



Timing is everything: Effects of day and night warming on predator functional traits

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ABSTRACT

Global mean temperature is increasing; however, when evaluated on a local scale, much of the documented warming is happening asymmetrically, with temperatures increasing either during the day or at night. Yet, most experimental studies increase temperatures constantly throughout a 24 h day, or disproportionately during the daytime, which could lead to a mismatch between evaluated and predicted warming. We conducted three experiments to evaluate how timing of warming (constant, day, or night) influences development, predation, and respiration rates of predators. Lady beetles were reared from egg to adulthood in one of four different temperature treatments. Next, we compared aphid consumption rates by adult lady beetles that were reared in different conditions. Finally, all individuals were exposed to a single similar temperature to evaluate respiration rates. Warming treatment affected many of the measured predator traits, including hatching success, development times, predation rates, and respiration rates, but the direction and magnitude of effects differed among traits measured. Our results demonstrate that the timing of warming has ecologically-relevant effects and that future studies should strive for more realistic warming treatments to increase their predictive power and reliability of results.

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1. Introduction

Climate change is expected to have effects on species and their ecological interactions, but predicting these effects is difficult because of complex interspecific interactions within communities (Blois et al., 2013; Davis et al., 1998; Urban et al., 2016; Van der Putten et al., 2010). While initial climate change work largely emphasized the implications for plants and resulting bottom-up effects on other species (Koricheva and Hayes, 2018), recent work has revealed that top trophic-levels are often more sensitive to alterations in natural climatic patterns (Ripple and Van Valkenburgh, 2010; Voigt et al., 2003), and their responses can have important top-down effects (Zarnetske et al., 2012). Specifically, climate warming can influence predator functional traits, such as consumption rates (Wu et al., 2011), and cause habitat shifts (Barton and Schmitz, 2009), which can then affect lower trophic levels. Thus, understanding how predators are affected by climate warming is fundamental to our understanding of communities in the Anthropocene.

Despite increasing numbers of published examples on the effects of warming on food webs (Barton, 2017), it remains unclear how well experimental studies predict effects of actual climate change. Effects of

temperature are context-dependent, so subtle differences between experimental treatments and actual climate warming could produce misleading inferences about effects of warming (Hoover and Newman, 2004; Langley et al., 2018; Thompson et al., 2013; Wolkovich et al., 2012). While evaluating every nuance of warming within an ecosystem is logistically infeasible, incorporating variability and realistic temporal patterns of temperature is an important step towards more realistic experiments.

Recent investigations demonstrate that Earth is not warming at a constant rate, but is instead warming in complex ways that can have a similarly complex influence on ecological responses (Buckley and Huey, 2016; Clark et al., 2014; Stoks et al., 2017). Empirical observations and climate models have revealed that warming is occurring asymmetrically within a 24 h day, with night temperatures generally increasing more than day temperatures (Davy et al., 2017; Easterling et al., 1997; Vose et al., 2005). Whereas the direct effect of night-warming on plant communities has been investigated (Peng et al., 2013; Peraudeau et al., 2015; Turnbull et al., 2002; Volder et al., 2007; Yang et al., 2016), effects on higher trophic levels are less frequently examined. Thus, our current understanding of how predators and their top-down effects are influenced by climate change is based on over-simplified warming treatments that disproportionately warm during the daytime or increase temperatures constantly throughout a 24 h day. This is troublesome because the few empirical examples that exist (Barton and

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Schmitz, 2018; Miller et al., 2017), as well as metabolic theory (Speights et al., 2017), suggest that day and night warming may have contrasting effects on predators.

A predator's influence within a food web is dependent on myriad traits, including generation time, body size, consumption rate, and metabolic demands (Gravel et al., 2016; Schmitz, 2017). Since these functional traits can be influenced by temperature (Englund et al., 2011; McGill et al., 2006; Uiterwaal and DeLong, 2018) and could respond differently when the timing of warming is shifted, it is important to learn how asymmetric warming could impact predator life-history characteristics. For example, longer predator generation times could lead to increased body size and, therefore, increased prey consumption. However, a decreased rate of development also could expand the time an individual is vulnerable to predation. Understanding how predators will respond to climate change is not just a theoretical exercise, but may also be important for applied issues including the biological control of pest species (Logan et al., 2003; Thomson et al., 2010).

For example, aphids are among the most common herbivorous pests and are often controlled by a suite of predators (Koch, 2003; Losey and Denno, 1998; Luff, 1983). Lady beetles provide an important ecosystem service by consuming aphids and controlling their populations, which can reduce reliance on chemical pesticides to prevent crop damage (Ragsdale et al., 2011). In particular, the multicolored Asian lady beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae), has been shown to consume proportionally more aphids than other coccinellids (Costamagna and Landis, 2007; Finlayson et al., 2010; Lucas et al., 2002), making them an influential biocontrol agent of many aphid species. Because these lady beetles have become an integral part of agroecosystems and integrative pest management strategies, understanding effects of warming on their functional traits and ability to control aphids has important agricultural applications.

Lady beetles and aphids are commonly used in climate warming experiments and modeling efforts (Abbott et al., 2014; Lamana and Miller, 1998; Zhang et al., 2014), and these established studies can be used to generate useful predictions and among-study comparisons. For example, lady beetle egg survival and the time spent in egg and early developmental stages are influenced by constant and variable temperature treatments (Lamana and Miller, 1998; Zhang et al., 2014), which could influence their susceptibility to intraguild predation (Pell et al., 2008) and alter predator populations. Because these species can be studied in the laboratory, they are especially useful for investigating nuanced effects of warming on predator traits.

Our study examines how different patterns of warming impact lady beetle functional traits that are fundamental to top-down control of their prey. We conducted three sets of experiments (Development, Predation, and Metabolic; Fig. A. 1 'Experiments') to evaluate several predictions. First, we evaluated the effect of four different warming scenarios (ambient, constant, day, or night) on lady beetle development rates from egg to adulthood, as well as survival and final body mass. Secondly, we asked the question: are there differences in predator foraging rates between individuals reared in, or introduced to, different warming environments? For this experiment we measured predation rates of the reared adult lady beetles under each warming scenario to evaluate effects of warming and acclimation. Finally, to evaluate how different temperature scenarios may influence predator's ability to tolerate extreme heating events, we measured lady beetle respiration rates at high temperatures. Overall, these experiments allow us to test the hypotheses that the timing of warming matters for predators and their prey, and those different functional traits may be affected by warming in different ways.

2. Methods

All experiments were conducted in plant growth chambers (Percival, Model: E41L2C8, Perry, Iowa, USA) at Mississippi State University in Spring 2018. The four temperature treatments were ambient with mean: 19 °C (14/24 °C, min/max) and three warming treatments

with means 23 °C: constant (18/28 °C), day (14/32 °C), and night warming (22/24 °C) (Table A. 1). The ambient temperature represented the lady beetles thermal optimum (Soares et al., 2003; Wang et al., 2009), and a mean temperature increase of 4 °C represented the predicted mean global temperature increase (IPCC, 2014). All growth chambers were programmed to maintain 40% relative humidity and a 12/12 h light/dark cycle. Temperature increases occurred at rate of 1 °C every 2.5 min and temperature decreases occurred at a rate of 1 °C every minute. Lady beetles and pea aphids, *Acyrtosiphon pisum* (Homoptera: Aphididae), were obtained from laboratory colonies maintained at Mississippi State University. The lady beetle colony was established with wild-caught individuals from locations near Starkville, MS, and was supplemented each year to maintain genetic variation and natural phenotypes. Because pea aphids are parthenogenic, all aphids in the study were genetically identical clones (clonal line a2a; pink/red color). Aphid colonies were maintained on fava bean plants (Windsor variety; Johnny's Selected Seeds Winslow, Maine, USA).

2.1. Experiment 1: developmental traits

To determine how warming influenced survival and development rates, we raised lady beetles from egg to adulthood in four temperature treatments. To produce eggs for the experiment, ten adult lady beetles were placed in each of two plastic containers (five males and five females in each container) and fed *ad libitum* for two days to encourage mating. We then determined sex of each lady beetle by the shape of the distal margin of the fifth visible abdominal sternite (male: concave, female: convex; (McCornack et al., 2007). Individual females were placed in individual petri dishes with mesh tops and fed aphids daily. Females were checked daily until eggs were observed, at which time we separated the egg mass and placed single eggs into individual petri dishes. Individual eggs were placed in each petri dish to combat egg or larvae cannibalism. We randomly assigned each individual petri dish containing one egg to one of four growth chambers and ensured relatively equal numbers of eggs from each brood placed into each growth chamber. Mother was recorded on each petri dish so that we could use it as a random effect in our statistical analyses. In total, 93, 96, 152, and 94 eggs were placed in the ambient, constant, day, and night-warming treatments, respectively. Due to low hatching success in day-warming treatments, we included more replicates in this treatment to ensure adequate numbers of larvae for subsequent analyses. A total of eight mothers were represented in trait analyses, with only 2 of 8 not being represented in all temperature treatments.

To reduce potential chamber effects, we reassigned the temperatures of the growth chambers every three days, and the lady beetles were moved accordingly (Fajer et al., 1991). After hatching, 26 1st instar larvae from each treatment were selected for continued monitoring and fed pea aphids daily *ad libitum* throughout the experiment. All additional lady beetles were removed from the experiment. We used 26 individuals because this was the lowest number produced (day-warming treatment) and we wanted to continue with a balanced experimental design. Lady beetles were checked every day to record development progress. The dates of the following developmental events were recorded: egg laid, egg hatched (1st instar larvae), molt to 2nd, 3rd, and 4th instar, pupation, and eclosion of adult. To obtain lady beetle body size, we weighed each lady beetle the day following eclosion. One individual from the ambient, constant, and day-warming, and two individuals from the night-warming, treatment did not reach adulthood and were excluded from the development analyses.

2.2. Experiment 2: predation traits

One week after eclosion, we began experiments to measure adult foraging rates in the four temperature treatments. We used 24 adult lady beetles from each rearing treatment (Experiment 1), randomly assigning six beetles from each treatment to all four treatments in a

fully-crossed design. At this time, sex was not considered when assigning individuals to foraging treatments. However, we did account for weight and that was used as a covariate in all statistical analyses. We fed them aphids *ad libitum* for five days in the newly-assigned foraging treatment before beginning the experiment. To standardize satiation levels among individuals, we removed all remaining aphids from petri dishes and starved the lady beetles for 24 h. We then placed 20 aphids into each petri dish for 2 h before returning to count and remove any remaining live aphids. This process was completed on three consecutive days for each lady beetle. We returned each lady beetle to their original rearing treatment after the completion of the predation trials. In the day and night-warming foraging treatments, only four and five individuals, respectively, were represented from the night-warming reared treatment. The constant foraging treatment had only four individuals represented from the constant and ambient-warming reared treatments.

2.3. Experiment 3: metabolic traits

To determine if rearing conditions influenced acclimation and how lady beetles respond to high temperatures, we measured their respiration rates at 32 °C, which is equivalent to the highest temperature present in our experiments (*i.e.*, the maximum temperature in the day-warming treatment). After the lady beetles were returned to their rearing treatment following the completion of experiment 2, all lady beetles, still in their individual petri dishes, were placed in a common room temperature environment (~23 °C) for 24 h. Lady beetles were returned to their rearing treatment to provide time for the completion of all predation experiments (different temperature treatments reached 'one week after eclosion' at different times). We did this to standardize the starting thermal conditions of all individuals, thereby isolating rearing conditions (experiment 1) as the explanatory variable. At the conclusion of all experiments, we determined the sex of each remaining lady beetle.

At 5-min intervals, we returned each lady beetle to a growth chamber set to 32 °C to measure respiration rates. Each lady beetle was placed into a respiration chamber (G113 Flow-Through Chamber, 1.6 cm ID × 10 cm L; Qubit Biology Inc., Kingston, Ontario, Canada) attached to a stop flow-through system, with an airflow rate of 200 mL/min, and an infrared CO₂ analyzer (Stoltz et al., 2012) (Q-S151 model, 1 ppm resolution; Qubit Biology Inc., Kingston, Ontario, Canada). Lady beetles were placed inside the respiration chamber 5 min after returning to the growth chamber. The tube was flushed with ambient air and then sealed for 3 min before the system was flushed again, and CO₂ peaks were recorded. The CO₂ analyzer readings were converted using the formula: $VCO_2 \text{ (stop flow)} = \text{integral}(CO_2) * \text{flow} / (\text{stop time}) / 1000$ (Rogowitz and Chappell, 2000).

2.4. Statistics

Data were analyzed in the R statistical programming environment (R Core Team, 2016). We used a generalized linear mixed effect model (GLMM) with binomial error distribution to analyze lady beetle hatching success. Rearing temperature treatment was a four-level (ambient, constant, day, or night-warming) fixed effect, and egg mother was used as a random effect. We used a linear mixed-effects model (LMER) to analyze lady beetle development time and body weight. For the development analysis, rearing temperature was a four-level fixed effect, and individual lady beetle was treated as a random effect. Post-hoc tests (least square means) were separated based on life stage (1st, 2nd, 3rd, and 4th instar, pupae, and adult). For the body weight analysis, rearing temperature was a four-level fixed effect, and mother was treated as a random effect. Since sex was not determined for all lady beetles that developed, sex was not included in this model. However, a supplemental analysis on those lady beetles that survived all experiments, revealed no interaction between sex and temperature on lady beetle weight (Fig. A. 2) Additionally, a chi-square test was used to determine that there were equal numbers of male and female

remaining lady beetles in all temperature treatments (Fig. A. 3). We used a GLMM with binomial error distribution to analyze lady beetle aphid consumption. Rearing and foraging temperature treatments were four-level (ambient, constant, day, or night-warming) fixed effects, weight was a continuous covariate, and individual lady beetle was a random effect because each lady beetle's aphid consumption was measured on three separate days. Post-hoc tests (least square means) were used to compare differences between the four-level temperature treatments as a whole and then based on rearing and foraging temperature treatments. We used a linear model (LM) to analyze beetle respiration rate. Weight was used as a continuous covariate for the analysis and rearing-temperature treatment was treated as a four-level factor.

Inferences from these models are based on likelihood ratio tests that compared models with and without target fixed effects and interactive effects. When needed, least square methods were used to complete post-hoc Tukey tests. Model assumptions were evaluated visually using QQ plots, residual plots, and likelihood profiles, as appropriate.

3. Results

3.1. Experiment 1: developmental traits

3.1.1. Hatching success

Rearing temperature treatment had a significant effect on lady beetle hatching success ($df = 3$, $\text{Chisq} = 83.8$, $p < 0.001$; Fig. 1). Post-hoc tests revealed that day-warming reared lady beetles had reduced hatching success ($\bar{x}=32\%$) when compared to ambient- ($\bar{x}=78\%$; $P < 0.0001$), constant- ($\bar{x}=62\%$; $P < 0.0001$), and night-warming ($\bar{x}=74\%$; $P < 0.0001$) reared lady beetles. Lady beetles reared in constant-warming treatments had lower hatching success than those reared in ambient conditions ($P = 0.053$).

3.1.2. Development time

Rearing temperature had a significant effect on lady beetle development time ($df = 3$, $\text{Chisq} = 35.8$, $P < 0.001$; Table 1). For 1st instar through pupation we detected an effect of warming, but no differences in development time, based on timing of warming. However, we did see effects in the egg stage and total development (egg-adult).

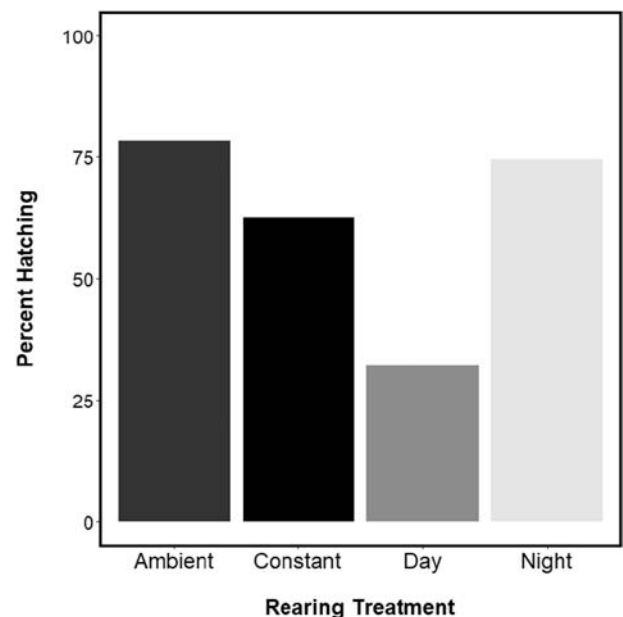


Fig. 1. Hatching success. Egg hatching success for each temperature treatment displayed as a percentage. Bars represent mean hatching success. A significant reduction was observed between day and all other treatments (all $P < 0.0001$), as well as between constant and ambient treatments ($P = 0.053$).

Table 1

Development time (days) for each life stage. Figure insets display mean \pm SE with letters denoting significant differences between warming treatments ($P < 0.05$).

Life Stage	Does warming matter?	Does the timing of warming matter?	contrast	t ratio	p value	
Egg	y	y	ambient - constant	11.103	<.0001	
			ambient - day	5.047	<.0001	
			ambient - night	10.947	<.0001	
			constant - day	-6.056	<.0001	
			constant - night	-0.042	1	
			day - night	5.952	<.0001	
1st Instar	y	n	ambient - constant	5.417	<.0001	
			ambient - day	4.432	0.0001	
			ambient - night	5.199	<.0001	
			constant - day	-0.985	0.7583	
			constant - night	-0.162	0.9985	
			day - night	0.812	0.8485	
2nd Instar	y	n	ambient - constant	4.139	0.0004	
			ambient - day	2.365	0.0909	
			ambient - night	3.902	0.001	
			constant - day	-1.774	0.2922	
			constant - night	-0.195	0.9974	
			day - night	1.561	0.4059	
3rd Instar	y	n	ambient - constant	4.875	<.0001	
			ambient - day	4.225	0.0003	
			ambient - night	4.142	0.0004	
			constant - day	-0.65	0.9153	
			constant - night	-0.684	0.9031	
			day - night	-0.04	1	
4th Instar	y	n	ambient - constant	2.331	0.1005	
			ambient - day	4.451	0.0002	
			ambient - night	2.691	0.0429	
			constant - day	2.119	0.1568	
			constant - night	0.384	0.9806	
			day - night	-1.713	0.3243	
Pupae	y	n	ambient - constant	13.121	<.0001	
			ambient - day	11.79	<.0001	
			ambient - night	11.792	<.0001	
			constant - day	-1.331	0.5464	
			constant - night	-1.179	0.6418	
			day - night	0.137	0.9991	
Egg-Adult	y	y	ambient - constant	19.655	<.0001	
			ambient - day	16.419	<.0001	
			ambient - night	18.472	<.0001	
			constant - day	-3.236	0.0097	
			constant - night	-0.965	0.7694	
			day - night	2.235	0.1238	

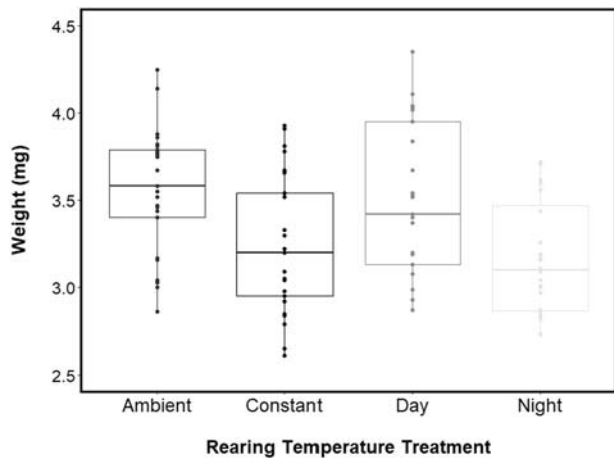


Fig. 2. Adult lady beetle weight. Adult lady beetle weight (mg) as a function of rearing temperature. Lady beetles in constant and night-warming treatments weighed significantly less than ambient conditions ($P < 0.05$), but other pairwise comparisons were not significant.

Time to hatching was 13.5, 29.7, and 29.7% longer in the ambient-warming treatment (4.44 ± 0.11 d) compared to the day- (3.84 ± 0.07 d; $P < 0.001$), constant- (3.12 ± 0.06 d; $P < 0.001$), and night-warming treatments (3.12 ± 0.06 d; $P < 0.001$), respectively. Eggs in

day-warming treatments had 18.7 and 19.7% longer development periods compared to constant- ($P < 0.001$) and night-warming treatments ($P < 0.001$), respectively. We found no difference between the constant- and night-warming treatments.

Total development time was 20.6, 24.8, and 23.6% longer in the ambient-warming treatment (26.2 ± 0.23 d) compared to the day- (20.8 ± 0.17 d; $P < 0.001$), constant- (19.7 ± 0.17 d; $P < 0.001$), and night-warming treatments (20.0 ± 0.37 d; $P < 0.001$), respectively. Lady beetles in day-warming treatments took 5.28% longer to develop than lady beetles in constant-warming treatments ($P = 0.009$). We found no significant differences between other treatment combinations.

3.1.3. Body weight

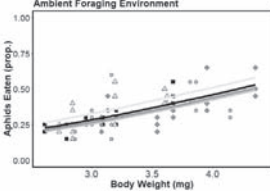
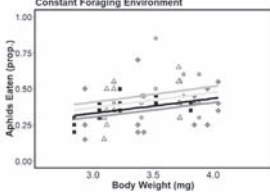
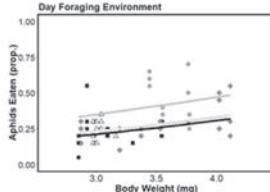
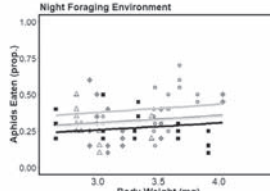
Lady beetle weight differed significantly among rearing-temperature treatment ($df = 3$, $\text{Chisq} = 17.3$, $P = 0.0006$; Fig. 2). Post-hoc test revealed an 8.57 and 11.4% significant reduction in constant- ($P = 0.012$) and night-warming ($P = 0.002$) lady beetle weight compared to those lady beetles reared in ambient conditions. Additionally, our results showed that lady beetles reared in day-warming treatments tended to weigh 8.57% more than those reared in night-warming conditions ($P = 0.060$).

3.2. Experiment 2: predation traits

Lady beetle aphid consumption differed based on rearing-temperature treatment ($df = 3$, $\text{Chisq} = 12.2$, $P = 0.006$; Table 2),

Table 2

Proportion of aphids eaten by adult lady beetles when placed in foraging environments. Lines represent predicted values (binomial error distribution) for each rearing treatment. For all figure insets individuals reared in ambient conditions are represented by dark grey circles, constant conditions are represented by black squares, day conditions are represented by light grey triangles. Ambient and night-warming foraging environments contrasts were left blank because no significant differences observed between the rearing treatments.

Foraging Environment	Does rearing treatment matter?	Rearing Environment contrasts	z-ratio	p-value	
Ambient	n				
Constant	y	ambient - constant	1.924	0.2178	
		ambient - day	2.807	0.0258	
		ambient - night	0.979	0.7612	
		constant - day	0.678	0.9054	
		constant - night	-0.993	0.7534	
Day	y	day - night	-1.705	0.3208	
		ambient - constant	2.789	0.0274	
		ambient - day	2.618	0.0439	
		ambient - night	2.022	0.1799	
		constant - day	-0.476	0.9644	
Night	n	constant - night	-0.512	0.9563	
		day - night	-0.036	1	

foraging treatment ($df = 3$, $\text{Chisq} = 13.2$, $P = 0.004$), and body weight ($df = 1$, $\text{Chisq} = 21.1$, $P < 0.001$). Aphid consumption by lady beetles placed in ambient- and night-warming foraging conditions showed no effect of lady beetle rearing treatment. Aphid consumption by beetles placed in day- and constant-warming foraging conditions showed effects of lady beetle rearing treatment ($P = 0.041$ and $P = 0.047$, respectively). Post-hoc tests revealed that in day- and constant-warming foraging conditions, ambient reared beetles consumed more aphids than day reared beetles ($P < 0.05$). In day-warming foraging conditions, ambient reared beetles consumed more than constant-warming reared beetles ($P < 0.05$).

3.3. Experiment 3: metabolic traits

Lady beetle respiration ($\mu\text{L}/\text{min}$) had a positive relationship with body weight ($df = 1$, $F = 32.9$, $P < 0.001$; Fig. 3), and differed based on rearing-temperature treatment ($df = 3$, $F = 3.88$, $P = 0.012$). Post-hoc tests revealed a significant respiration increase in the ambient-warming compared to the day-warming reared lady beetles ($P = 0.008$), but other pairwise interactions were not significantly different.

4. Discussion

We found evidence from a laboratory experiment that climate warming can impact predator functional traits that are important in top-down control of prey. Importantly, our results consistently show that different types of warming (constant, day, and night) have different effects on predators. This is concerning because most climate change studies use constant- or day-only warming treatments (Speights et al., 2017), whereas much of climate warming is driven by higher night temperatures (Davy et al., 2017). Thus, our study suggests that warming treatments that are inconsistent with actual climate change could lead to incorrect conclusions about how predators will respond to warming.

Warming as a whole (ambient versus warming treatments) influenced all ten of the traits we evaluated, and the timing of warming

Table 3

The effect of warming and timing of warming on each evaluated trait.

Trait	Does warming matter?	Does the timing of warming matter?
Hatching success	y	y
Development		
Egg	y	y
1st	y	n
2nd	y	n
3rd	y	n
4th	y	n
Pupae	y	n
Adult Body Mass	y	y
Foraging environment	Does rearing warming treatment matter?	Does the type of rearing warming treatment matter?
Foraging		
Ambient	n	
Constant	y	y
Day	y	y
Night	n	
Respiration		
32 °C	y	y

mattered for five of the ten (Table 3). When comparing day- and night-warming treatments specifically, the largest effects appeared to be at the egg stage, and then effect size decreased with age. Importantly, inferences about how warming affects lady beetles are different depending on treatment. Inferences from our day-warming results may be that predator populations will decline due to decreased hatching success and increased development time that could expose them to intra- and interspecific predation (Chen and Chen, 2018), but this has yet to be explored. However, our night-warming treatments seemed to benefit lady beetles, having no effect on hatching success, yet allowing them to develop faster. While reduced generation time could increase population growth rates, lady beetles in night-warming treatments were also smaller at adulthood, and we do not know how that may affect fitness. Smaller body size did not seem to reduce their ability to control pests, as lady beetles reared in night-warming treatments ate as many aphids as lady beetles from other treatments. Our results show that the timing of warming can impact these predators in important ways that may affect predictions of how climate change affects top-down control.

In addition to increases in mean warming, Earth's climate is becoming more variable and experiencing more extreme heating events (Thompson et al., 2013). We hypothesized that asynchronous warming could make predators more or less susceptible to the effects of extreme events. For example, if temperatures are warming asynchronously more in daytime, it may help predators to acclimate to variable extreme high temperature events. Indeed, we found that lady beetles from day-warming treatments had the lowest respiration rates when exposed to a high temperature. In contrast, our results suggest that night-warming predators were at a disadvantage when exposed to a high temperature, as they tended to have higher respiration rates than day-warming predators. Although nighttime warming did not benefit lady beetles by preparing them for extreme heating, predators from this treatment did not appear more stressed than those from ambient treatments.

Ecologists have been tasked with understanding and helping to predict effects of climate change. Unfortunately, it is impossible to study the responses of all organisms to increasing temperatures, and therefore a trait-based approach to developing generalizable predictions conducted in a controlled laboratory environment is essential. Despite some limitations, our work illustrates two important points. First, different traits respond to warming in different ways, thus making the net effect of warming difficult to predict. Consequently, it is essential that ecologists think critically about the traits that we study and the implications of our assumptions about remaining traits. Second, the timing of warming

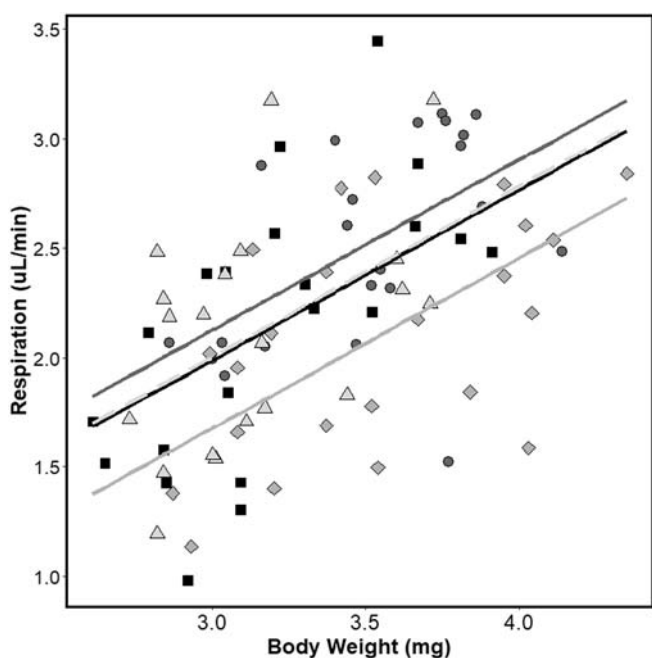


Fig. 3. Lady beetle respiration. Respiration rate as a function of weight (mg) and rearing temperature (ambient – dark grey circles, constant – black squares, day – grey diamonds, night – light grey (dashed line) triangles). Lines represent predicted values and symbols represent raw data. Lady beetles reared in ambient conditions had increased respiration rates compared to day-warming reared lady beetles ($P < 0.05$).

matters. Creating warming treatments is difficult (Speights et al., 2018), especially in the field, yet the nuances of warming are influential. While asymmetrical warming is not found equally across the globe, nighttime warming is commonly driving increased mean temperatures. As such, we encourage researchers to consider the timing of warming treatments carefully so that we can better understand effects of climate warming.

Declaration of competing interest

None.

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Author contributions

CJS and BTB developed the ideas for this research and designed the methods. CJS collected and analyzed the data. CJS and BTB interpreted the data, wrote the manuscript, and have given final approval for publication.

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Appendix A. Supplementary data

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