, HATCH under FLA-ENY-005691.

Appendix A

Table A1
Correlation matrix for female body size principal component analysis. PW = pronotum with; HL = head length; FFL = front femur length; HFL = hind femur length; HFW = hind femur width; HFA = hind femur area; HTA = hind tibia area.

	PW	HL	FFL	HFL	HFW	HFA	HTA
PW	1						
HL	0.727	1					
FFL	0.878	0.795	1				
HFL	0.914	0.776	0.931	1			
HFW	0.810	0.617	0.773	0.847	1		
HFA	0.867	0.764	0.871	0.914	0.868	1	
HTA	0.823	0.765	0.875	0.864	0.747	0.866	1

Table A2
Principal components resulting from seven female body size measurements (pronotum with; head length; front femur length; hind femur length; hind femur width; hind femur area; hind tibia area). With a minimum eigenvalue of 1 criterion, only the first is extracted.

Component	Eigenvalue	% of Variance Explained
1	5.953	85.036
2	0.412	5.887
3	0.212	3.026
4	0.177	2.524
5	0.107	1.530
6	0.088	1.252
7	0.052	0.745

Table A3
Female body size principal component analysis component 1 (PC1) factor loadings. PW = pronotum with; HL = head length; FFL = front femur length; HFL = hind femur length; HFW = hind femur width; HFA = hind femur area; HTA = hind tibia area.

	Component 1 Factor Loadings
PW	0.934
HL	0.840
FFL	0.950
HFL	0.970
HFW	0.878
HFA	0.954
HTA	0.921

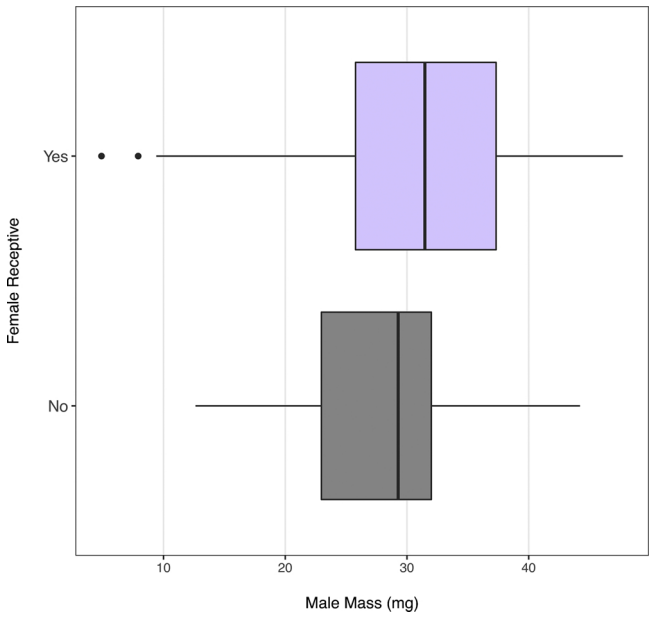

Fig. A1. Female receptivity and male mass. Boxplot showing male mass for females that were (lilac) and were not (gray) receptive: median; hinges at first and third quantiles; whiskers to minimum and maximum values (up to 1.5 times the interquartile range below or above the hinges); and outliers beyond whiskers.

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