

Warming experiments test the sensitivity of an endangered butterfly across life history stages

Lainey V. Bristow (✉ lainey.bristow@gmail.com)

University of Notre Dame

Ralph Grundel

Geological Survey

Jason D.K. Dzurisin

Colorado State University

Grace C. Wu

University of California, Santa Barbara

Yudi Li

University of California, Davis

Andrew Hildreth

University of California, Los Angeles

Jessica J. Hellmann

University of Minnesota

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Abstract

Introduction: The Karner blue butterfly (*Lycaeides melissa samuelis*, KBB) is a federally listed endangered species living in disjunct habitats within the Midwest and Eastern United States. As a hostplant specialist and an ectotherm, the KBB is likely to be susceptible to effects of climate change.

Aims/Methods: We undertook warming experiments to explore the temperature sensitivity of various KBB life history stages and traits. Over a two-year period, we exposed all KBB life stages to temperature increases of +2, +4, and +6 °C above historic (1952-1999) mean temperatures. We analyzed the effect of these treatments on life history parameters likely related to fitness and population size, including development time, voltinism, degree-day accumulation, body weight, and morphology.

Results: Warming treatments resulted in earlier emergence and accelerated development, leading to additional generations. Warming also decreased the number of degree-days required to initiate spring hatch and eclosion. As treatment warmth increased, adult body mass, length, and area decreased and voltinism increased. Females with lower adult mass and smaller body size produced fewer eggs.

Discussion: Our data suggest a trade-off between accelerated development and body size. Furthermore, an increase in adult mass and abdominal area was associated with reduced fecundity.

Implications for Insect Conservation: Changes in development time and voltinism may affect phenological overlap with the species' obligate host plant (*Lupinus perennis*), which could negatively affect completion of the final generation's lifecycle in a given year, and thus that year's overwintering population size.

Introduction

Recent status and trend evaluations of various insect groups suggest significant declines in population abundance and landscape occupancy (Gardiner and Didham 2020; Hallmann et al. 2020; Montgomery et al. 2020; Powney et al. 2019; Roth et al. 2020; Sánchez-Bayo and Wyckhuys 2019; Stepanian et al. 2020; Theng et al. 2020; van Klink et al. 2020; Wagner 2020). However, time series of multiple species' abundances often suggest that abundance and occupancy trends are variable among species and that species, not surprisingly, respond differently to the various threats associated with declines (Crossley et al. 2020; Didham et al. 2020). This reminds us that to understand and predict population change, we need to study demographic response to changing conditions.

Between 1992–2014, the Karner blue butterfly (*Lycaeides melissa samuelis*, KBB) was monitored and studied at the Indiana Dunes National Park (INDU) in northwest Indiana, USA. During this period, KBB populations declined steadily (despite extensive habitat management by the National Park Service (NPS)) and the species went regionally extinct by 2014. During the final phase of decline, we documented a relationship between spring and summer temperatures and rapid decline (Patterson et al. 2020). Historically high spring temperatures were associated with phenological mismatching between larvae

and the larval hostplant (lupine, *Lupinus perennis*). High summer temperatures were associated with early senescence of the hostplant, decreasing hostplant availability for developing summer larvae. The result of these two climate related effects was a swift final decline of the KBB. Intensive landscape management by the NPS during the final two decades of the INDU population also provided increased habitat and resources for the KBB, suggesting that habitat availability was not the prime cause of decline. To understand warming effects further, we undertook a series of laboratory studies to examine how changes in mean temperatures might have affected KBB populations leading up to its end-stage precipitous decline (Erenler et al. 2020).

The KBB is a lupine-feeding specialist butterfly and federally-listed endangered species under the Endangered Species Act in the United States (U.S.) and is extirpated in Canada (Clough 1992). The KBB benefits from landscapes with heterogeneous canopy cover that enhance microclimatic diversity (Grundel et al. 1998b). Similarly, lupine population viability benefits from heterogeneous microscale temperature, moisture, shade, and soil conditions (Pavlovic and Grundel 2009).

KBB populations have been extirpated in several U.S. states and Canada between 1990–2015. Comparative analyses suggest the Karner blue is a climate sensitive species (Thurman et al. 2020; Li 2020); however, we lack an experimental – and therefore mechanistic – understanding of how climate and extinction risk might be linked. Weather patterns have been associated with population extinctions in other butterflies (Pollard 1991; Saunders et al. 1991; McLaughlin et al. 2002). As a specialist species with isolated regional populations and limited dispersal ability (Hess and Hess 2015; Knutson et al. 1999), the KBB is unlikely to shift, on its own, to alternative habitats in response to changing conditions. Therefore, examining the potential sensitivity of remaining KBB populations to climate change, and its overall adaptive capacity is valuable to quantify climate sensitivity and inform management practices (Fuller 2008 and Thurman et al. 2020).

In this study, we focus on temperature as a primary variable because much of the ecology and evolution of ectotherms is influenced by temperature. At the INDU, where the KBB used in this study originated, annual mean temperature has increased about 2 °C in the last century (Monahan and Fisichelli 2014), with an increase of 6 °C by the end of the 21st century projected under certain high-emissions scenarios (Hayhoe et al. 2010). Studies on other butterfly species suggest that this amount of warming could affect many critical life history parameters (Cornelissen 2011; Nooten et al. 2014; Villalpando et al. 2009).

Temperature has a direct effect on many physiological processes in insects (Gotthard 2001). We performed experimental tests of warming on different life history stages of the KBB, focusing on life history parameters affecting fitness and population size, to understand possible mechanisms by which climate change might affect KBB populations (García-Barros 2000; Nylin and Gotthard 1998). Different temperature treatments were used to test five predictions, based on published observations of how temperature has affected other insects.

Prediction 1: Exposure to higher temperatures will result in accelerated development (Sibly and Atkinson 1994). Specifically, warming will decrease developmental time during the larval and pupal stages. At

warmer temperatures, insects spend less time developing when diapause is facultatively affected by temperature rather than being solely dependent on light and dark cycles (McWatters and Saunders 1998). Additionally, higher temperatures increase growth rates and shorten developmental time before eclosion (Atkinson 1994; Davies 2019; Kingsolver 1989; Van der Have and De Jong 1996).

Prediction 2: Warming will cause earlier egg hatching, possibly cued by a more rapid accumulation of degree days. Developmental changes lead to changes in insect phenology (Cayton et al. 2015), and growing degree-days (GDD) provide a metric linking climatic conditions and insect phenology (Nufio et al. 2010). Changes in diurnal temperature ranges can substantially change degree-day accumulation and alter insect life history (Chen et al. 2015; Patterson et al. 2020). If individuals are exposed to warmer temperatures, accumulation of GDD will occur in fewer days (Tobin et al. 2008) and this could trigger earlier processes such as egg hatching. Rapid accumulation of GDD could shorten diapause duration (Hahn and Denlinger 2007; Kerr et al. 2020; Leather et al. 1995).

Prediction 3: Warming temperature will delay the onset of diapause, which, in this species, allows for additional generations (Davies 2019; Schiesari and O'Connor 2013). Typically, KBB eggs laid in mid-summer (e.g., July) by second- or summer-generation females enter diapause that is broken the following spring. However, field observations suggest extra KBB generations occur occasionally when second generation eggs develop into adults in the summer they are produced rather than diapause until the following spring (2012). This could increase population abundance within that year, but shifted voltinism patterns introduce the risk of encountering sudden heat or cold stress outside the normal period when adults would be present, potentially decreasing fitness. Additional generations could also expose larvae from extra generations to lower quality or absent host plant or nectar resources. Since KBB larval fitness and survival is dependent on the timing and quality of its host plant (Grundel et al. 1998a), it is important to understand what causes additional generations.

Prediction 4: Warmer temperatures will produce smaller KBB. Body size is a product of development time and mass accumulation (Davidowitz et al. 2004). However, accelerated development rate can decrease metabolic rate and enzyme kinetics (Atkinson 1994; Gotthard 2001) and therefore reduce adult size (Van der Have and De Jong 1996). Bergmann's Rule suggests that endothermic and ectothermic organisms in warmer climates tend to have smaller adult body sizes than in cooler climates (Bergmann 1847). Body size influences many aspects of an organism's physiology (Kingsolver and Huey 2008) and a larger body can enhance overall performance and fitness (Peters and Peters 1986).

Prediction 5: Warming and accelerated development result in reduced fitness (i.e., reduced egg production or fecundity). The complex relationship between temperature and development creates a size-dependent allocation tradeoff in ectotherms (Berger et al. 2008; Gotthard 2001). Previous insect studies have found that larger females often exhibit higher fecundity (Clifford and Boerger 1974; Hough and Pimentel 1978; Tyndale-Biscoe and Hughes 1969), and some have linked this to the mother's extended development (Kamm 1972; Masaki 1972). If adult females become smaller with exposure to higher temperatures, their fecundity could decrease in response (Berger et al. 2008; Kozłowski 1992).

Methods

Karner blue life history and distribution

Historically, the KBB occurred between approximately 41°-46° N latitude and 93°-71° W longitude, extending from Minnesota to Maine in the United States and Southern Ontario, Canada (USFWS 2003). The KBB is typically bivoltine across its range, overwintering as eggs that hatch in early spring, often in April, and producing a first generation of adults in May and June. These first-generation adults breed and produce a second generation of adults from about June-August. Females from that second generation lay eggs that overwinter, restarting the cycle. At the time of this study, the KBB was present in disjunct populations in the Midwest and Northeast U.S. states of Wisconsin, Michigan, Indiana, New Hampshire, and New York, with Wisconsin and Michigan supporting the largest populations (USFWS 2012; Hess & Hess 2015). The Indiana laboratory populations used in the present study were derived from an Indiana population that was reported as extinct in the wild by 2014 (Patterson et al. 2020).

Temperature Treatments

We performed warming experiments in the laboratory throughout the life cycle of the KBB. An experimental colony was started from 75 second-generation pupae from an INDU captive-rearing program and 30 wild-caught second-generation females from two INDU locations (Inland Marsh (Tolleston Dunes) and Miller Woods). Treatments were maintained for generations from the fall of 2010 to summer of 2011 (Year 1) and again from fall of 2011 to summer of 2012 (Year 2). Year 2 individuals were direct descendants of Year 1 individuals such that diapaused offspring from final generations of Year 1 were first-generation individuals in Year 2.

KBBs were reared in two types of experimental growth chambers, depending on the temperature required. During the growing season and until late fall (October), they were reared in Conviron® MTR30 environmental chambers that have a thermal operating envelope between 10 and 45°C. When the experimental temperature treatment fell below the lower operating temperature of the Conviron (10°C), we transferred the eggs to custom-constructed, remotely-monitored “cold simulation chambers” constructed from consumer-grade chest freezers. The cold simulation chambers were capable of a colder thermal profile (-32 to 10°C). Temperatures were controlled by a laptop executing a MATLAB (MATLAB 2010) script running on a continuous loop. When temperature deviated from set conditions, the freezer’s compressor was turned on or off to regulate towards the desired temperature. All chambers were held within + 0.4 and - 0.4°C of set point. In both chamber types, we simulated daylight patterns or photoperiod using full-spectrum, cold cathode, 60-watt light bulbs.

For our control temperature treatment, termed “+0°C”, we used historic mean temperatures from 1952–1999. Historic temperatures were set from daily mean minimum and maximum temperatures using data from a weather station within the current boundary of INDU at Ogden Dunes, Indiana from 1952–1989 and at the INDU Headquarters in Porter, Indiana from 1989–1999. We set the minimum temperature based on the mean minimum 1952–1999 temperature near sunrise, and we set the maximum, based on

the mean maximum 1952–1999 temperature in the late-afternoon. We increased the temperature hourly from the minimum and decreased temperatures from the maximum according to average hourly rates of temperature change observed during the day from the 1952–1999 data. We maintained a given daily mean temperature pattern for a ten-day interval and then reset the pattern to reflect temperatures observed within the next ten-day interval.

In addition to the + 0°C historic control, we exposed groups of KBB individuals to three warming treatments of + 2°C, + 4°C, and + 6°C above the historic hourly temperatures. These levels approximate climate projections in the Midwest region for the years 2040, 2070, and 2100, respectively, based on higher emissions scenarios (Hayhoe et al. 2010). Such temperatures also reflect extreme conditions in the nearer future (Shaver et al. 2000; Walther et al. 2002).

Colony Maintenance and Data Collection

Each year of experimentation began with first-generation overwintering eggs that were laid in mid to late summer of a year, would subsequently overwinter, and hatch in spring of the next calendar year. Eggs were held in small plastic containers and monitored daily in the spring for hatching. Larvae were removed and placed in individual small transparent plastic cups with lupine leaves. We harvested large, green leaves that were not yet withering from wild lupines at INDU and lupine grown in a greenhouse. All treatments were fed from the same batch of collected or grown leaves. Since lupine senesces as the summer progresses, lupine quality may have declined for larvae later in the experiment, but equally among treatments.

Upon pupation, we weighed and placed individuals into containers with an appropriate substrate to aid in proper eclosion positioning. Individuals were monitored daily for signs of pupation marked by the darkening of the pupal case.

Once adults emerged, we recorded eclosion date, adult mass, and sex, marked their wings with identifying information and took high-resolution photos of left, right and ventral perspectives of each adult. We used ImageJ (Schneider et al. 2012) to measure total wing area, snout-ventral length, thorax-ventral length, and abdominal area in the photographs (length in cm or area in cm²).

Based on previous KBB rearing protocol, we fed adults sex-specific nectar solutions that contained specific concentrations of honey, water, and other nutrients (<https://www.fws.gov/midwest///endangered/insects/kbb/pdf/KBBPropagationManual2010Nov.pdf>). We mated adults, avoiding near relative, to the extent possible, within each temperature treatment group. To enhance likelihood of mating, we temporarily removed adults from environmental chambers and exposed them to natural light and humidity. Mating cages, with males and females, were placed in a greenhouse or outdoor setting with both natural and artificial nectar resources.

To collect eggs, mated females were placed in oviposition cages. We recorded the number of eggs laid by each female. Egg containers were placed in the same temperature treatment as their mother.

Not every individual in the experiment was tracked to death because excess individuals were returned to the field. This thinning process precluded us from making demographic calculations.

Data Analysis

We used growing degree days (GDD) as a metric to evaluate development differences among treatments. GDD describe the number of thermal units an organism accumulates between a minimum or maximum temperature, measuring total accumulated degrees ($^{\circ}\text{C}$) (Damos and Savopoulou-Soultani 2011; Hodgson et al. 2011; Nufio et al. 2010). Accumulation was based on hourly temperatures. We evaluated the temperature and degree-day accumulation at egg hatch and calculated an overall mean for each of the four treatments (Year 1, first flight only). We calculated the total accumulated degree-days above 9°C at eclosion for Year 1 individuals, based on observations of spring KBB larvae (Swengel 1995).

We analyzed development and life stage timing from the two years of our experiments. We examined how emergence and life stage timing differed among temperature treatments and generations using analysis of variance (ANOVA) (R Core Team 2014). These data were used to compare likely timing of KBB generations in relation to lupine emergence, flowering, and senescence records derived from a year-long lupine phenology field study (Dirig 1994). Linear regressions and analysis of variance (One-Way ANOVA) were calculated with SYSTAT 13 (Systat Software, San Jose, CA) and R (R Core Team 2014). We grouped all generations and treatment data within a year and then performed regression and ANOVA. We performed post hoc pairwise comparisons following oneway ANOVA in SYSTAT using Tukey and Bonferroni tests to test for significant differences (F-ratios, p-values < 0.05) among groups. We examined development rates, degree-day accumulation, body mass, and morphological attributes for male-female differences within each temperature treatment and found none (p-value > 0.05). Therefore, we combined male and female data when examining effects of temperature on these variables. We examined how emergence and life stage timing differed among temperature treatments and generations by plotting the average dates for each life stage by treatment.

For predicting adult mass and egg production response variables, we used generalized linear models in SYSTAT, including three categorical explanatory variables (temperature, four levels; generation, three levels; sex, two levels) and two continuous variables (development duration, adult mass). Confidence and prediction interval outputs were used to plot lower and upper 95% confidence levels (LCL and UCL, respectively).

Results

Development Time (prediction 1)

The warming treatments accelerated development in both years, with development time (total number of days from hatch to eclosion) being significantly shorter as treatment temperature increased (Year 1, Fig. 1a, $p < 0.0001$, $F = 84.713$, $R^2 = 0.17$, $df = 3$, $n_{\text{adults}} = 1311$; Year 2: Fig. 1b, $p < 0.0001$, $F = 54.294$, $R^2 = 0.19$,

df = 3 n_{adults} = 704). Individuals in the second generation within each year experienced shorter mean development times compared to those in the first generation (Year 1: Appendix S1; Year 2, Fig. 1b).

Egg Hatching and Degree-Day Accumulation (prediction 2)

In both years, warmer treatments produced significantly earlier average first generation (spring) hatch dates than the + 0°C historic control (Table 1). Mean hatching dates were between 9–21 days earlier for + 6°C eggs than for + 0°C eggs in the two years.

In Year 1, individuals in the warming treatments accumulated significantly fewer degree-days above the thermal developmental minimum temperature of 9 °C at egg hatch than did the control (Fig. 2a) (prediction 2), with decreasing degree-day accumulation in higher temperature treatments. Mean temperatures at hatch are within 1–3°C among treatments (Fig. 2b), but significantly different among most treatments.

When combining all generations, warming treatments accumulated significantly fewer degree-days at eclosion (Appendix S2). First-generation individuals accumulated more degree-days above 9°C at eclosion than in second and third generations (Appendix S2).

Phenology (prediction 3)

Earlier hatching led to additional generations beyond the typical bivoltine (two generation) life history. All warming treatments produced a third generation (+ 2°C, + 4°C, + 6°C) in both years. A partial fourth generation occurred in Year 1 in the + 6°C treatment as larvae, although no larvae (n_{larvae} = 5) pupated in that treatment. In Year 2, there was a fourth generation for the + 6°C treatment only (n = 41 fourth generation individuals) that successfully pupated and resulting adult females mated and laid eggs.

Within the historic temperature scenario (+ 0°C, Fig. 3a), wild lupine phenology and availability of lupine host leaves aligns with larval emergence, even in the second generation. Earlier hatch dates and extra generations in the warming treatments (Table 1) extended the total KBB generation season (i.e., time from first generation egg hatch to last generation adult emergence). For both Year 1 and Year 2, the + 6°C treatment larvae individuals experienced an extended summer period, likely beyond the typical lupine growth season (Fig. 3c).

Table 1 Average spring hatch date by year and treatment. Symbols indicate treatments and flight that are statistically significant (ANOVA p < 0.05) from other treatments within that year (Year 1 with *, Year 2 with ^)

Year	+ 0	+2	+4	+6
1	April 20 *	April 19 *	April 9 *	March 28 *
2	April 24 ^	April 19	April 19	April 15 ^

Body Mass and Morphology (prediction 4)

There was a statistically significant effect of temperature (oneway ANOVA, $p = 0.04$, $F = 4.4$, $R^2 = 0.01$, $df = 3$, $n_{\text{pupae}} = 1157$) and generation ($p < 0.0001$, $F = 436.9$, $R^2 = 0.43$, $df = 2$, $n_{\text{pupae}} = 1157$) on pupal mass. Pupal mass decreased in the warming treatments (Appendix S3).

In Year 1, adult mass decreased for the + 4°C and + 6°C treatments compared to the control (Fig. 4a). In Year 2, adult mass decreased for the + 6°C treatment compared to the control (Fig. 4b). In both years, adult mass was lowest in the highest temperature treatment, + 6°C.

Combining all treatments and flights in Year 1, we found that individuals with higher degree-day accumulation at eclosion had higher adult mass (Fig. 5).

Like adult mass, a maximum was reached in the body size measurements at the intermediate temperatures. There was a slight increase in wing area, abdomen area, snout-ventral length, and thorax-ventral length in the + 2°C and + 4°C treatments compared to + 0°C (Fig. 6a-d). The + 6°C treatment showed a significant decrease in body size when compared to the control, except for wing area. In addition to these differences in response to warming, there was a statistically significant decline in body size measurements with from the first to subsequent generations. Body size measurements for generation four were always lower than those of generation two, and generation two was always lower than generation one. (Fig. 6e-f).

Reproduction (prediction 5)

Egg production per female did not differ significantly among generations or treatments in either year (Year 1, treatment $p = 0.44$ and generation $p = 0.12$, $n_{\text{adult females}} = 225$; Year 2, treatment $p = 0.70$ and generation $p = 0.23$, $n_{\text{adult females}} = 79$). Therefore, we combined females from all treatments and flights within the year to evaluate an overall trend among these variables from our prediction. There was a statistically significant positive correlation between adult mass and degree-day accumulation. As female mass decreased, the number of eggs produced per female decreased (Fig. 7a; Fig. 7b). Heavier females with larger abdominal area produced more eggs (Fig. 7c). Bonferroni-adjusted significance suggested that adult mass correlation with egg count was statistically significant.

Discussion

The Karner blue butterfly is a well-studied species that is sensitive to, and benefits from, microclimatic variation (Grundel and Pavlovic 2007; Grundel et al. 1998a, b). Despite early recognition of the role that temperature variation plays in enhancing both the value of these landscapes for population viability and of the quality of foraging resources, concerted conservation management centered on maximizing hostplant and nectar resources and microclimatic variation has not always prevented local extirpations (Patterson et al. 2020). This disconnect between providing both needed microclimatic variation and seeming provision of abundant food resources, yet ultimate lack of success of management efforts is

consistent with assessments that classify the KBB as exhibiting relatively low adaptive capacity (Thurman et al. 2020). That assessment emphasized the species' limited climatic niche breadth and habitat specialization as examples of limited ability to withstand environmental change, especially climate change.

This management track record suggests that ample resources might not equate to population viability. This may be related to phenological mismatching between larvae and hostplants that can render even abundant hostplant resources temporally unavailable to the butterfly. Understanding how this mismatch might arise is therefore important for KBB conservation. The prospect that the species and its hostplant might successfully coevolve under climate change to prevent this mismatch may be central to prolonged suitability of some historic KBB sites for conservation. In this study, we examined how rising temperatures might affect the timing and rate of development for the Karner blue and thereby affect this species' ability to maintain synchrony with its obligate hostplant, wild lupine. Further, we tested five predictions about the effect of warming key demographic parameters. All five of our predictions were supported but with non-linear effects across treatments.

In the laboratory colony studied, warmer temperatures were associated both with faster development that accelerated with each generation within the yearly cycle of reproduction and with earlier egg hatching. With increasing rearing temperatures, the number of degree-days to KBB egg hatching decreased, suggesting developmental acceleration, the number of generations per year increased, and adult mass decreased. As generations per year increased beyond two, larvae in third and fourth generations were likely to be present when the hostplant was no longer present or of poor quality. As temperatures increased, adult mass was lowest, and body dimensions were smallest, for the highest temperature treatment (+ 6°C). Heavier females produced fewer eggs, suggesting a demographic penalty at highest temperatures. These trends have been documented in other *Lepidoptera* (Huey and Kingsolver 1993; Kingsolver and Huey 2008; Diamond et al. 2014; Miller-Rushing et al. 2010).

Many of these trends can be maladaptive demographically since earlier egg hatching can result in phenological mismatching with the larval hostplant (Patterson et al. 2020), extra generations beyond the nominal two generations per year may mean larvae are present after most hostplants are senescent, and body mass, which was positively correlated with egg production in females, decreased at the highest temperatures compared to the lowest temperature, suggesting lowered fecundity. While the Karner blue produces at least two generations per year, the hostplant is a perennial that emerges in early spring, likely before eggs hatch so that food is available for the subsequent larvae. But if the rate of advancement in time of spring egg hatching with rising temperatures is faster than for lupine emergence, as we saw in the final stages of extinction of the Karner blue at Indiana Dunes National Park (Patterson et al. 2020, Ault et al 2013), the response to increased temperatures in the first generation can be seen as maladaptive or overly sensitive to changes in temperature. Results from the current study suggest hatching would occur around the third week in April under historic mean temperatures but as early as late March with a 6°C increase in mean temperatures.

We also found a decrease in body weight and size in response to increased temperature, with some variation among treatments (prediction 4, Figs. 4 and 6). For pupal weight, each warming treatment resulted in individuals that weighed less than the control. There were slight increases in adult mass and body size for both intermediate (+ 2°C, + 4°C) temperature treatments. However, despite these increasing trends, there appeared to be a thermal limit to increasing body size with warming; perhaps the + 6°C temperature was too warm to maintain growth in body mass through larval and pupal developmental stages. The highest temperature treatment could have detrimental effects on larval development. If KBB has an upper thermal threshold for growth, high temperatures may inhibit healthy development and adult body mass.

Many experiments have been conducted to understand the relationship between temperature and size in Lepidoptera, and these have resulted in the discovery of a complex, interaction between the two. We predicted that mass would decrease with warmer temperature treatments (Bergmann 1847) because catabolism (i.e., metabolic processes that tear down biomolecules) has a higher temperature coefficient than anabolism (i.e., processes that build biomolecules), and thus an increase in temperature should increase growth rate and reduce final size (Johnston and Bennett 2008; Perrin 1988). This metabolic model may apply to the warmest + 6°C treatment but does not appear to apply to the other warming treatments (Johnston and Bennett 2008). The + 2°C and + 4°C individuals were larger and heavier than the control, however, accelerated development could be increasing the metabolic activity and thus molecular or tissue damage, which could have negative effects on internal physiology and function that we cannot detect from our experiments (Atkinson 1994).

An important question arises from the sensitivities we observed: do the increased number of individuals in additional generations compensate for fewer eggs laid per smaller female? To illustrate the effects of extra generations, consider Y fertile females with 100% survivorship, each producing X offspring per generation. Each additional generation increases the population size of that growing season by a factor of X . With two generations, the population is $Y \cdot X^2$, where the exponent represents exponential population growth. In four generations, the population would be $Y \cdot X^4$. Thus, additional generations add substantially more individuals. However, if one or more of those generations experiences high mortality, such as due to decreased host plant quality or abundance, these population gains could be eliminated.

Within our experiment in Year 1, fourth generation individuals in the + 6°C treatment did not reach pupation. Not only would these individuals be excluded from the total population count, but also there would be no offspring produced by them the following spring. In this way, unfavorable conditions, such as extreme heat (i.e., late summer) or poor-quality host plants (i.e., mismatched phenology and senesced lupine), could outweigh a tradeoff between population gains from extra voltinism and heightened mortality risk (Cayton et al. 2015). In Year 2 where the fourth generation successfully developed and bred, having fewer individuals in the additional generations might increase the level of inbreeding, which can have direct, compounding effects on genetic diversity and overall population fitness (Frankham 1996; Soulé 1986).

Increased temperatures led to more generations, and additional generations led to smaller KBBs. Smaller females produced significantly fewer eggs than larger females, as has been observed in other insects (Clifford and Boerger 1974; Hough and Pimentel 1978; Tyndale-Biscoe and Hughes 1969) (prediction 5, Fig. 7). Smaller females laying fewer eggs combined with high mortality in the final generation could decrease the number of offspring that hatch the following season. Although we did not observe statistically significant differences in average body mass between the two experimental years, if this phenomenon continued over several years, reduced per capita fecundity could decrease population size. Yet, if additional generations allow for further reproductive output while minimizing life stage mortality, these effects could be compensated by increasing population size within a single growing season.

Assumptions and Future Research

Several further questions are raised by our experiment. Future studies could evaluate the possible effects of climate change on the larval host plant (Patterson et al. 2020) and other key resources. Any nectar-mediated effect of climate change would likely occur through the entire flower community or several dominant nectar species (in contrast to a single larval host species). Moreover, other interactions or environmental factors could be important (e.g., ant symbiosis, predation, and parasitism). Temperature variation and changes in precipitation should also be evaluated in relation to warming. Our experiments did not measure or control for humidity in the chambers, which could be a potential consideration for future studies (Bai et al. 2015). Lastly, Fuller (2008) analyzed likely KBB life history tables and concluded overwinter egg survivorship had a dominant role in KBB demography.

Additional research could evaluate the percentages of eggs that made it to adults in each generation. It would be helpful to evaluate how frequently KBBs might make it to those third and fourth generations, not just that it happens. Data collection could include percent of eggs making it to adulthood and percent of eggs that break diapause in the late summer or early fall to start an additional generation, which could indicate if going for a third or fourth generation is likely to be a drain on the population. These results could help inform a demographic model prediction using the exponential contribution, plus the decline in fecundity to predict a maximal population change related to extra broods with increased temperatures. This maximal change could not likely be expressed in a laboratory setting with a continually provided food source, since survival in the field is likely to be negatively affected by lack of a host plant or nectar sources.

Management Implications

As climate regimes are shifting away from historic conditions, land managers might need to create new management strategies that reduce species exposure and sensitivity to climate change and increase adaptive capacity (Sgrò et al. 2011). Early in the conservation history of the Karner blue butterfly, researchers and managers recognized a critical role for providing microclimatic variation that would enable a variety of interactions between timing of insect and hostplant development and changes in hostplant quality to coexist on the landscape (Grundel and Pavlovic 2007; Grundel et al. 1998a, b; Lane 1999; Maxwell 1998). Despite this recognition, the critical role of phenological matching (Kharouba et al.

2018; Lindén 2018) was underappreciated in part because we lack the tools to accurately align the species in this critical interaction. Although management actions that centered on manipulating the thermal and soil moisture environments via canopy cover manipulation (Grundel et al. 1998a, b) were pursued at the INDU, the range of microclimates made available do not appear to be sufficient; perhaps they are not sufficient to accelerate the emergence of the hostplant sufficiently to catch up with the earlier egg hatching under warming or to slow senescence sufficiently to prolong quality hostplant resources that are needed for additional KBB generations. Those mismatches, and possible reduced fecundity, are the key implications of our study for KBB conservation, and new management strategies are needed to address them. (Nadeau and Urban 2019; Olivieri et al. 2015)

Declarations

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Author Contributions: L.V. Bristow wrote the main manuscript text and prepared the figures. All authors reviewed the manuscript.

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Figures

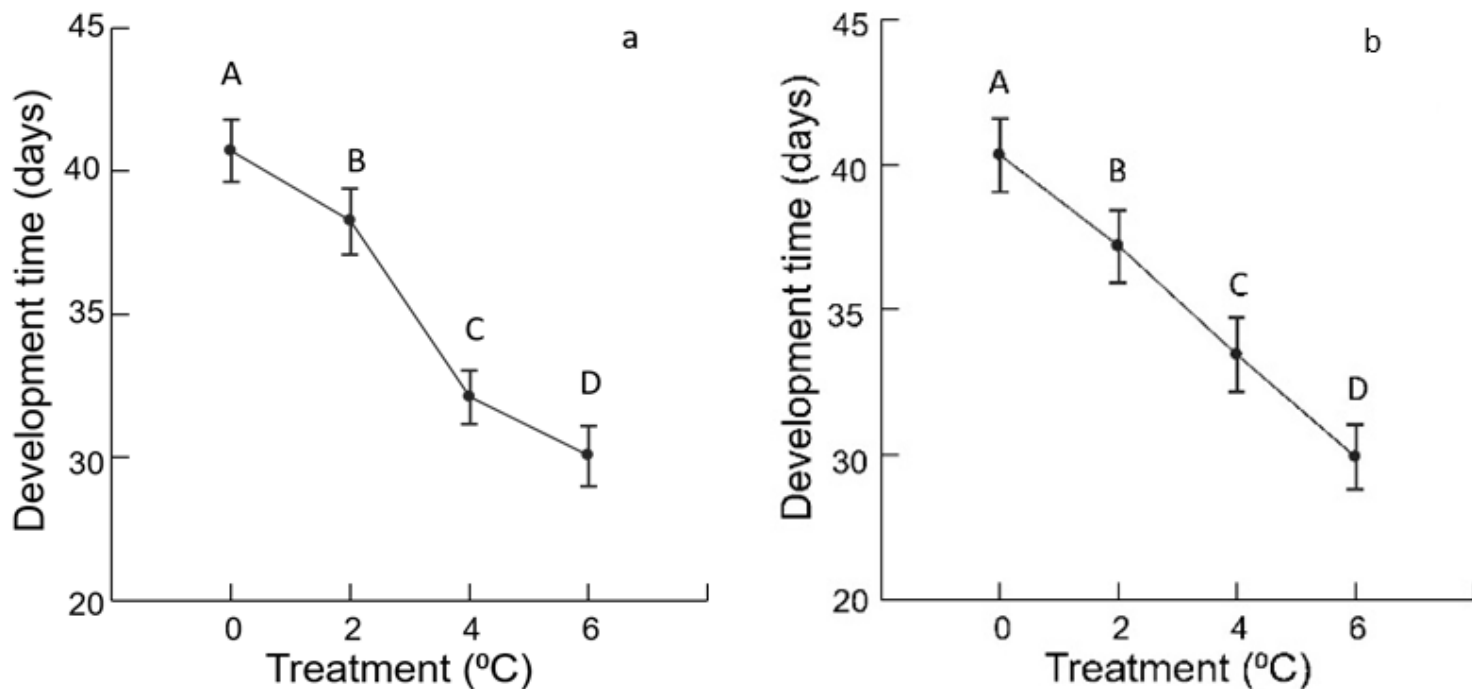


Figure 1

Average development time (days as larvae and pupae) by treatment and each year (Year 1: 1a, $p < 0.0001$, $F = 84.7$, $R^2 = 0.17$, $df = 3$, $n = 1311$; Year 2: 1b, ANOVA $p < 0.0001$, $F = 54.3$, $R^2 = 0.2$, $df = 3$, $n = 704$). Error bars represent SD ($p < 0.05$). Treatments labeled with different uppercase letters are significantly different

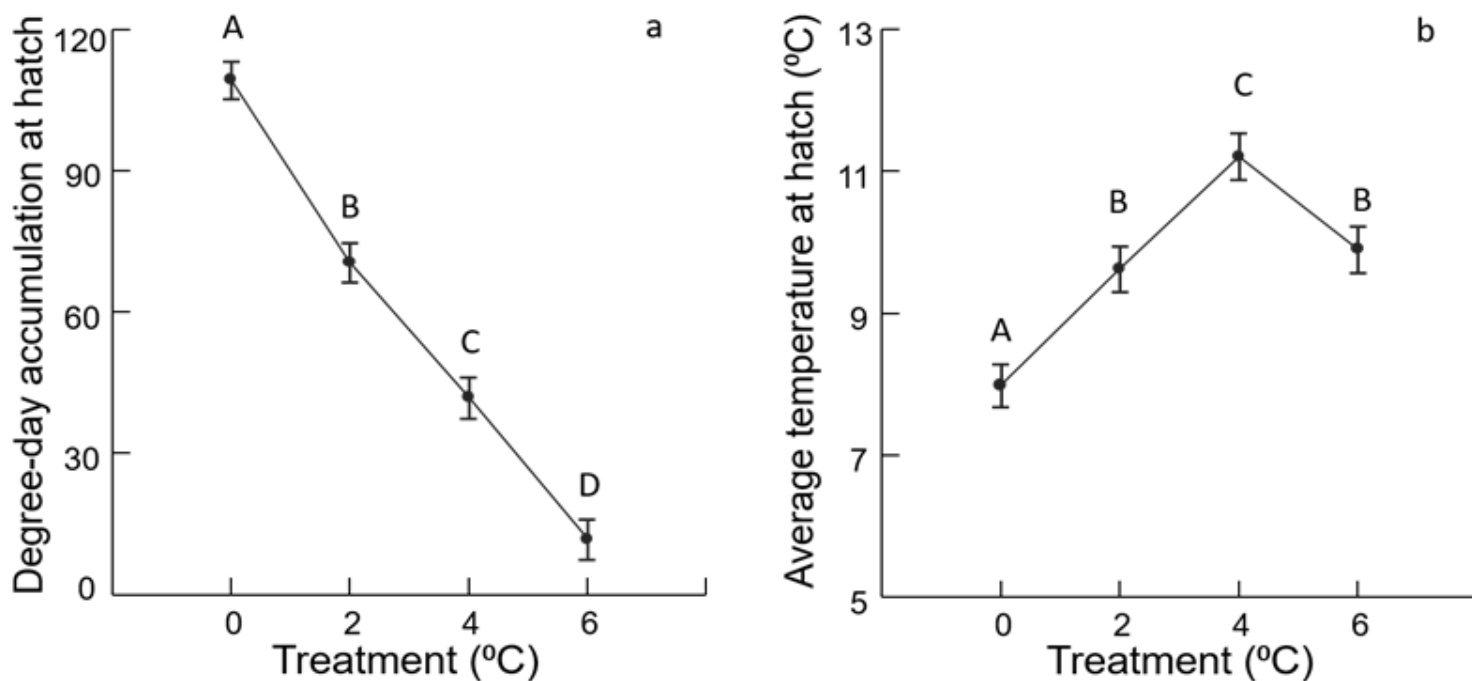


Figure 2

Average degree-day accumulation at hatch by treatment (2a, ANOVA $p < 0.0001$, $F = 395.3$, $R^2 = 0.84$, $df = 3$, $n = 477$) and average temperature at hatch by treatment (2b, ANOVA $p < 0.0001$, $F = 95.9$, $R^2 = 0.4$, $df = 3$, $n = 479$) for Year 1 individuals. Error bars and uppercase letters follow Fig. 1

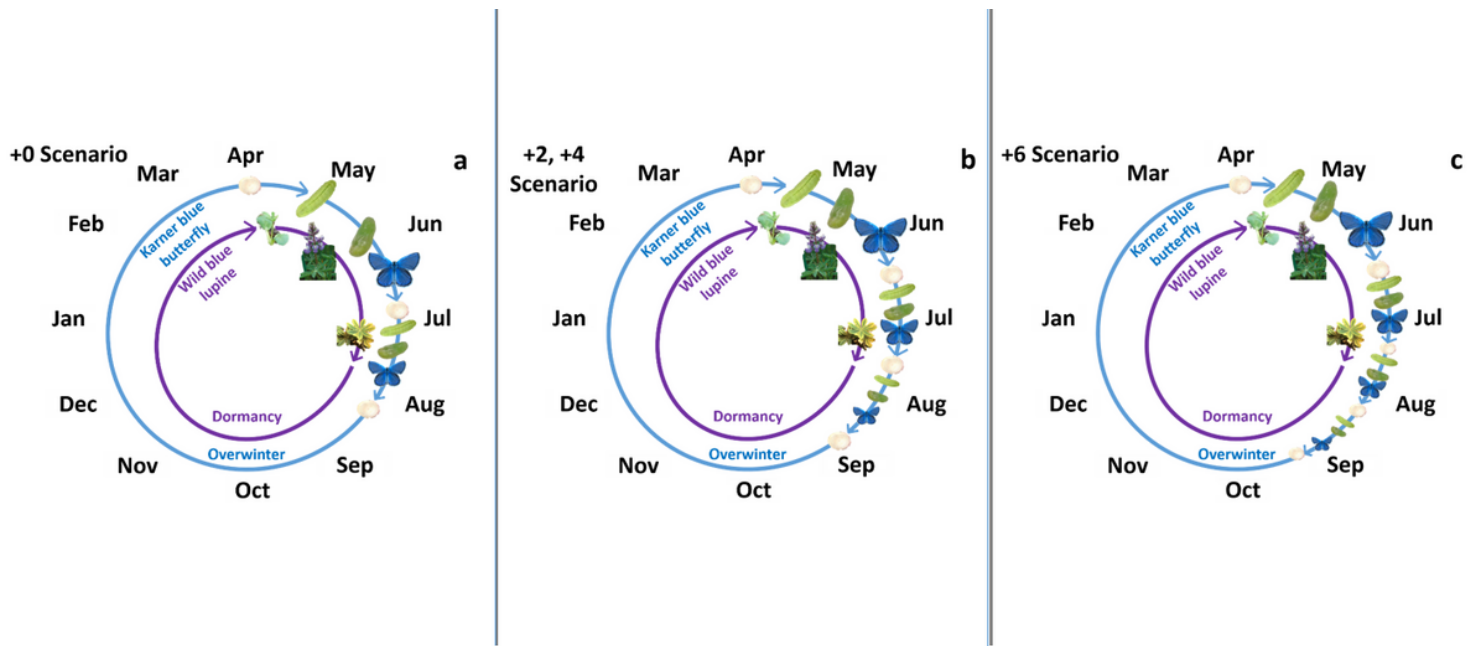


Figure 3

Timing of each flight in the +0, +2 and +4, and +6 climate change scenarios (a, b, c) using Year 2 KBB data, in relation to lupine phenology records from Dirig 1994. Each KBB symbol represents the average date for start of that life stage (egg, larva, pupa, adult) among treatments. The three symbols for lupine correspond to: emergence, flowering, senescence

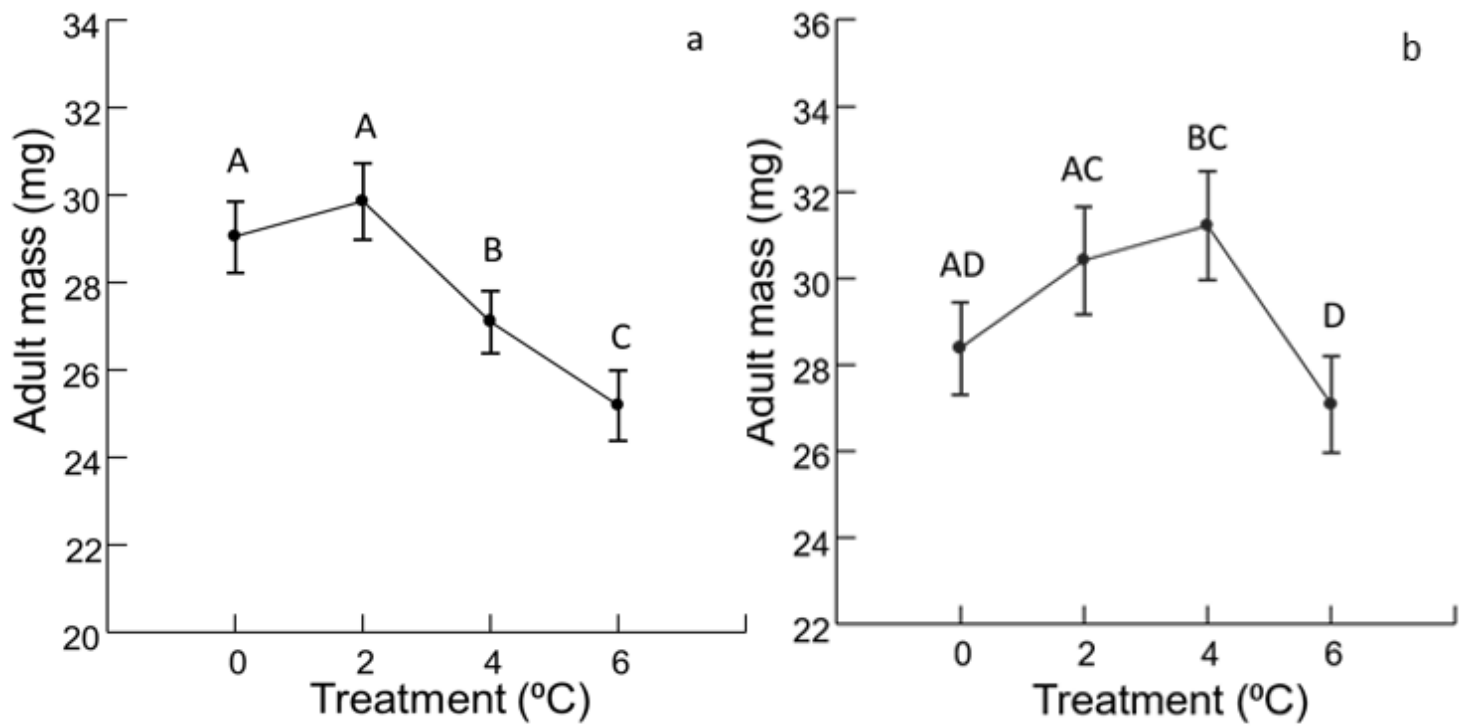


Figure 4

Adult mass (mg) by treatment in Year 1 (4a, ANOVA, $p < 0.0001$, $F = 24.38$, $R^2 = 0.05$, $df = 3$, $n = 1,307$), and Adult mass in Year 2 (4b, ANOVA $p < 0.0001$, $F = 10.136$, $R^2 = 0.04$, $df = 3$, $n = 768$). Error bars and uppercase letters follow Fig. 1

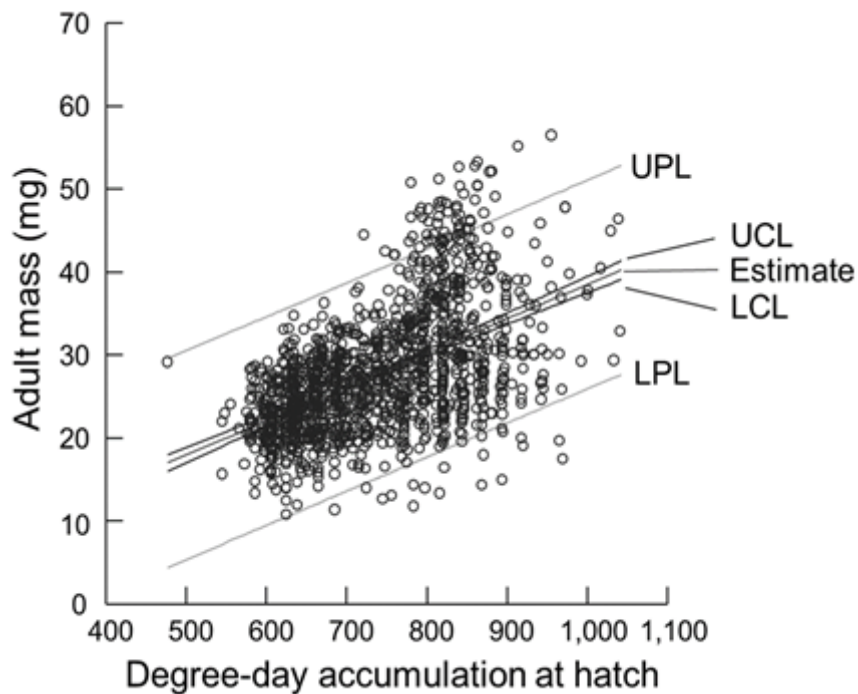


Figure 5

Adult mass by degree-day accumulation at hatch for Year 1 individuals (Linear Regression, $p < 0.0001$, $r = 0.52$, $F = 513.11$, $R^2 = 0.28$, $df = 1$, $n = 1,288$). Lower and upper confidence levels (LCL and UCL, respectively), and lower and upper prediction levels (LCL and UCL respectively)

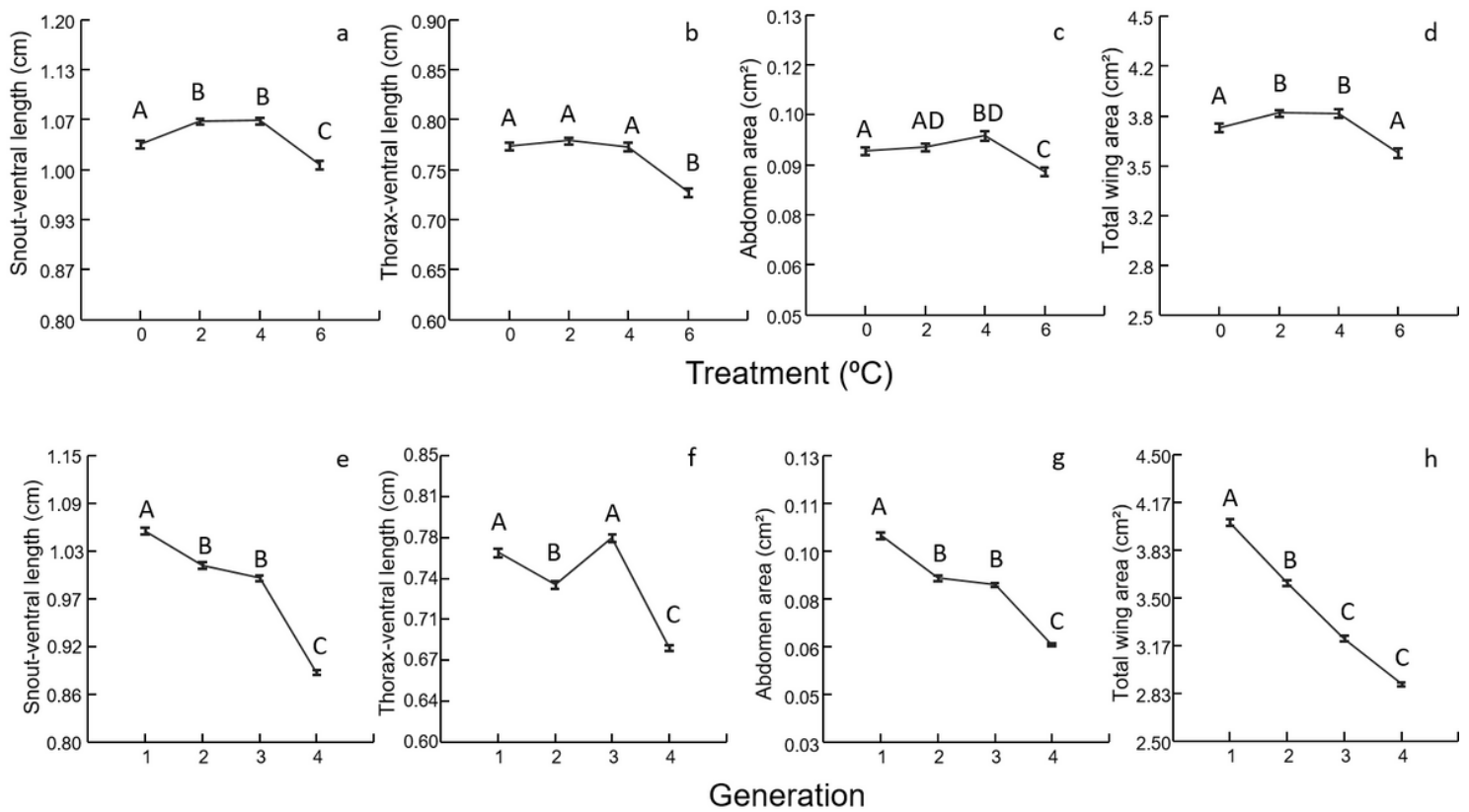


Figure 6

Body size measurements plotted for treatment (6a: snout-ventral length, ANOVA $p < 0.0001$, $F = 23.10$, $R^2 = 0.09$, $df = 3$; 6b: thorax-ventral length, ANOVA $p < 0.0001$, $F = 30.8$, $R^2 = 0.11$, $df = 3$; 6c: abdominal area, ANOVA $p < 0.0001$, $F = 11.49$, $R^2 = 0.05$, $df = 3$; 6d: total wing area, ANOVA $p < 0.0001$, $F = 11.52$, $R^2 = 0.09$, $df = 3$, $n = 694$), and generation (6e: snout-ventral length, ANOVA $p < 0.0001$, $F = 101.79$, $R^2 = 0.3$, $df = 3$; 6f: thorax-ventral length, ANOVA $p < 0.0001$, $F = 60.274$, $R^2 = 0.2$, $df = 3$; 6g: abdominal area, ANOVA $p < 0.0001$, $F = 113.92$, $R^2 = 0.32$, $df = 3$; 6h: total wing area, ANOVA $p < 0.0001$, $F = 448.10$, $R^2 = 0.63$, $df = 1$, $n = 694$;) for Year 2 individuals. Error bars and uppercase letters follow Fig. 1

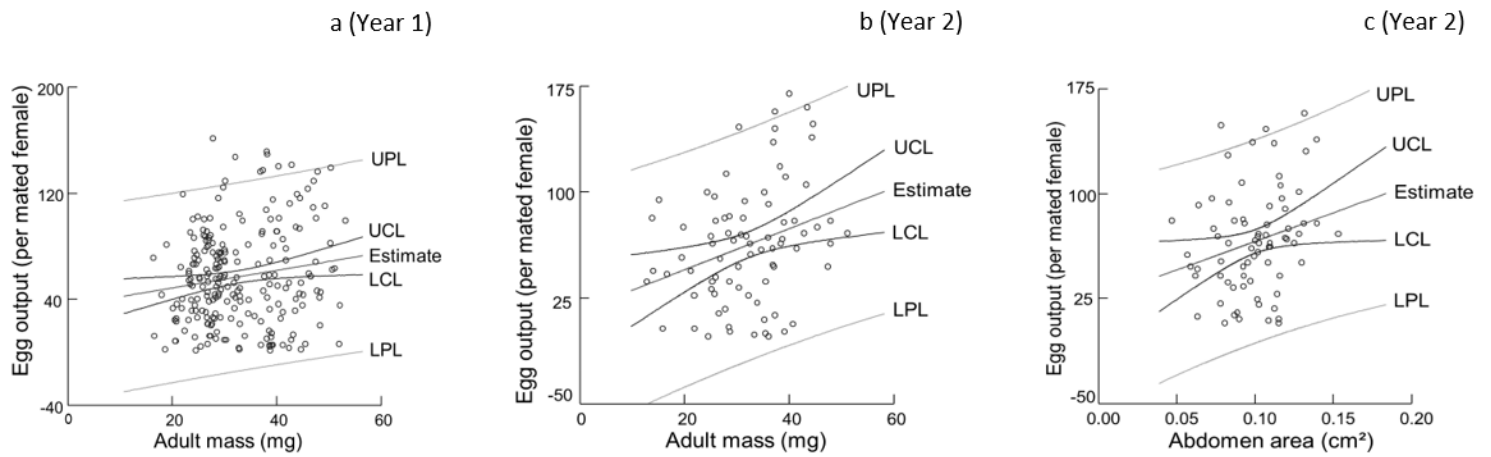


Figure 7

Egg output per mated female plotted against their corresponding adult mass for Year 1 (7a) (Linear Regression $p = 0.02$, $F = 5.05$, $r = 0.15$, $R^2 = 0.02$, $df = 1$, $n = 225$) and Year 2 (7b) (Linear Regression $p = 0.0078$, $F = 7.45$, $r = 0.31$, $R^2 = 0.08$, $df = 1$, $n = 79$). Number of eggs laid plotted with female abdominal area for Year 2 individuals only (7c) (ANOVA $p = 0.03$, $F = 4.424$, $df = 1$, $r = 0.26$, $R^2 = 0.06$, $n = 74$). Lower and upper confidence levels (LCL and UCL, respectively), and lower and upper prediction levels (LCL and UCL respectively)

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