

RESEARCH ARTICLE

Experimental warming in the field delays phenology and reduces body mass, fat content and survival: Implications for the persistence of a pollinator under climate change

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Funding information

NSF, Grant/Award Number: DGE 11-43953; Danish National Research Foundation

Handling Editor: Caroline Williams

Abstract

1. Climate change is rapidly altering thermal environments across the globe. The effects of increased temperatures in already warm environments may be particularly strong because organisms are likely to be near their thermal safety margins, with limited tolerance to additional heat stress.
2. We conduct an in situ field experiment over 2 years to investigate the direct effects of temperature change on an early-season solitary bee in a warm, arid region of the Southwestern USA. Our field experiment manipulates the thermal environment of *Osmia ribifloris* (Megachilidae) from larval development through adult emergence, simulating both previous cooler (c. 1950; nest boxes painted white) and future warmer (2040–2099; nest boxes painted black) climate conditions. In each year, we measure adult emergence phenology, linear body size, body mass, fat content and survival.
3. Bees in the warming treatment exhibit delayed emergence phenology and a substantial increase in phenological variance. Increases in temperature also lead to reductions in body mass and fat content. Whereas bees in the cooling and control treatments experience negligible amounts of mortality, bees in the warming treatment experience 30%–75% mortality.
4. Our findings indicate that temperature changes that have occurred since c. 1950 have likely had relatively weak and non-negative effects, but predicted warmer temperatures create a high stress thermal environment for *O. ribifloris*. Later and more variable emergence dates under warming likely compromise phenological synchrony with floral resources and the ability of individuals to find mates. The consequences of phenological asynchrony, combined with reductions in body mass and fat content, will likely impose fitness reductions for surviving bees. Combined with high rates of mortality, our results suggest that *O. ribifloris* may face local extinction in the warmer parts of its range within the century under climate change.
5. Temperature increases in already warm ecosystems can have substantial consequences for key components of life history, physiology and survival. Our study provides an important example of how the responses of ectothermic insects to

temperature increases in already warm environments may be insufficient to mitigate the negative consequences of future climate change.

KEYWORDS

bees, ectotherms, heat stress, insects, *Osmia*, phenological mismatch, pollinator declines, warming experiment

1 | INTRODUCTION

Temperature is universally important for organisms (Hill, Wyse, & Anderson, 2016), and the thermal environment of a diversity of organisms is changing rapidly because of global climate change (IPCC 2014; Parmesan & Yohe, 2003). For ectothermic insects, temperature is a principal determinant of metabolism and developmental rates, and therefore, increases in temperature have a variety of consequences. Warmer temperatures associated with climate change lead to earlier phenological events, shrinking body size and reduced survival in insects (e.g. Bartomeus et al., 2011; Bowden et al., 2015; Gordo & Sanz, 2005; Penick, Diamond, Sanders, & Dunn, 2017; Sgolastra, Bosch, Molowny-Horas, Maini, & Kemp, 2010; Stange & Ayres, 2010). These responses to climate warming may have ramifications for insect population dynamics, species interactions, ecosystem function, and the local persistence or extinction of insect species (e.g. Burkle, Marlin, & Knight, 2013; Deutsch et al., 2008; Forrest & Chisholm, 2017; Kingsolver, 1989; Sheridan & Bickford, 2011).

Understanding the consequences of warming is especially timely for insect pollinators, given the importance of pollination services in concert with their documented global declines (Biesmeijer, 2006; Burkle et al., 2013; Garibaldi et al., 2013; Ollerton, Winfree, & Tarrant, 2011). It has been difficult to study the effects of climate warming on insect pollinators because relatively few long-term datasets exist that allow researchers to link pollinator ecology to changes in climate (Bartomeus et al., 2011; Burkle et al., 2013; Kudo & Ida, 2013; Ogilvie et al., 2017). The responses of some insect pollinators to warming have been investigated under simplified laboratory conditions (Bosch & Kemp, 2003, 2004; Fründ, Zieger, & Tschardtke, 2013; Sgolastra et al., 2011) and others with short-term observational studies (Forrest & Chisholm, 2017; Kudo, Nishiwaki, Kasagi, & Kosuge, 2004). However, field experiments that manipulate temperature conditions on a meaningful aspect of the pollinator's life cycle are conspicuously lacking and can provide a more predictive understanding of the direct effects of temperature change. Experiments that incorporate daily variation are likely to be particularly insightful because temperature variation itself can make insects more vulnerable to climate change (Kingsolver, Diamond, & Buckley, 2013; Paaijmans et al., 2013).

The effects of warming should be particularly strong when the temperature of the environment is near the optimal temperature of the organism (i.e. a narrow thermal safety margin; Deutsch et al., 2008). For this reason, ectothermic organisms in

already warm ecosystems may have especially limited tolerance to additional heat stress (Araújo et al., 2013; Hoffmann, Chown, & Clusella-Trullas, 2012; Huey et al., 2009; Kingsolver et al., 2013; Sunday, Bates, & Dulvy, 2011). Ectothermic insects are predicted to be particularly vulnerable to heat stress associated with future climate change at mid-latitudes (Hoffmann et al., 2012; Kingsolver et al., 2013). Furthermore, within these mid-latitude regions, insects living in characteristically warm environments like deserts may already face stressful temperature conditions (Vale & Brito, 2015).

We developed an experimental approach to investigate the direct effects of temperature variation associated with climate change on a mid-latitude, early-season pollinating bee (*Osmia ribifloris*; Megachilidae) under field conditions in the Southwestern USA. Temperatures for this region of the USA have already warmed by c. 1.55°C since 1949 and are projected to become increasingly warmer in the coming decades (Brusca et al., 2013; Garfin, Jardine, Merideth, Black, & LeRoy, 2013; IPCC 2014). Our field experiment manipulates the thermal environment experienced by *O. ribifloris* from larval development through adult emergence (Figure 1), simulating both previous cooler (c. 1950) and future warmer (2021–2099) climate conditions (Table 1), while simultaneously incorporating daily and day-to-day temperature variation. We examine how temperature directly influences several important components of *O. ribifloris* life history and physiology: adult emergence phenology, body size (linear size and mass), adult fat content and survival. This experimental set-up allows us to investigate how this early-season pollinator may have responded to warming over the past several decades, and how it may respond to continued warming in future.

2 | MATERIALS AND METHODS

2.1 | Study site and natural history

This experimental study was conducted in a warm, arid region of the Southwestern USA in the Santa Catalina Mountains, c. 40 km north-east of Tucson, Arizona (32°20'17.2248"N, 110°42'57.3228"W; 1,500 m a.s.l.). This dry, mid-elevation ecosystem sits at the intersection of open oak woodland and desert grassland, and is dominated by *Bouteloua curtipendula*, *Quercus emoryi*, *Q. arizonica* and *Arctostaphylos pungens* (Brusca et al., 2013; Whittaker & Niering, 1964). Our study population represents the lower elevation limits of *Osmia ribifloris* in the Santa Catalina Mountains.

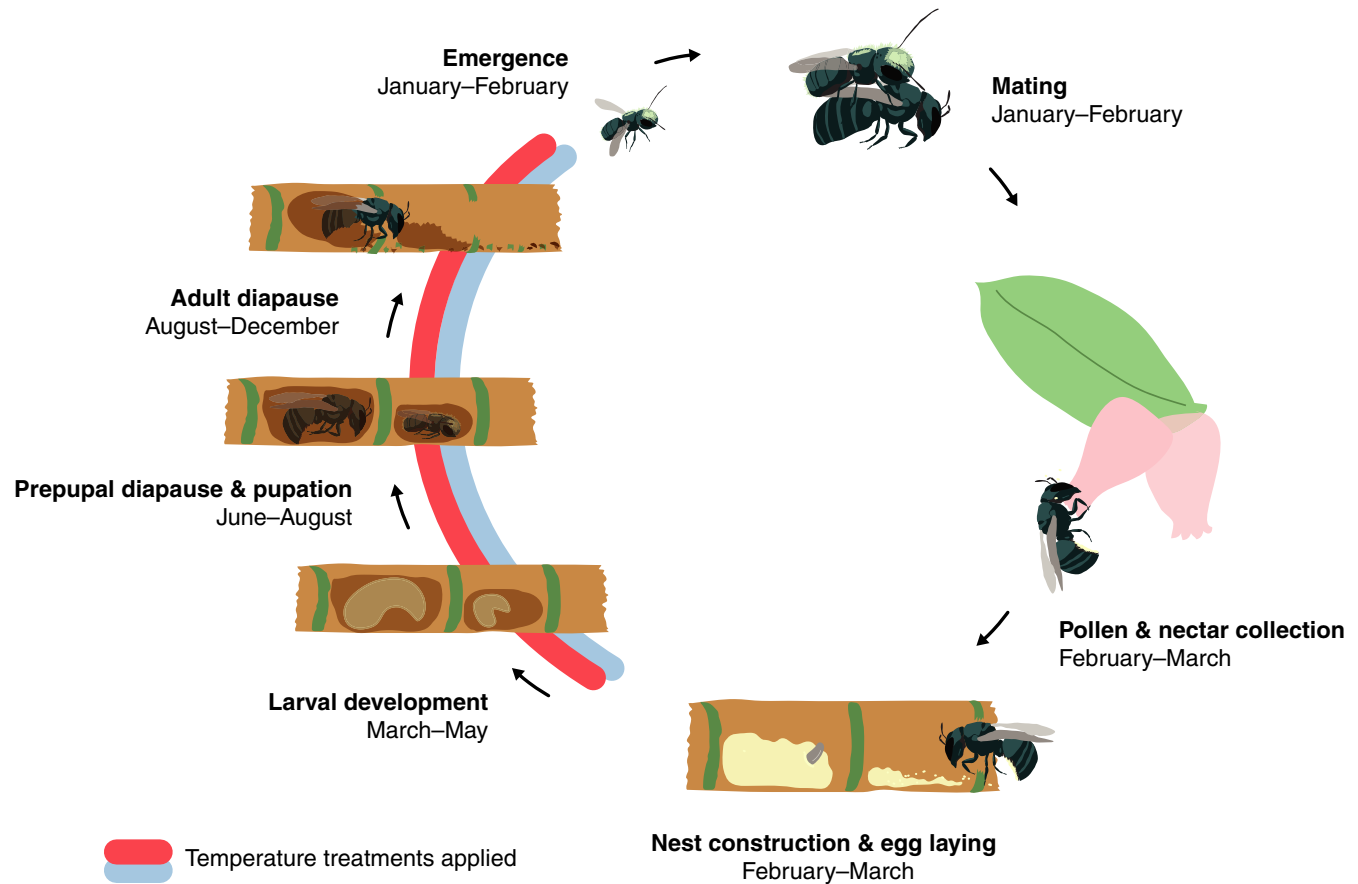


FIGURE 1 Life cycle of the solitary bee *Osmia ribifloris* in the study area near Tucson, Arizona, USA, in the Santa Catalina Mountains. Parts of the life cycle during which the temperature manipulation was applied are highlighted with red and blue. Note that members of the genus *Osmia* undergo a period of prepupal diapause as well as a period of adult diapause. Illustration by P.J.C

Osmia ribifloris biedermaui (Megachilidae, hereafter *Osmia ribifloris*) is a solitary bee native to the Western USA, with a patchy distribution (Rust, 1986). Female bees construct nests of masticated leaf pulp within existing wooden cavities. In our study population, females lay eggs in early spring, and larval development proceeds through spring until summer; larvae enter a state of prepupal diapause during summer, and following pupation in mid-late summer, adults remain inside the cocoon and enter a second diapause until emergence in January or February (e.g. Sgolastra, Kemp, Maini, & Bosch, 2012; Figure 1). Emergence in this population is notably earlier than other Western populations of *O. ribifloris* (which emerge in March and April; Krombein, 1967; Rust, 1986). Because nests are sealed and built inside wooden cavities that are shielded from sunlight and precipitation, temperature is the primary abiotic factor regulating development and phenology. Unlike most members of the genus, *O. ribifloris* is oligolectic, foraging and provisioning larvae with one or a few pollen resources (Haider, Dorn, Sedivy, & Müller, 2014; Rust, 1986). *Osmia ribifloris* emergence and nesting coincide with flowering of *A. pungens*; at this time of the season virtually no other floral resources are available until after nest-building is complete. Therefore, in our study population, females exclusively visit and collect pollen for larval provisions from *A. pungens* (point-leaf manzanita, Ericaceae).

2.2 | General experimental protocol

Our experiment has four stages: collection of newly completed nests from unmanipulated nest boxes and transfer to experimental nest boxes; exposure to experimental temperature treatments in the field from April until emergence in the following year; monitoring emergence phenology; and collection of emergent bees and postprocessing in the laboratory to measure adult body size and fat content.

2.3 | Natural population nesting phenology

In December 2012, a series of 30 artificial trap-nest boxes were equally distributed across three nearby sites (10 boxes per site). Nest boxes were constructed from wooden blocks to mimic natural nesting habitat (following Torchio, 1990); Megachilidae bees readily accept and build nests inside of these artificial wood nest boxes (also referred to as trap-nests). Thirty holes were drilled in each nest box, measuring 6 mm in diameter and 12 cm in length; each hole was lined with a paper drinking straw to allow for nest removal. Once per week, from January until the end of March, all active nests were scored with a start and end date.

Once population-level nesting was complete, a random sample of nests constructed during the population's peak nesting activity

TABLE 1 Historic (1950–2011) and projected (2021–2099) temperature change in the Desert Southwestern USA compared to mean temperature differences between experimental treatments and controls (cooling in relation to historic temperatures and warming in relation to projected temperatures). Historic temperature change is based on Brusca et al. (2013) for Tucson, Arizona, USA, and projected temperature changes are based on high emission (A2) scenarios reported in Garfin et al. (2013) for the Desert Southwestern USA; values represent lower and upper bounds

Year range	Historic or projected Δ °C	Mean temperature Δ °C relative to control			
		Cooling		Warming	
		2013	2014	2013	2014
1950–2011	–1.5 to 0.0	–0.9	–1.1	–	–
2021–2050	+1.0 to +2.0	–	–	+1.8	–
2041–2070	+1.8 to +3.3	–	–	–	+2.6
2070–2099	+2.8 to +5.0	–	–	–	–

Note. Note that the mean difference in warming relative to the control in 2013 falls within the upper bounds of the 2021–2050 temperature projections and the lower bounds of the 2041–2070 projections.

was relocated to the experimental temperature treatments nearby. Here, we define peak nesting as the two successive weeks with the greatest nesting activity. We limited our collection of nests to this peak nesting period to minimize effects with which the timing of nest construction may be correlated (e.g. floral resource availability). These nests were then randomly assigned to experimental temperature treatments.

2.4 | Temperature experiment

We established three temperature treatments in the field during the 2013–2014 season (2013 or year 1) and 2014–2015 season (2014 or year 2): warming, control and cooling. Bees were exposed to these temperature treatments from the early stages of larval development until adult emergence (Figure 1). The warming treatment consisted of nest boxes painted with flat black acrylic latex-based paint (Figure 2); because a black surface absorbs more radiant energy, this treatment warms internal nest temperatures. The control treatment consisted of identical nest boxes painted with a transparent acrylic paint (Figure 2); these resemble the unmanipulated nest boxes placed in the wild nearby. The cooling treatment differed between the 2 years of the experiment, although treatment effects were similar (see Section 3). In the first year of the experiment, nest boxes were affixed with wooden shade structures; in the second year of the experiment, nest boxes were painted with a white, reflective acrylic latex-based roof paint (Figure 2; Elastek, Tucson, AZ). Both cooling treatments reduced the absorption of radiant energy (via shading or reflection), which cools internal nest temperatures. The roofs of nest boxes in all treatments were painted with the same white, reflective paint to



FIGURE 2 Photograph of the field experiment at the study site near Tucson, Arizona, USA, in the Santa Catalina Mountains during the 2014–2015 season. Black nest boxes represent the warming treatment, white nest boxes represent the cooling treatment, and natural wood nest boxes represent the control treatment. Each nest box contains three individual nests relocated from nearby “unmanipulated” nest boxes; individual nests were randomly assigned to each nest hole across treatments. Each set of three nest boxes includes all three treatments, representing an experimental block

prevent excessive heating and to prevent any top-down temperature gradients within each nest box. Because the experiment is set up under naturally variable field conditions, the temperature manipulations incorporate day-to-day variation in temperature. All nest boxes contained an internal compartment to house a temperature data logger (HOBO, Onset, Bourne, MA, USA); data loggers were placed inside a subset of the manipulated nest boxes (2013, $n = 6$; 2014, $n = 15$) to monitor their internal temperatures during the course of the experiment.

During year 1 of the experiment, nine total experimental nest boxes were set up with three replicates within each treatment. Each experimental nest box contained a single vertical column of five nest holes. In April 2013, 45 nests were relocated from the unmanipulated nest boxes to the experiment. During year 2 of the experiment, 30 total experimental nest boxes were set up with 10 replicates within each treatment. Each experimental nest box had three vertical holes drilled into it. In April 2014, 90 nests were relocated from the unmanipulated nest boxes to the experiment.

In both years, the experiment was set up on an exposed hillside within an approximate 15×15 m area (Figure 2). All nest box holes were oriented approximately south. Within each experimental block, nest boxes were attached to a wooden structure similar to a sawhorse, and the centre of each nest box was c. 0.5 m from the ground (Figure 2). Treatments were set up in a fully randomized block design.

2.5 | Response variables

In early December, prior to adult emergence, all nests were affixed with a clear plastic vial to capture emerging bees (following Forrest & Thomson, 2011). Emergence was monitored every 3 days from the beginning of January until March, until emergence ceased. All emergent adults were collected, sexed and brought back to the laboratory for processing.

Body size was quantified with two measurements: (a) a linear measure of body size that is analogous to length (intertegular span, which is the distance between a bees' wing tegulae, measured to the nearest 0.01 mm; Cane, 1987), and (b) body mass (measured to the nearest 0.001 mg). Before weighing, all emergent bees were dried at 50°C for 5 days until reaching constant mass. Although linear body size and body mass may be strongly correlated, body mass can respond to changes in temperature while linear body size remains unchanged (Chown & Gaston, 2010), making it important to measure both variables.

Adult fat content upon emergence provides further insight into the underlying cause of any changes in body size. For example, warmer temperatures may lead to reductions in body mass because of depletion of fat bodies. Adult fat content was measured on a subset of adult bees. Dry bees were placed in three 24-hr changes of methyl-chloroform to extract lipids and then were redried and reweighed (Folch, Lees, & Sloane Stanley, 1957; Raubenheimer & Simson, 2003). The difference between the initial dry weight and dry weight following lipid extraction divided by the initial dry weight provides a measure of overall adult fat content (i.e. proportion body fat). Our measure quantifies total lipid content, rather than only storage lipids (fat bodies) and therefore provides an overestimate of storage lipids (Williams, Thomas, MacMillan, Marshall, & Sinclair, 2011). Nevertheless, in the related taxon, *Osmia lignaria*, total adult fat content evaluated via methyl-chloroform extraction is strongly related to storage lipid content (Sgolastra et al., 2011). Importantly, our method of lipid extraction does not introduce bias into our results: Any relative differences in adult fat content among treatments should be robust.

After emergence was complete (i.e. no newly emerging bees for at least two consecutive weeks), all nests were examined for the presence of dead bees (prepupa, pupa and adults). Sex could not be determined on all dead specimen because some were underdeveloped.

2.6 | Data analysis

Differences in temperature among treatments were analysed using linear mixed effects models in R 3.3.1 (*lme4* package in R; Bolker et al., 2009; Bates et al., 2015; R Core Team, 2016). The effectiveness of our temperature treatments were investigated using both mean temperature and daily maximum temperature. Day of year was included in each model as a random intercept to account for the repeated measure of daily temperature readings. Pairwise comparisons among treatments were examined using Tukey's Honest Significant Difference (HSD) method.

Differences in *O. ribifloris* response variables among treatments were analysed using linear mixed effects models with each bee nest nested within experimental block as a random intercept term. Each model also included a treatment \times sex interaction. Years were analysed separately because the number of bee nests and the design for the cooling treatment differed across years. After determining the presence of an overall treatment effect in each model for each response variable, pairwise comparisons among treatments for each sex in each year were examined using Tukey's HSD method. Sexes were analysed separately because sex-specific differences in response variables were expected: Male solitary bees emerge before females, and female bees tend to be larger than males. For adult emergence phenology, we additionally examined differences in variance among treatments using the nonparametric Brown-Forsythe Levene test for homogeneity of variances for each sex in each year. When unequal variance was detected, mean differences in emergence phenology among treatments for each sex in each year were examined using a linear mixed effect model that accounted for unequal variance (*nlme* package in R; Pinheiro, Bates, DebRoy, & R Core Team, 2016). Mortality (proportion of dead bees per nest, weighted by the total number of bees per nest) was analysed using a generalized linear mixed effect model with a binomial error distribution.

3 | RESULTS

Warming increased the mean daily internal temperature of the nest boxes by 1.8–2.6°C relative to controls; cooling decreased mean daily temperature by 0.9–1.1°C relative to controls (2013–2014, $F = 3,556$, $p < 0.0001$; 2014–2015, $F = 18,395$, $p < 0.0001$; Table 1). These *mean daily temperature* differences agree well with previous climate conditions (cooling treatment = c. 1950) and projected climate conditions (warming treatment = c. 2021–2099) (Table 1). However, because the experimental treatments are most effective at altering temperature during daylight hours, the treatment effects may also be realized via comparison of *daily maximum temperatures*. Here, warming increased daily maximum temperatures by on average 3.6°C during the first year of the experiment and 6.6°C during the second year of the experiment, relative to controls; cooling decreased daily maximum temperatures by on average 2.9°C during the first year of the experiment and 2.7°C during the second year of the experiment (2013–2014, $F = 4,458$, $p < 0.0001$; 2014–2015, $F = 21,349$, $p < 0.0001$; Figure 3 and Supporting Information Figure S1). Air temperatures in the area were variable across the 2 years of the experiment, with 2014 (i.e. the 2014–2015 season) ranking as the second warmest year on record since the 1980s and 2013 ranking as the 7th warmest year (Supporting Information Table S1).

During the 2013–2014 season, 163 bees emerged from 32 nests, amounting to 58, 54 and 21 bees from the control, cooling and warming treatments, respectively. During the 2014–2015 season, 131 bees emerged from 55 nests, amounting to 71, 79 and 13 bees from the control, cooling and warming treatments, respectively (Supporting Information Table S2). Bees did not emerge from

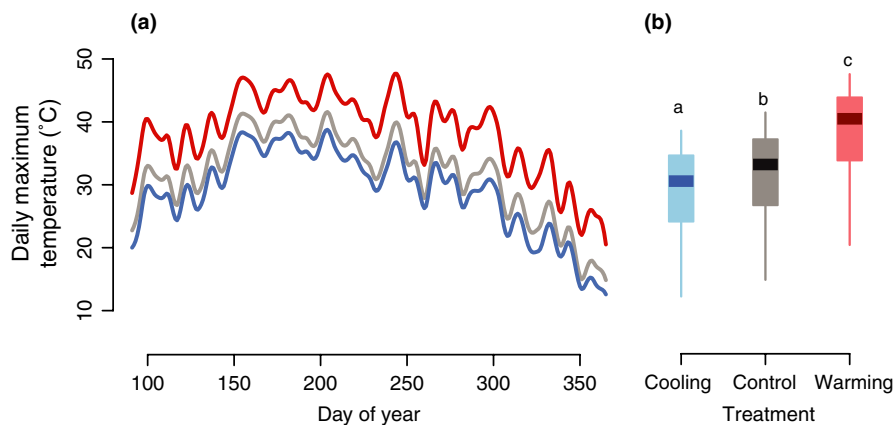


FIGURE 3 Experimental treatment effects measured with data loggers inside of nest boxes for the 2014–2015 season. (a) Daily maximum temperatures are from the approximate time of nest completion, April 2014, until the approximate time of adult emergence, January 2015. The lines for each treatment are slightly smoothed using a spline function for illustrative purposes (smoothing parameter = 0.35). (b) Box plots of daily maximum temperature for each treatment. Thick horizontal lines represent medians, with box edges representing the 25th and 75th percentile and whiskers representing minimum and maximum (within 1.5 times the interquartile range). Different lower case letters indicate pairwise significant differences at $p < 0.001$. Experimental treatment effects for the 2013–2014 season are qualitatively similar and are provided in Supporting Information Figure S1

all nests for several reasons: because of mortality that occurred during the temperature experiment, because of parasitism (by *Sapyga angustata*; P.J.C. unpublished data) that occurred during nest construction prior to nest transfer into the experiment, because bees left some nests vacant during nest construction (as defence against parasitism, e.g. Tepedino, McDonald, & Rothwell, 1979) or some combination thereof.

There was no treatment \times sex interaction for any response variable in either year, indicating that the effect of treatment did not depend on sex (Table 2). As expected, sex had a significant effect on all response variables (Table 2), and further analysis of results is shown separately for the sexes for each year of the experiment. Model coefficients for all pairwise comparisons for each response variable are provided in Supporting Information Table S3.

Warmer nesting temperatures delayed emergence date for both males and females, whereas cooler nesting temperatures had a weaker and more variable effect (Table 2 and Supporting Information Table S3; Figure 4). For males, warming led to a 9.8-day delay in the timing of emergence relative to the control in year 1, and a 23.1-day delay in year 2; for females, warming led to a 23.0-day delay in emergence in year 1 and a 4.9-day delay in year 2. For males, cooling led to a 6.6-day delay relative to the control in year 1, and a 2.9-day advance in year 2; for females, cooling led to a 5.1-day delay relative to the control in year 1, and a 2.1-day advance in year 2.

Warmer nest temperatures increased variance in emergence date, whereas cooling had a weaker and less consistent effect (Figure 4; Supporting Information Table S3). In most cases (except females in year 2), variance in emergence date differed across treatments (males, year 1: $F = 6.91$, $p < 0.003$; males, year 2: $F = 10.27$, $p < 0.001$; females, year 1: $F = 4.91$, $p = 0.019$; females, year 2: $F = 0.79$, $p = 0.457$). For males, warming led to a 5.5-fold and a 3.4-fold increase in variance in emergence relative to the control in years 1 and 2, respectively (Figure 4;

TABLE 2 Model coefficients for all response variables from generalized linear mixed effects models (except phenological variance, which is conducted on each sex in each year separately). p -values were estimated using the Kenward–Rogers approximation with type II sums of squares; type II sums of squares were used because we did not detect a significant treatment \times sex interaction

Response variable	2013–2014		2014–2015	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Emergence phenology				
Treatment	17.63	<0.0001	16.26	<0.0001
Sex	10.425	0.002	50.68	<0.0001
Treatment \times sex	1.24	0.299	0.471	0.626
Linear body size				
Treatment	2.70	0.080	1.95	0.149
Sex	526.78	<0.0001	970.10	<0.0001
Treatment \times sex	0.05	0.951	0.245	0.783
Body mass				
Treatment	8.283	<0.001	30.265	<0.0001
Sex	134.22	<0.0001	298.80	<0.0001
Treatment \times sex	0.023	0.978	1.451	0.240
Fat content				
Treatment	34.21	<0.0001	41.94	<0.0001
Sex	24.39	<0.0001	21.25	<0.0001
Treatment \times sex	1.613	0.211	0.338	0.714
Survival				
Treatment	9.392	<0.001	24.22	<0.0001

Supporting Information Table S3). For females, warming led to a 3.7-fold increase in variance in emergence in year 1 (Figure 4; Supporting Information Table S3). In contrast, cooling only significantly altered

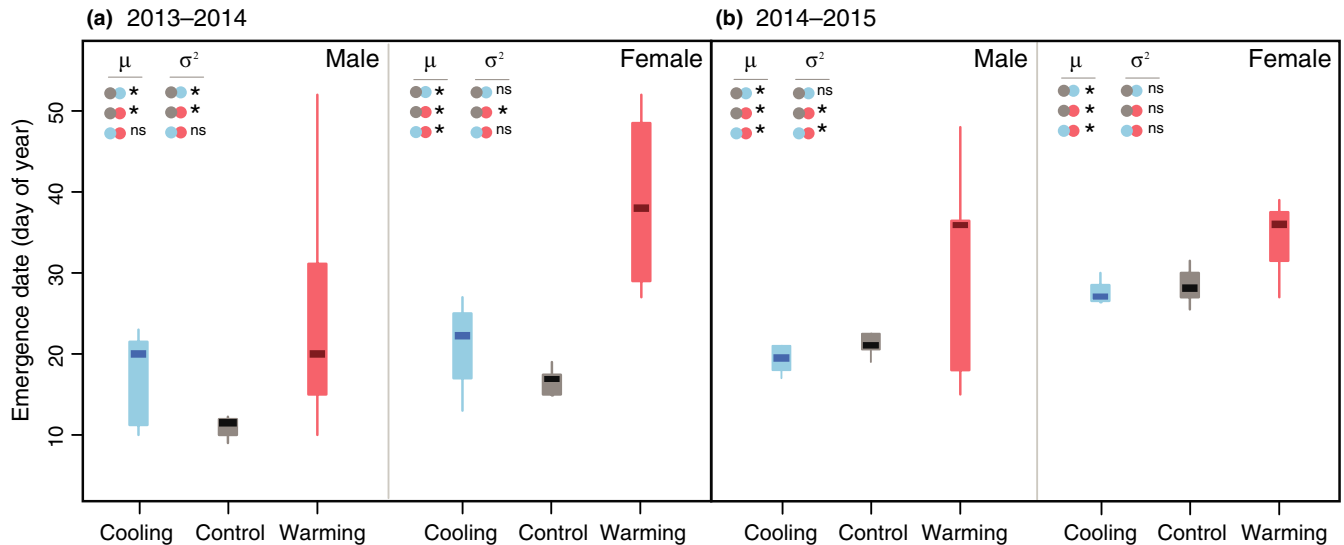


FIGURE 4 Adult emergence phenology for *Osmia ribifloris* males and females in three temperature treatments from the 2 years of the experiment for (a) the 2013–2014 season, and (b) the 2014–2015 season. For each year of the experiment, nesting occurs in year t (e.g. 2013) and emergence occurs in year $t+1$ (e.g. at the very start of 2014). Pairwise comparisons of means and variance are shown as insets. Asterisks represent significant differences at $p < 0.05$, and “ns” indicates no statistical difference (i.e. “not significant”). Box plot details are described in Figure 3. See Table 2 and Supporting Information Table S3 for all model coefficients

variance in emergence relative to the control in males in year 1 (by two-fold; Figure 4; Supporting Information Table S3).

Experimental nest temperatures had no effect on adult linear body size (Table 2; Figure 5). In contrast, adult body mass responded to temperature manipulation across all sexes and years (Table 2; Figure 5). For males, warming led to a 24.4% decrease in body mass relative to the control in year 1, and a 30.1% decrease in year 2; for females, warming led to a 14.4% decrease in body mass in year 1 relative to the control, and a 29.2% decrease in year 2. Cooling had an overall weaker effect in the opposite direction. For males, cooling led to a 9.0% increase in body mass relative to the control in year 1, and a 15.6% increase in year 2; for females, cooling led to a 6.3% increase in body mass relative to the control in year 1, and an 8.4% increase in year 2.

Warmer nest temperatures consistently led to a decline in adult fat content for both sexes, whereas cooling had no effect (Table 2; Figure 5). For males, warming led to a 45.0% reduction in fat content in year 1 relative to the control, and a 39.0% reduction in year 2; for females, warming led to a 47.2% reduction in fat content in year 1, and a 55.5% reduction in year 2. In contrast, bees in the cooling treatments experienced either a modest increase in fat content or no change at all relative to the control. For males, cooling led to a 7.3% increase in fat content relative to the control in year 1, and a 24.5% increase in fat content in year 2; for females, cooling led to a 6.8% decrease in fat content relative to the control in year 1, and a 20.0% increase in fat content relative to the control in year 2.

Experimental warming had a strong and negative effect on bee survival (Table 2; Figure 6). Mortality was greatest in the warmest treatments, reaching 30.3% in year 1, and 73.4% in year 2. In contrast, mortality was very low in both control and cooling treatments:

zero in year 1 and <5% in year 2 in controls, and <4% in year 1 and <1% in year 2 in the cooling treatment. In both years of the experiment, the majority of the observed mortality in the warming treatment occurred among adult bees [75.0% (12/16 dead bees) and 55.1% (27/49 dead bees), in years 1 and 2, respectively]. The remaining bees died during prepupal or pupal stages.

4 | DISCUSSION

Using an in situ field experiment to investigate the direct effects of temperature on *Osmia ribifloris*, an early-season solitary bee, we find consistent evidence that increases in temperature of a couple of degrees Celsius on average, and several degrees in terms of daily maximum, can have substantial consequences for key components of its life history and physiology. Responses of *O. ribifloris* to cooling suggest that the temperature changes that have occurred since c. 1950 until the present have had relatively weak and non-negative effects. However, the shifts in phenology, reductions in body mass and fat content, and increases in mortality in response to warming indicate that *O. ribifloris* is likely to approach the limits of its thermal safety margins under future climate change in the Southwestern USA, with implications for its long-term viability.

The consistently delayed emergence phenology, accompanied by a considerable increase in phenological variance, is likely driven by a combination of longer summers and shorter, warmer winters in the warming treatments (Figure 3; Supporting Information Figure S1). In the complete absence of winter conditions in a laboratory experiment, bees of the related taxon *O. lignaria* exhibit delayed emergence, in part owing to a lack of winter chilling (Sgolastra et al., 2010,

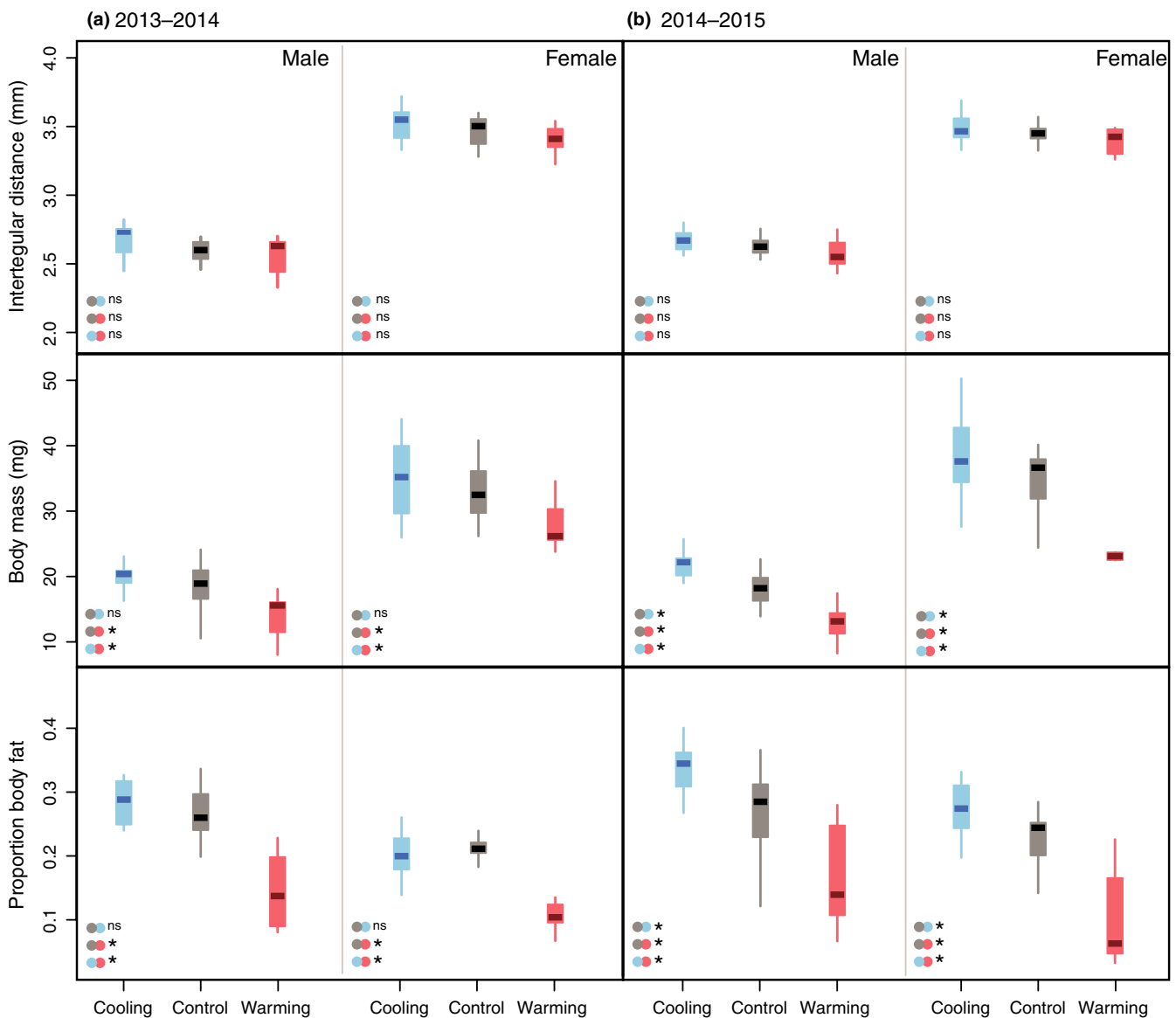


FIGURE 5 Response of linear body size (intertegular span), body mass and adult fat content of *Osmia ribifloris* to temperature manipulation from (a) the 2013–2014 season and (b) the 2014–2015 season. Pairwise comparisons of means are shown as insets. Asterisks represent significant differences at $p < 0.05$, and “ns” indicates no statistical difference. Box plot details are described in Figure 3. See Table 2 and Supporting Information Table S3 for all model coefficients

2011). Laboratory experiments also show that longer summers and warmer winters can desynchronize population-level emergence, whereas cool temperatures during winter can synchronize emergence among individuals (Sgolastra et al., 2012; Tauber, Tauber, & Masaki, 1986). In our mid-latitude study system, cool winter conditions are exceedingly brief (Brusca et al., 2013; Garfin et al., 2013), leaving a narrow margin during which *O. ribifloris* can meet any winter chilling required to terminate adult diapause and to synchronize population-level emergence. Therefore, increased temperatures in the warming treatment may disrupt the physiological processes underlying emergence phenology. In contrast to our results, increases in temperature across the globe generally lead to earlier phenological events for many organisms, including bees (e.g. Bartomeus et al., 2011; CaraDonna, Iler, & Inouye, 2014; Gordo & Sanz, 2005;

Høye, Post, Meltofte, Schmidt, & Forchhammer, 2007; McKinney et al., 2012; Parmesan, 2007; Thackeray et al., 2010). Many of these studies reporting advanced phenology are from relatively cooler, temperate ecosystems that experience pronounced winter. In the context of these general phenological advancements, our results are suggestive of a nonlinear response to warming (sensu Iler, Høye, Inouye, & Schmidt, 2013), in which warming leads to phenological delays rather than advancements in extreme conditions. Regardless of the mechanism underlying the changes in mean phenological events and their variance, these phenological responses have the potential to compromise temporal synchrony with floral resources and the ability of individuals to find potential mates. Bees that fail to emerge during the flowering period of the single primary floral resource available during early spring (point-leaf manzanita,

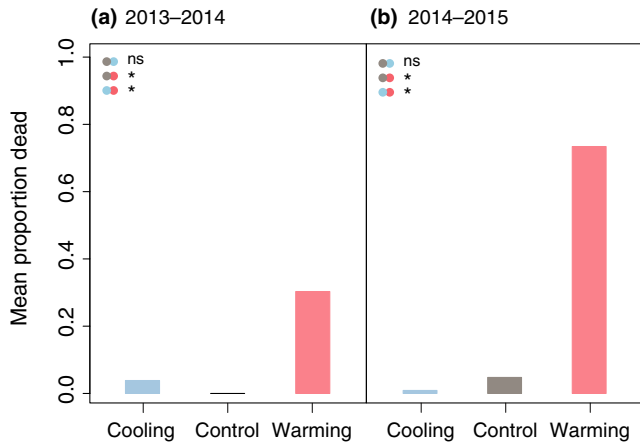


FIGURE 6 Mortality of *Osmia ribifloris* in three temperature treatments from (a) the 2013–2014 season and (b) the 2014–2015 season. Bars represent the mean proportion of dead bees per nest for each treatment. See Table 2 and Supporting Information Table S3 for all model coefficients

A. pungens) have virtually no food resources. Therefore, phenological synchrony with this floral resource should be paramount for population viability of *O. ribifloris*. Long-term data from the same location show that the point-leaf manzanita is flowering later in recent years (1984–2013; Crimmins, Crimmins, & Bertelsen, 2010; Rafferty, Bertelsen, & Bronstein, 2016), but the climate cues driving these delays are different than those for *O. ribifloris*. Our experiment suggests that *O. ribifloris* emergence (and subsequent nesting activity) is driven by temperature, whereas long-term data suggest that point-leaf manzanita flowering is driven by the timing of winter precipitation (later winter rains lead to later flowering; Crimmins et al., 2010). These two climate factors are not correlated ($r = -0.21$, $p = 0.09$) in this region, indicating that maintenance of phenological synchrony is not guaranteed under future climate change conditions. The increase in variance of adult emergence phenology may further challenge the ability of *O. ribifloris* individuals to be well-timed with both floral resources and potential mates. In sum, these phenological responses to future climate change conditions may predispose these adult bees to low resource levels, low mate availability or both, each of which may negatively affect fitness.

In addition to altering emergence phenology, warming also leads to considerable reductions in body mass and fat content (Figure 5). If warming has the strongest effect during larval development, we expect to see smaller adult linear body size (intertegular span) and reduced body mass. Instead, we find that linear body size remains constant across all temperature treatments, whereas body mass and fat content exhibit sharp declines in response to warming (Figure 5), suggesting that increases in temperature have the greatest effect during adult diapause. Indeed, Megachilid bees that enter diapause as adults lose weight rapidly during diapause, especially at warmer temperatures, but are otherwise insensitive to temperature changes during prepupal dormancy (Bosch, Sgolastra, & Kemp, 2010; Sgolastra et al., 2010, 2011). Therefore, the warming experienced during adult diapause in our experiment, and the concomitant lack of winter chilling during

this part of the life cycle, likely elevates the metabolism of *O. ribifloris*, reducing body mass via depletion of fat stores at a much faster rate compared to the temperatures experienced in control and cooling treatments. These reductions in body mass and fat content likely have strong direct effects on bee fitness. Within a given population, smaller insects generally have lower reproductive fitness (Kingsolver & Huey, 2008), and laboratory studies of other *Osmia* spp. show that adult bees with less mass have less fat stores, shorter life spans and lower adult vigour (all of which are approximate measures of fitness; Bosch & Kemp, 2003, 2004; Kemp & Bosch, 2005).

Although there is a suite of negative consequences related to shifts in phenology and changes in body mass and fat content, a high proportion of bees fail to survive the warming treatment altogether (Figure 6). There is also considerable interannual variation in mortality in the warming treatments, likely due to interannual temperature variation: 2014 is the second warmest year on record at our study site (Supporting Information Table S1), and this may exacerbate the effect of experimental warming on bee mortality compared to 2013. A longer duration of warmer summer conditions, combined with a shorter duration of cooler winter conditions, compromises the ability of *O. ribifloris* to regulate the onset and termination of adult diapause in addition to its metabolic activity during adult diapause (Sgolastra et al., 2010, 2011). The high levels of mortality in our experiment are consistent with the result of complete or near-complete depletion of fat stores during adult diapause owing to elevated metabolic activity under warmer conditions and insufficient chilling (Bosch et al., 2010; Sgolastra et al., 2011). In sum, these thermal stresses in the warming treatment likely lead to a cascade of physiological consequences that ultimately cause mortality (e.g. Sgolastra et al., 2011).

Our findings suggest that unless *O. ribifloris* can rapidly adapt to projected temperature increases in the future, it may face local extirpation in the warmer parts of its range within the century. Although warming leads to high levels of mortality, not all bees perish under increased temperature conditions. This leaves an opportunity for rapid adaptation to increases in temperature during nesting. However, *O. ribifloris* is likely near the limits of its thermal safety margins at our study site, and most terrestrial ectotherms have limited potential to increase their upper thermal tolerance limits (Araújo et al., 2013; Hoffmann et al., 2012). Being near an upper thermal safety margin also limits the ability of plasticity to compensate for environmental changes. The consequences of phenological asynchrony of adult emergence, along with reductions in body mass and fat content, will likely impose considerable challenges for surviving bees. If local extirpation does occur in the warmer parts of the geographic range of *O. ribifloris*, future warming could ultimately lead to range contractions towards higher elevations and latitudes.

Here, we show that temperature increases in an already warm, mid-latitude ecosystem can lead to a variety of responses in an early-season pollinating bee. However, these responses do not appear to mitigate the stressful thermal conditions imposed by the increases in temperature and may lead to the eventual local extirpation of this species. These findings illustrate the need for continued research on a diversity of organisms and from a diversity of ecosystems in

order to appropriately predict and understand organism responses to future climate change.

ACKNOWLEDGEMENTS

We thank M. Price and N. Waser for assistance with fieldwork, discussion of experimental design and constructive comments on an early draft of the manuscript; U. Basinger for assistance with early-season fieldwork; C. CaraDonna for engineering and construction advice; D. Papaj for suggestions on experimental design; J. Bronstein for helpful discussions during the early stages of the project; the Iler + CaraDonna Lab Group for comments on the manuscript; and A. Classen and N. Sanders for laboratory space and support at the University of Copenhagen. We additionally thank C. Fox, C. Williams and two anonymous reviewers for constructive feedback on the manuscript. This work was supported by NSF grant DGE 11-43953 to P.J.C., and additional funding was provided by the Danish National Research Foundation to P.J.C.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

P.J.C. conceived and designed the project; P.J.C. obtained funding for the project; P.J.C., J.L.C. and A.M.I. conducted fieldwork; P.J.C. & A.M.I. conducted laboratory work; P.J.C. analysed data; P.J.C. wrote the first draft of the manuscript; and all authors contributed to revisions.

DATA ACCESSIBILITY

The primary data used in this study are archived at the Dryad Digital Repository: <https://doi.org/10.5061/dryad.tv8v1d6> CaraDonna, Cunningham, and Iler (2018).

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REFERENCES

- Araújo, M. B., Ferri-Yáñez, F., Bozinovic, F., Marquet, P. A., Valladares, F., & Chown, S. L. (2013). Heat freezes niche evolution. *Ecology Letters*, 16, 1206–1219. <https://doi.org/10.1111/ele.12155>
- Bartomeus, I., Ascher, J. S., Wagner, D., Danforth, B. N., Colla, S., Kornbluth, S., & Winfree, R. (2011). Climate-associated phenological advances in bee pollinators and bee-pollinated plants. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 20645–20649. <https://doi.org/10.1073/pnas.111559108>
- Bates, D., Maechler, M., Bolker, B., Walker, S., Bojesen Christensen, R., Singmann, H., & Dai, B. (2015). *Package 'lme4'*. 1–95.
- Biesmeijer, J. C. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*, 313, 351–354. <https://doi.org/10.1126/science.1127863>
- Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J.-S. S. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution*, 24, 127–135. <https://doi.org/10.1016/j.tree.2008.10.008>
- Bosch, J., & Kemp, W. P. (2003). Effect of wintering duration and temperature on survival and emergence time in males of the orchard pollinator *Osmialignaria* (Hymenoptera: Megachilidae). *Environmental Entomology*, 32, 711–716. <https://doi.org/10.1603/0046-225X-32.4.711>
- Bosch, J., & Kemp, W. P. (2004). Effect of pre-wintering and wintering temperature regimes on weight loss, survival, and emergence time in the mason bee *Osmia cornuta* (Hymenoptera: Megachilidae). *Apidologie*, 35, 469–479. <https://doi.org/10.1051/apido:2004035>
- Bosch, J., Sgolastra, F., & Kemp, W. P. (2010). Timing of eclosion affects diapause development, fat body consumption and longevity in *Osmia lignaria*, a univoltine, adult-wintering solitary bee. *Journal of Insect Physiology*, 56, 1950–1958.
- Bowden, J. J., Eskildsen, A., Hansen, R. R., Olsen, K., Kurle, C. M., & Høye, T. T. (2015). High-Arctic butterflies become smaller with rising temperatures. *Biology Letters*, 11, 20150574. <https://doi.org/10.1098/rsbl.2015.0574>
- Brusca, R. C., Wiens, J. F., Meyer, W. M., Eble, J., Franklin, K., Overpeck, J. T., & Moore, W. (2013). Dramatic response to climate change in the Southwest: Robert Whittaker's 1963 Arizona Mountain plant transect revisited. *Ecology and Evolution*, 3, 3307–3319.
- Burkle, L. A., Marlin, J. C., & Knight, T. M. (2013). Supplementary materials: Plant-pollinator interactions over 120 years: Loss of species, co-occurrence, and function. *Science*, 339, 1611–1615. <https://doi.org/10.1126/science.1232728>
- Cane, J. H. (1987). Estimation of bee size using intertegular span (Apoidea). *Journal of the Kansas Entomological Society*, 60, 145–147.
- CaraDonna, P. J., Cunningham, J. L., & Iler, A. M. (2018). Data from: Experimental warming in the field delays phenology and reduces body mass, fat content, and survival: Implications for the persistence of a pollinator under climate change. *Dryad Digital Repository*, <https://doi.org/10.5061/dryad.tv8v1d6>
- CaraDonna, P. J., Iler, A. M., & Inouye, D. W. (2014). Shifts in flowering phenology reshape a subalpine plant community. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 4916–4921. <https://doi.org/10.1073/pnas.1323073111>
- Chown, S. L., & Gaston, K. J. (2010). Body size variation in insects: A macroecological perspective. *Biological Reviews*, 85, 139–169. <https://doi.org/10.1111/j.1469-185X.2009.00097.x>
- Crimmins, T. M., Crimmins, M. A., & Bertelsen, C. D. (2010). Complex responses to climate drivers in onset of spring flowering across a semi-arid elevation gradient. *Journal of Ecology*, 98, 1042–1051. <https://doi.org/10.1111/j.1365-2745.2010.01696.x>
- Deutsch, C. A., Tewksbury, J. J., Huey, R. B., Sheldon, K. S., Ghalambor, C. K., Haak, D. C., & Martin, P. R. (2008). Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 6668–6672. <https://doi.org/10.1073/pnas.0709472105>
- Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissue. *Journal of Biological Chemistry*, 28, 2077–2080.
- Forrest, J. R. K., & Chisholm, S. P. M. (2017). Direct benefits and indirect costs of warm temperatures for high-elevation populations of a solitary bee. *Ecology*, 98, 359–369. <https://doi.org/10.1002/ecy.1655>
- Forrest, J. R. K., & Thomson, J. D. (2011). An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows. *Ecological Monographs*, 81, 469–491. <https://doi.org/10.1890/10-1885.1>
- Fründ, J., Zieger, S. L., & Tschardtke, T. (2013). Response diversity of wild bees to overwintering temperatures. *Oecologia*, 173, 1639–1648. <https://doi.org/10.1007/s00442-013-2729-1>

- Garfin, G., Jardine, A., Merideth, R., Black, M., & LeRoy, S. (2013). *Assessment of climate change in the southwest United States*. Washington, DC: Island Press. <https://doi.org/10.5822/978-1-61091-484-0>
- Garibaldi, L. A., Steffan-Dewenter, I., Winfree, R., Aizen, M. A., Bommarco, R., Cunningham, S. A., ... Klein, A. M. (2013). Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science*, 339, 1608–1611. <https://doi.org/10.1126/science.1230200>
- Gordo, O., & Sanz, J. J. (2005). Phenology and climate change: A long-term study in a Mediterranean locality. *Oecologia*, 146, 484–495. <https://doi.org/10.1007/s00442-005-0240-z>
- Haider, M., Dorn, S., Sedivy, C., & Müller, A. (2014). Phylogeny and floral hosts of a predominantly pollen generalist group of mason bees (Megachilidae: Osmiini). *Biological Journal of the Linnean Society*, 111, 78–91. <https://doi.org/10.1111/bij.12186>
- Hill, R. W., Wyse, G. A., & Anderson, M. (2016). *Animal physiology*, 4th ed. Sunderland, MA: Sinauer Associates, Inc..
- Hoffmann, A. A., Chown, S. L., & Clusella-Trullas, S. (2012). Upper thermal limits in terrestrial ectotherms: How constrained are they? *Functional Ecology*, 27, 934–949.
- Høye, T. T., Post, E., Meltøfte, H., Schmidt, N. M., & Forchhammer, M. C. (2007). Rapid advancement of spring in the high arctic. *Current Biology*, 17, 449–451. <https://doi.org/10.1016/j.cub.2007.04.047>
- Huey, R. B., Deutsch, C. A., Tewksbury, J. J., Vitt, L. J., Hertz, P. E., Álvarez Pérez, J., & Garland, T. (2009). Why tropical forest lizards are vulnerable to climate warming. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 276, 1939–1948. <https://doi.org/10.1098/rspb.2008.1957>
- Iler, A. M., Høye, T. T., Inouye, D. W., & Schmidt, N. M. (2013). Non-linear flowering responses to climate: Are species approaching their limits of phenological change? *Philosophical Transactions of the Royal Society B*, 368, 20120489. <https://doi.org/10.1098/rstb.2012.0489>
- IPCC (2014). *Climate change 2014: Synthesis report*. Intergovernmental Panel on Climate Change, Geneva, Switzerland.
- Kemp, W. P., & Bosch, J. (2005). Effect of temperature on *Osmia lignaria* (Hymenoptera: Megachilidae) prepupa-adult development, survival, and emergence. *Journal of Economic Entomology*, 98, 1917–1923. <https://doi.org/10.1093/jee/98.6.1917>
- Kingsolver, J. G. (1989). Weather and the population dynamics of insects: Integrating physiological and population ecology. *Physiological Zoology*, 62, 314–334. <https://doi.org/10.1086/physzool.62.2.30156173>
- Kingsolver, J. G., Diamond, S. E., & Buckley, L. B. (2013). Heat stress and the fitness consequences of climate change for terrestrial ectotherms. *Functional Ecology*, 27, 1415–1423. <https://doi.org/10.1111/1365-2435.12145>
- Kingsolver, J. G., & Huey, R. B. (2008). Size, temperature, and fitness: Three rules. *Evolutionary Ecology Research*, 10, 251–268.
- Krombein, K. V. (1967). *Trap-nesting wasps and bees: Life histories, nests, and associates*. Washington, DC: Smithsonian Press.
- Kudo, G., & Ida, T. Y. (2013). Early onset of spring increases the phenological mismatch between plants and pollinators. *Ecology*, 94, 2311–2320. <https://doi.org/10.1890/12-2003.1>
- Kudo, G., Nishiwaki, Y., Kasagi, T., & Kosuge, S. (2004). Does seed production of spring ephemerals decrease when spring comes early? *Ecological Research*, 19, 255–259. <https://doi.org/10.1111/j.1440-1703.2003.00630.x>
- McKinney, A. M., Caradonna, P. J., Inouye, D. W., Barr, B., Bertelsen, C. D., & Waser, N. M. (2012). Asynchronous changes in phenology of migrating Broad-tailed Hummingbirds and their early-season nectar resources. *Ecology*, 93, 1987–1993. <https://doi.org/10.1890/12-0255.1>
- Ogilvie, J. E., Griffin, S. R., Gezon, Z. J., Inouye, B. D., Underwood, N., Inouye, D. W., & Irwin, R. E. (2017). Interannual bumble bee abundance is driven by indirect climate effects on floral resource phenology. *Ecology Letters*, 20, 1507–1515. <https://doi.org/10.1111/ele.12854>
- Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, 120, 321–326. <https://doi.org/10.1111/j.1600-0706.2010.18644.x>
- Paaajmans, K. P., Heinig, R. L., Seliga, R. A., Blanford, J. I., Blanford, S., Murdock, C. C., & Thomas, M. B. (2013). Temperature variation makes ectotherms more sensitive to climate change. *Global Change Biology*, 19, 2373–2380. <https://doi.org/10.1111/gcb.12240>
- Parmesan, C. (2007). Influences of species, latitudes and methodologies on estimates of phenological response to global warming. *Global Change Biology*, 13, 1860–1872. <https://doi.org/10.1111/j.1365-2486.2007.01404.x>
- Parmesan, C., & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421, 37–42. <https://doi.org/10.1038/nature01286>
- Penick, C. A., Diamond, S. E., Sanders, N. J., & Dunn, R. R. (2017). Beyond thermal limits: Comprehensive metrics of performance identify key axes of thermal adaptation in ants. *Functional Ecology*, 31, 1091–1100. <https://doi.org/10.1111/1365-2435.12818>
- Pinheiro, J., Bates, D., DebRoy, S., & R Core Team. (2016). *nlme: Linear and nonlinear mixed effects models. R package version 3.1* (pp. 1–336). Retrieved from <https://CRAN.R-project.org/package=nlme>
- R Core Team. (2016). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <https://www.R-project.org/>
- Rafferty, N. E., Bertelsen, C. D., & Bronstein, J. L. (2016). Later flowering is associated with a compressed flowering season and reduced reproductive output in an early season floral resource. *Oikos*, 125, 821–828. <https://doi.org/10.1111/oik.02573>
- Raubenheimer, D., & Simson, S. J. (2003). Nutrient balancing in grasshoppers: Behavioural and physiological correlates of dietary breadth. *Journal of Experimental Biology*, 206, 1669–1681. <https://doi.org/10.1242/jeb.00336>
- Rust, R. W. (1986). Biology of *Osmia* (*Osmia*) *ribifloris* Cockerell (Hymenoptera: Megachilidae). *Journal of the Kansas Entomological Society*, 59, 89–94.
- Sgolastra, F., Bosch, J., Molowny-Horas, R., Maini, S., & Kemp, W. (2010). Effect of temperature regime on diapause intensity in an adult-wintering Hymenopteran with obligate diapause. *Journal of Insect Physiology*, 56, 185–194. <https://doi.org/10.1016/j.jinsphys.2009.10.001>
- Sgolastra, F., Kemp, W., Buckner, J., Pitts-Singer, T., Maini, S., & Bosch, J. (2011). The long summer: Pre-wintering temperatures affect metabolic expenditure and winter survival in a solitary bee. *Journal of Insect Physiology*, 57, 1651–1659. <https://doi.org/10.1016/j.jinsphys.2011.08.017>
- Sgolastra, F., Kemp, W. P., Maini, S., & Bosch, J. (2012). Duration of prepupal summer dormancy regulates synchronization of adult diapause with winter temperatures in bees of the genus *Osmia*. *Journal of Insect Physiology*, 58, 924–933. <https://doi.org/10.1016/j.jinsphys.2012.04.008>
- Sheridan, J. A., & Bickford, D. (2011). Shrinking body size as an ecological response to climate change. *Nature Climate Change*, 1, 401–406. <https://doi.org/10.1038/nclimate1259>
- Stange, E. E., & Ayres, M. P. (2010). Climate change impacts: Insects. In *Encyclopedia of life sciences (ELS)*. Chichester, UK: John Wiley & Sons, Ltd.
- Sunday, J. M., Bates, A. E., & Dulvy, N. K. (2011). Global analysis of thermal tolerance and latitude in ectotherms. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 278, 1823–1830. <https://doi.org/10.1098/rspb.2010.1295>
- Tauber, M., Tauber, C., & Masaki, S. (1986). *Seasonal adaptations of insects*. Oxford, UK: Oxford University Press.

- Tepedino, V. J., McDonald, L. L., & Rothwell, R. (1979). Defense against parasitization in mud-nesting hymenoptera - can empty cells increase net reproductive output? *Behavioral Ecology and Sociobiology*, 6, 99–104. <https://doi.org/10.1007/BF00292555>
- Thackeray, S. J., Sparks, T. H., Frederiksen, M., Burthe, S., Bacon, P. J., Bell, J. R., ... Wanless, S. (2010). Trophic level asynchrony in rates of phenological change for marine, freshwater and terrestrial environments. *Global Change Biology*, 16, 3304–3313. <https://doi.org/10.1111/j.1365-2486.2010.02165.x>
- Torchio, P. F. (1990). *Osmia ribifloris*, a native bee species developed as a commercially managed pollinator of highbush blueberry. *Journal of the Kansas Entomological Society*, 63, 427–436.
- Vale, C. G., & Brito, J. C. (2015). Desert-adapted species are vulnerable to climate change: Insights from the warmest region on earth. *Global Ecology and Conservation*, 4, 369–379. <https://doi.org/10.1016/j.gecco.2015.07.012>
- Whittaker, R. H., & Niering, W. A. (1964). Vegetation of the Santa Catalina Mountains, Arizona. I. Ecological classification and distribution of species. *Journal of the Arizona Academy of Science*, 3, 9–34. <https://doi.org/10.2307/40021924>
- Williams, C. M., Thomas, R. A., MacMillan, H. A., Marshall, K. E., & Sinclair, B. E. (2011). Triacylglyceride measurement in small

quantities of homogenised insect tissue: Comparisons and caveats. *Journal of Insect Physiology*, 57, 1602–1613. <https://doi.org/10.1016/j.jinsphys.2011.08.008>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: CaraDonna PJ, Cunningham JL, Iler AM. Experimental warming in the field delays phenology and reduces body mass, fat content and survival: Implications for the persistence of a pollinator under climate change. *Funct Ecol*. 2018;32:2345–2356. <https://doi.org/10.1111/1365-2435.13151>