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The Effects of Climate Warming on Plant-Herbivore Interactions

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FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

THE EFFECTS OF CLIMATE WARMING ON PLANT-HERBIVORE INTERACTIONS

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

BIOLOGY

by

Nathan P. Lemoine

2015

To: Dean Michael R. Heithaus
College of Arts and Sciences

This dissertation, written by Nathan P. Lemoine, and entitled The Effects of Climate Warming on Plant-Herbivore Interactions, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Florida International University, 2015

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CHAPTER V

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DEDICATION

I dedicate this dissertation to Sasha DoS. KoB. CoS. Lemoine, who helped me through the toughest parts and sat patiently while I typed and typed and typed.

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ABSTRACT OF THE DISSERTATION
THE EFFECTS OF CLIMATE WARMING ON PLANT-HERBIVORE INTERACTIONS

by

Nathan P. Lemoine

Florida International University, 2015

Miami, Florida

Professor Deron E. Burkepile, Major Professor

Rising temperatures associated with climate change will alter the fundamental physiological processes of most ectothermic species. Drastic changes in catabolic and anabolic reaction rates exert strong effects on growth, reproduction, and consumption rates that cascade up through all levels of the biological hierarchy. This dissertation determined how climate warming might alter the important relationship between plants and insect herbivores, as mediated through changes in herbivore physiology. Consumption and fitness increased with temperature for almost all consumers. However, all consumers also exhibited a critical temperature, beyond which consumption declined rapidly through metabolism continued to increase. This mismatch in metabolic demands and energy intake reduced consumer fitness at high temperatures. Furthermore, increased metabolic nitrogen demand can induce nitrogen limitation in insect herbivores at high temperatures. These basic physiological changes can modify the way herbivores interact with plants in a number of ways. For example, the Japanese beetle, *Popillia japonica*, altered its feeding behavior on numerous host plant species, depending on host plant quality. Unfortunately, the effects of temperature on plant-herbivore interactions will be difficult to predict, as there was no predictable relationship between consumption and temperature across numerous plant-herbivore pairs. Finally, rising temperatures disrupt insect herbivore control of plant fitness, thereby altering one of the most important components of plant-herbivore interactions. Thus, climate change will fundamentally change the nature of plant-herbivore interactions in the future.

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CHAPTER I
INTRODUCTION

Climate change poses one of the most significant threats to natural ecosystem structure and function. Carbon cycling, nitrogen remineralization, primary and secondary production, food web stability, community resistance to invasions, and species extinction rates are some of the numerous ecosystem processes that stand to be profoundly altered by ongoing rapid changes in climate (Bellard et al. 2012, Dukes and Mooney 1999, Melillo et al. 2002, Petchey et al. 1999). In particular, climate change imposes considerable changes on important trophic interactions within a community (Barton et al. 2009, Voigt et al. 2003). Given that such trophic interactions control community structure and nutrient cycling in most communities (Belovsky and Slade 2000, Metcalf et al. 2014, Nitschke et al. 2014), predicting the effects of climate change on these interactions has become an active and important area of research (Cahill et al. 2013). Unfortunately, despite a considerable surge in climate-related research over the past decade, we currently lack an accurate, mechanistic understanding of the role of environmental conditions in determining important trophic interactions within food webs.

One popular tool for predicting the effects of climate change on species interactions is the Metabolic Theory of Ecology (MTE) (West et al. 1997). Metabolic Theory posits that temperature controls most fundamental physiological processes in ectothermic organisms by controlling rates of metabolic reactions (Gillooly et al. 2001). By controlling metabolic reactions, temperature should therefore control numerous aspects of an individual's physiology and ecology, such as rates of individual growth, mortality, reproduction, and consumption (Brown et al. 2004). In fact, MTE makes specific, quantitative predictions about the rate at which metabolic reactions should occur at higher temperatures (Gillooly et al. 2001). These predictions make MTE a valuable tool for modeling how climate change, in particular rising temperatures, might alter population dynamics and food web structure in the future. Indeed, researchers have extended traditional consumer-resource population models by incorporating temperature-dependence of multiple parameters, which allows these models to predict how rising temperatures will influence plant-herbivore or predator-prey interactions (O'Connor et al. 2011). However, the accuracy of these predictions hinges on the accuracy of MTE as a predictive framework.

In order to assess the utility of MTE as a predictive framework for climate change, I experimentally test the assumptions and predictions of MTE in Chapter II using the sea urchin *Lytechinus variegatus* coupled with a meta-analysis of numerous other consumer species. Metabolic Theory proposes that metabolic rates and consumption rates both increase exponentially with temperature at similar rates (Brown et al. 2004). I found that, although metabolism of most ectothermic species does increase exponentially with temperature, consumption almost always displays a marked, unimodal curve. The mismatch of metabolism and consumption at high temperatures led to reduced consumer growth and fitness at the upper end of the temperature range for each species, which is the critical range for predicting the effects of climate change on consumer population dynamics and trophic interactions. Thus, many of MTEs fundamental assumptions appear to have little biological basis and do not account for the unimodal relationship between fitness and temperature for most species.

Another assumption of MTE is that energy (*i.e.* carbon) primarily determines the relationships between consumption, growth, and temperature (Stern 2004). However, consumption and growth of consumers are regulated by a number of other important macronutrients, such as nitrogen and phosphorus (Stern and Elser 2002). In particular, nitrogen is a primary constituent of most proteins and therefore necessary for both somatic growth and cellular maintenance (Stern and Elser 2002). The costs of cellular maintenance increase at higher temperatures due to more rapid protein denaturing and synthesis (Somero 2011), indicating that rising temperatures might induce nitrogen-limitation in numerous herbivores. Yet we know relatively little about the way dietary nitrogen content interacts with temperature to structure the performance and feeding behavior of herbivores (Cross et al. 2015). In Chapter III, I used the lepidopteran *Spodoptera exigua* as a model organism to assess how dietary nitrogen and temperature jointly regulate herbivore feeding behavior and growth. I found that increased metabolic nitrogen demands at high temperatures drove *S. exigua* to increase consumption of low-quality diets by almost 300%. These results suggest that the rising costs of metabolic

nitrogen demands due to climate warming demands impose considerable constraints on the feeding behavior of ectothermic herbivores.

In addition to nitrogen, plants also contain numerous secondary metabolites that can affect herbivore feeding behavior and performance (Agrawal 1998). Indeed, generalist herbivores must account for both nutrition and defensive compounds when choosing which plant species to consum (Cruz-Rivera and Hay 2003). Furthermore, temperature modifies the efficacy of chemical defenses, which often become less effective against generalist herbivores at higher temperatures (Stamp and Osier 1998, Stamp and Yang 1996, Stamp et al. 1997). Therefore, rising temperatures due to climate change might alter the way generalist herbivores forage among various plant species. In Chapter IV, I determined how temperature alters both herbivore feeding behavior and herbivore feeding preferences among nine plant species using the Japanese beetles *Popillia japonica*. I found that *P. japonica* growth and consumption rates both increased at high temperatures, but only on high nitrogen plants. Furthermore, rising temperatures caused *P. japonica* to forage on a restricted subset of plant species. Foraging choices were not related to nutrient content. Rather, this trend was driven the secondary metabolites of two plant species, *Vitis vulpina* and *Rosa multiflora*, becoming more attractive to *P. japonica* at high temperatures.

Interestingly, *P. japonica* exhibited different behavior from *S. exigua* in Chapter II, failing to increase consumption of low-quality diets at high temperatures. These inverse responses led me to question whether there were general trends for predicting the effects of temperature on consumption rates among many plant-herbivore pairs based on plant nutritional quality. In Chapter V, I evaluated how temperature affects consumption rates for 14 plant-herbivore pairs. I found that there was high variability in the shape of thermal response curves of consumption, both among and within herbivore species. Across all combinations, nutritional content explained almost no variation in the shape of thermal response curves. However, within species, consumption rates increased with temperature more rapidly on high-nitrogen plants, as was observed for *P. japonica* in Chapter IV.

Chapters II – V examine short-term impacts of warming on plant herbivory rates. The assumption of these chapters is that changes in plant herbivory rates due to climate change ultimately impact plant fitness. The effects of insect herbivores on plant fitness have been well-studied (Strauss and Agrawal 1999), and several studies have documented direct effects of warming on plant fitness (Aerts et al. 2004, Prasad et al. 2002, Totland 1999). However, the ways in which warming modifies herbivore effects on plant fitness remain unknown. In Chapter VI, I used *in situ* warming infrastructure to factorially manipulate both temperatures and herbivore presence on the common evening primrose, *Oenothera biennis*. I found that, under ambient temperatures, herbivores induce fitness trade offs in *O. biennis*, wherein plants exposed to herbivores produce more, albeit lighter, seeds to compensate for reductions in fruit numbers. Crucially, warming negated almost all effects of herbivores on plant fitness. These results suggest that the nature, and ultimate consequences, of plant-herbivore interactions may look dramatically different in future climates.

In conclusion, this dissertation examines the mechanisms by which climate change influences plant-herbivore interactions. I found that one of the dominant models used to predict the effects of climate change, MTE, breaks down towards the upper end of species' thermal tolerances. Moreover, temperature interacts with dietary nitrogen content to constrain the responses of herbivores to rising temperatures due to increased metabolic nitrogen demand. These increased demands lead to numerous different thermal response curves of both consumption and growth, both among and within species. Finally, warming ultimately negated any effect of herbivores on plant fitness by constraining plant reproductive characteristics to a relatively small subset of potential outcomes. Thus, this work considerably advances our knowledge with respect to the effects of temperature on important evolutionary and ecological interactions.

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CHAPTER II

TEMPERATURE-INDUCED MISMATCHES BETWEEN METABOLISM AND CONSUMPTION

REDUCE CONSUMER FITNESS

Abstract

As physiological processes of ectotherms are coupled to environmental temperature, climate change will likely alter their fundamental biological rates, including metabolism, consumption, growth, and reproduction. Here we combine Metabolic Theory of Ecology (MTE) with metabolism and consumption measurements of a model organism, the urchin *Lytechinus variegatus* to test how climate change will affect consumer fitness. Unexpectedly, we found that metabolism and consumption exhibit different scaling relationships with temperature and are mismatched at high temperatures. This led to a dramatic reduction in ingestion efficiency and potentially consumer fitness. Using meta-analysis, we showed that such temperature-driven mismatches between consumption and metabolism are common across taxa and frequently lead to reduced consumer fitness. Our empirical and synthetic analyses identify a mechanism by which climate change reduces the fitness of ectotherm consumers that may be applied to a broad array of taxonomic groups. Moreover, we showed that the assumptions of MTE do not hold at temperatures near the upper range of species' thermal tolerances for a wide array of taxa. Models using MTE to predict the effects of climate change on consumer-resource dynamics may therefore be underestimating the consequences of rising temperatures on population and community dynamics.

Introduction

Increases in metabolism at high temperatures may have profound effects on all levels of the biological hierarchy (Gillooly et al. 2001, Brown et al. 2004). For example, increased metabolic rate can increase resource uptake by primary producers (Enquist et al. 2003) and consumption by species at higher trophic levels (Rall et al. 2010). Such changes may be responsible for restructuring competitive dominance hierarchies and increasing predation rates at moderate levels of warming (de Valpine and Harte 2001, Rall et al. 2010). Therefore, predicting how temperature-driven changes in physiology influence population dynamics and community structure is key to understanding the impacts of climate change.

The Metabolic Theory of Ecology (MTE, Brown et al. 2004) proposes that metabolic rates are constrained by thermodynamics and exhibit Arrhenius relationships with temperature, resulting in an exponential increase in metabolic rate with temperature (Gillooly et al. 2001). There is good evidence that metabolic rates increase exponentially with temperature within a species, supporting MTE (Gillooly et al. 2001, Clarke 2004). MTE further predicts that metabolic demand drives other vital biological rates, such as consumption, growth, and reproduction and that temperature scaling of these biological rates should match that of metabolism (Brown et al. 2004). Accordingly, MTE is a promising avenue for incorporating temperature-dependence in dynamic population models to predict the effects of climate change (Vassuer and McCann 2005, O'Connor et al. 2011).

However, equivalent scaling of metabolic rates and other vital biological rates used in these population models lacks empirical support (O'Connor et al. 2007), suggesting that these models may not be sufficient for predicting population responses to climate change. For example, simultaneous measurements of metabolism and consumption along temperature gradients have shown a mismatch between the temperature scaling of metabolism and consumption. Metabolism frequently increases more quickly with temperature than does consumption (Rall et al. 2010). Such temperature-driven mismatches may reduce ingestion efficiency, defined as the amount of carbon assimilated per unit of carbon respiration (Vassuer and McCann 2005). Reduced ingestion efficiencies may decrease individual fitness, as there is less energy available for growth and reproduction after basic cell maintenance costs have been considered. Thus, mismatches of metabolism and consumption at high temperatures suggest that climate change can decrease consumer fitness and lead to unstable population dynamics and possible consumer extinction (Vassuer and McCann 2005). By using published studies based on interspecific comparisons of metabolism and consumption, Vassuer and McCann (2005) concluded that consumption usually increases more rapidly than metabolism, thus unstable population dynamics are rare under climate change scenarios. However, if metabolism increases faster than consumption, as

intraspecific patterns suggest, the likelihood of consumer extinction under climate change increases substantially.

Here, we evaluated the effects of temperature on the performance of a common marine herbivore, the sea urchin *Lytechinus variegatus*. We asked three specific questions: (1) How does metabolism scale with temperature? (2) Does consumption scale with temperature in the same manner as metabolism? and (3) Does temperature affect the ingestion efficiency of *L. variegatus*? We also used meta-analysis to address two additional questions: (4) Are mismatches between metabolism and consumption common across taxa? and (5) What are the fitness consequences of such mismatches? In testing basic assumptions of MTE, we provide a mechanism for declines in fitness at high temperatures across a range of species.

Methods

Lytechinus variegatus is an herbivorous urchin found in seagrass beds throughout the Caribbean and Gulf of Mexico and can exert strong top-down control on seagrass biomass (Valentine et al. 2000). Thus, *L. variegatus* is an important study organism regarding herbivory research in marine systems (e.g. Prado and Heck 2011). Moreover, the physiology of tropical and subtropical species such as *L. variegatus* will likely be most affected by climate change (Dillon et al. 2010). Individuals were collected from Virginia Key, Florida (25.736° N, 80.156° W) from June–August 2011 and held in a flow-through tank at ambient temperatures (26.7 ± 1.1 °C, mean $\pm$ SE). Wet masses of urchins used in this study ranged from 30.59 – 256.46 g. Urchins were provided fresh *Thalassia testudinum* seagrass, their preferred food source, *ad libitum*.

For our metabolism measurements, one randomly chosen urchin was placed into each of five independent, recirculating seawater baths held at 26°C. Temperatures were adjusted to 20°, 23°, 26°, 29°, and 31°C gradually over 24 hours (< 1 °C change per hour), and respiration measured following Siikavuopio et al. (2008) (Appendix B). These temperatures were within the range of temperatures normally experienced by *L. variegatus* (Fig. A1). 31°C is the upper limit of temperatures at our collection site and will likely become more frequent under current climate

change projections. All temperatures were run simultaneously, with one replicate of each temperature run per day for five days ($n = 5$ replicates per temperature). Preliminary experiments showed that 24 hours allowed for sufficient metabolic adjustment (Fig. A2).

To measure consumption rates across the temperature gradient, urchins were randomly assigned to 1.6 L closed feeding arenas that were placed into water baths held at 26°C and were assigned the same temperatures used in the metabolism trials ($n = 12$ per temperature). Three replicates of each temperature were run per day. Grazing rates did not differ among days for each temperature, so data were pooled across days for analysis. Urchins were starved for 24 hours while acclimating to the new temperatures (Prado and Heck 2011). Fresh *T. testudinum* was blotted dry then weighed to obtain a pre-assay wet weight. Urchins were fed 2.00 ± 0.10 g. After 24 hours, any remaining seagrass was removed, blotted dry, and reweighed to get a post-assay wet weight. Consumption was estimated as the difference between pre-weight and post-weights. We excluded observations where $< 5\%$ of seagrass mass was consumed. *T. testudinum* wet weights were converted to dry weights using linear regression (Dry Weight = $0.228 \times$ Wet Weight, $p < 0.001$, $R^2=0.998$). Dry weights were converted to mg carbon consumed using estimates of carbon content in *T. testudinum* as 40% of dry weight (Prado and Heck 2011).

We measured the assimilation efficiency of *L. variegatus* at the same temperatures used in the respiration and consumption assays. Randomly chosen urchins were placed in feeding arenas held at ambient seawater temperatures and starved for five days, clearing the digestive tract of all fecal material (e.g. Prado and Heck 2011). Experimental arenas were then placed in water baths randomly assigned one of the five temperature treatments listed above ($n = 3$ per temperature). Seagrass consumption was estimated as described. Fecal material was siphoned from each feeding arena daily for five days after the feeding assay and dried to a constant weight at 60°C. Assimilation efficiency was estimated as $1 - \frac{\text{fecal weight}}{\text{consumption}}$ (Watts et al. 2011). Decreasing values indicate lower assimilation efficiency of food for maintenance, growth, or reproduction. Consumption was converted to mg C as described above.

Ingestion efficiency (I) is a unitless metric that provides an estimate of the excess carbon available after cellular maintenance costs have been considered. When $I > 1$, excess carbon is available for growth and reproduction; when $I < 1$, consumption is insufficient to meet metabolic demand. We calculated the predicted metabolic rate, R , of each urchin used in the consumption trials as $R = 1.61 \times 10^6 e^{0.46 \frac{-1}{kT}} M^{0.33}$ (see Results). We then estimated ingestion efficiency as $I = \frac{wC}{\lambda R}$, where w is assimilation efficiency (0.70, see Results), C is mg C consumed in 24 hours, R is mg C respired in 24 hours, and λ is a scaling coefficient relating basal metabolic rate (*i.e.* metabolic rate of inactive, fasting animals) to field metabolic rate (*i.e.* average metabolic rate in natural conditions) (Rall et al. 2010). Savage et al. (2004) determined that field metabolic rates are approximately three times higher than basal metabolic rates ($\lambda = 3$) for vertebrates. However, the concept of basal metabolic rate as used by many physiologists is not appropriate for invertebrates (Clarke and Fraser 2004). We therefore set $\lambda = 1$, indicating that field metabolic rates equal lab metabolic rates, providing a conservative estimate of I .

Data Analysis

MTE predicts exponential temperature relationships for respiration and consumption within species (Gillooly et al. 2001), but performance curves for many species show unimodal trends with temperature (Angilletta 2009). Therefore, we used Akaike Information Criteria corrected for small sample size (AICc) to evaluate five *a priori*-chosen temperature-scaling models for each response variable (respiration, consumption, assimilation efficiency, and ingestion efficiency): (1) exponential (MTE prediction), (2) Gaussian, (3) Brière1, (4) a linear relationship, and (5) a null model of no temperature effect. A Gaussian model is commonly used to evaluate thermal performance curves (Angilletta 2006). The Brière1 model (Brière et al. 1999) is an asymmetrical, unimodal curve that often provides the best description of thermal performance curves by allowing for a rapid drop-off beyond the thermal optimum (Shi and Ge 2010). A linear model was used to contrast the thermal response curves to a simple linear increase in biological rates with temperature. Finally, a null model of no temperature effect was

included to evaluate how well the other models compare to a model of no temperature effect (Hilborn and Mangel 1997). For exponential and Gaussian models, temperature was first converted to Kelvins to model the Arrhenius exponential function (Gillooly et al. 2001), whereas for linear and Briere¹ models, temperature was left in °C.

Each model initially included urchin mass as a potential covariate, but mass was removed if the 95% confidence interval of the parameter included 0 and the model re-evaluated. Models with $\Delta AICc > 2$ were considered to have poor support. We also calculated the posterior probability of each model to provide a plurality of model-choice criteria following Hilborn and Mangel (1997). We assumed that all models were equally probable *a priori* (i.e. flat priors). Posterior probabilities represent the probability that each model is the ‘true’ model, given the data. All results are reported as mean $\pm$ 1 SE.

Meta-Analysis

We examined whether fitness reductions due to mismatches between consumption and metabolism were common across taxa. We therefore conducted a literature search on ISI Web of Science using combinations of the key words *metab**, *resp**, *consumption*, *ingestion*, and *temperature*. We selected only manipulative lab experiments studies that: (1) simultaneously measured consumption and metabolism across three or more temperatures to allow for possible curvilinear effects, (2) used ectothermic species, and (3) manipulated only temperature within the range of temperatures naturally experienced by the study species. We found 9 studies encompassing 19 experiments, including the current one, that met these criteria. Additionally, 6 studies and 13 experiments also measured growth rates, allowing us to link mismatches between consumption and metabolism to growth (Appendix C).

As units and magnitudes of each parameter varied widely among experiments and taxa, we calculated the z-scores of metabolism, consumption, and growth rates within each study. Within each study, we added a constant to growth rates to ensure that only observations with negative growth had negative z-values so that standardized growth rates < 0 indicate loss of

mass. It did not affect the rank-order of observations within a study. We calculated the ratio of metabolism to consumption (M:C) after adding the same constant to every metabolism and consumption z-score to make all values positive across all studies. This preserved the rank-order of observations within and among studies while preventing complications due to differing positive and negative signs (Fig. A3). Increasing M:C indicates higher metabolic demand relative to consumption. This ratio does not indicate ingestion efficiency, as the units are in standard deviations, but expresses the relative strengths of metabolism and consumption. We regressed M:C against temperature and also regressed growth rates against M:C using linear mixed-effects models with species as a random factor. We chose the best model among null, linear, and quadratic models using AIC. All statistical analyses were conducted in R v2.13.

Results

Respiration rates increased from 0.02 ± 0.003 mg C per day to 0.04 ± 0.005 mg C per day as temperature increased from 20° to 31° C (Fig. 2.1A). This relationship was best described by the exponential function predicted by MTE (Table A1). The temperature exponent of respiration was estimated as 0.46 and the 95% confidence interval included 0.65 ($CI_{95\%} = 0.25 - 0.67$), the activation energy predicted by MTE (Allen et al. 2005). The Q_{10} of respiration for other urchin species ranged from 1.72 – 3.01, corresponding to activation energies of 0.37-0.76 (Gillooly et al. 2001, Siikavuopio et al. 2008). Our estimate of activation energy is therefore within the range of activation energies measured for other urchin species. Whole-organism respiration rates increased with body mass, although the confidence interval of the scaling parameter narrowly excluded zero ($CI_{95\%} = 0.01 - 0.63$, Table A1, Fig. A4).

Consumption rates increased from 26.35 ± 3.37 mg C per day to 40.38 ± 3.50 mg C per day between 20° and 29° C. At 31° C, consumption rates dropped rapidly to 28.62 ± 3.19 mg C per day (Fig. 2.1B). The Briere1 model best described this relationship (Table A1). The predicted maximum consumption rates were at 27.19°C. Consumption rates also increased with body mass ($CI_{95\%} = 0.07 - 0.43$, Table A1, Fig. A5).

Assimilation efficiency was relatively constant at 0.70 over all temperatures (Fig. 2.1C). Although the null model provided a slightly worse fit than the exponential or linear models (Table A1), the 95% confidence intervals of the temperature coefficient for both linear and exponential models included zero (linear $CI_{95\%} = -0.002 - 0.018$, exponential $CI_{95\%} = -0.02 - 0.20$), reducing these models to the null model. As confidence intervals narrowly included zero, assimilation efficiency may increase with temperature but the rate of change is negligible over the temperatures measured in this study. Also, assimilation efficiency did not scale with mass in either the linear ($CI_{95\%} = -0.003 - 0.009$) or exponential ($CI_{95\%} = -0.04 - 0.14$) models.

Consumption scaled equivalently with metabolism from 20 - 26° C, leading to relatively constant ingestion efficiency in these temperature ranges. Beyond 26° C, metabolism increased much more rapidly with temperature than did consumption, leading to a rapid decrease in ingestion efficiency (Fig. 2.1D). A Brière¹ model best described this pattern (Table A1). Ingestion efficiency did not vary with body mass (mass scaling parameter $CI_{95\%} = -0.12 - 0.16$). Though ingestion efficiency was still relatively high at 31° C ($\sim 366.55 \pm 40.13$), the rate of decrease was so severe that, when the curve is extrapolated, ingestion efficiency drops to ~ 72 at 32° C and becomes zero at $\sim 32.62^\circ$ C, temperatures well within climate change predictions.

Meta-Analysis

MTE predicts the metabolism:consumption ratio (M:C) to be temperature invariant or to decrease with increasing temperature. However, when pooled across species, M:C increased linearly with temperature (Fig. 2.2A, Table A2, $R^2 = 0.57$). Despite interspecific variation in the temperature ranges, metabolism increased more quickly with temperature than did consumption for all species except small *Strongylocentrotus droebachiensis* urchins, which showed a negative relationship (Fig. 2.2A). Importantly, increasing disparity between metabolism and consumption led to lower growth rates in all species for which data were available (Table A2, Fig. 2.2B, $R^2 = 0.58$). All but one observation of negative growth occurred at or above M:C = 1, indicating that organisms can lose mass if metabolism outpaces consumption (Fig. 2.2B).

Discussion

Rising temperatures will likely increase metabolic demand of individuals, especially ectotherms (Gillooly et al. 2001). In turn, rising metabolic costs may alter many vital biological rates, such as mortality, reproduction, and growth, thereby having profound impacts on community structure and ecosystem function (Allen et al. 2005). Our data support the prediction that temperature-driven changes in metabolic demand alter individual biological rates, but the relationships are more complex than exponential relationships proposed by MTE. A temperature-induced mismatch of metabolism and consumption dramatically reduced the ingestion efficiency of the urchin *L. variegatus*. Moreover, warming frequently led to higher metabolic rates relative to consumption across several species, resulting in reductions in fitness. Modest temperature increases predicted by climate change may therefore have severe consequences for an organism's fitness and population dynamics and potentially alter consumer-resource interactions.

One potential concern is that our short-term warming experiments do not allow for acclimatization of organisms and their metabolism that may happen over time. This would mean that we overestimated the mismatch between metabolism and consumption. However, it is unlikely that the unimodal consumption curve exhibited by *L. variegatus* in this study is a product of acute temperature stress. Consumption by *L. variegatus* exposed to temperatures ranging from 16 – 28° C for eight weeks showed a similar unimodal curve with a slow increase from 16° - 22° C (~ 2.2 – 2.5 g/day, respectively) and then a decrease in consumption at 28° C (~ 1.8 g/day, Watts et al. 2011), suggesting that a decrease in consumption rates is sustained over long time periods. Further, a recent meta-analysis found that 66% of species examined exhibited unimodal consumption curves with respect to temperature (Dell et al. 2011), suggesting that unimodal patterns of consumption may be the most common relationship with temperature.

Ingestion efficiency of *L. variegatus* declined by ~ 50% between 27 – 31° C due to the mismatch between metabolism and consumption at high temperatures (Fig. 2.1D). This decline in ingestion efficiency likely leads to lowered fitness as growth rates, gonad production, and gonad production efficiency of *L. variegatus* show a similar unimodal trend with temperature (Watts et al.

2011). If temperature increases to 32° C, ingestion efficiency drops to 72 and becomes zero at ~32.6° C, both temperatures are well within the 2° warming expected from climate change. While this pattern extrapolates slightly beyond our available data, the qualitative pattern is that slight warming at high temperatures drastically reduces energy for growth and reproduction. This could be especially important during summer spawning periods of *L. variegatus*, during which water temperatures already average 30 - 31 °C. Further, *L. variegatus* currently spends, on average, 50% of the year at or above 26° C when ingestion efficiency starts to decline and the energy available to growth and reproduction becomes less available. Under a conservative estimate of 2° C warming with climate change, *L. variegatus* would spend 66% of the year above 26° C, severely reducing the amount of time available for energy accumulation.

The negative effects of high temperatures on growth efficiency have long been known. For example, sockeye salmon, *Oncorhynchus nerka*, have slower growth rates at high temperatures that is likely driven by mismatches between consumption and metabolism nearly identical to those reported here (Brett 1971). That all but one size class of one species in our analyses exhibited mismatches between metabolism and consumption, and subsequently reduced growth rates, under experimental warming suggests that this is likely a general pattern among ectotherms. More recently, this mismatch between consumption and metabolism has been linked to declining fitness at high temperatures across a taxonomically diverse set of species, including reptiles, fishes, crustaceans, and arthropods (Angilletta 2009, Donelson et al. 2010). However, the physiological mechanism behind such fitness reductions has rarely been quantified.

One mechanism responsible for these patterns in fitness reduction at high temperatures is a mismatch between the costs of cellular maintenance and energy supply. Determinant growth of many organisms arises from more rapid increases in the cost of cellular maintenance than available energy supply with increasing body mass (West et al. 2001). Thus, a greater fraction of energy is devoted to maintenance rather than growth at high body sizes. Similarly, rising temperatures increase both the cellular demand for ATP and the fraction of ATP devoted to

cellular maintenance needed to repair damaged DNA and denatured proteins (Somero 2011). Moreover, ectotherms experience reduced aerobic scope (*i.e.* excess oxygen available for processes other than basal metabolism) at high temperatures (Pörtner and Knust 2007). Thus, the majority of assimilated energy at high temperatures is devoted to cellular maintenance and repair, leaving little excess energy for work (*i.e.* consumption, reproduction) or growth.

Models using MTE to predict the effects of climate change on consumer-resource interactions generally show that herbivore biomass declines at increased temperatures. Such models assume that consumption scales equivalently with metabolism (O'Connor et al. 2011) or use comparative studies among species to confirm that consumption increases more rapidly than metabolism as temperature increases (Vassuer and McCann 2005). However, comparative studies are based on interspecific comparisons at species' average body temperatures and may not represent intraspecific patterns. We show that, within a species, more rapid increases in consumption than metabolism may be the exception rather than the rule. Thus, previous models likely underestimated the consequences of climate change on consumer biomass if metabolism-consumption mismatches do induce unstable population cycles (Vassuer and McCann 2005).

MTE provides a promising avenue for predicting the effects of climate change on populations and communities, yet few studies evaluate its underlying assumptions regarding temperature-dependence of biological rates. If physiology-based population models such as MTE are used to predict the effects of climate change, we must define the conditions under which their assumptions are not met and their predictions incorrect (O'Connor et al. 2006). We demonstrated that some assumptions of MTE are not valid at the upper range of species' thermal tolerances, the range critical to predicting climate change effects. Specifically, mismatches between consumption and metabolism are widespread and frequently lead to reduced fitness in a variety of taxa. Such reductions in fitness may severely impact population dynamics and ecosystem function. Thus, thermal mismatches between metabolism and consumption should be explicitly considered when modeling the effects of climate change on populations and communities.

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Figure 2.1 – A) Relationship between whole-organism metabolic rates and temperature. Fitted line shows the exponential model. Respiration rates were mass corrected using the scaling parameter estimated by non-linear regression. B) Relationship between daily consumption rates and temperature. Fitted line shows the Brière1 model. Consumption rates have been mass corrected using the mass-scaling parameter estimated by nonlinear regression. C) Relationship between assimilation efficiency and temperature. Fitted line shows the null model. D) Relationship between ingestion efficiency and temperature. Fitted line shows the Brière1 model. For all panels, data points represent means $\pm$ SE.

Figure 2.2 – A) Relationship between the metabolism/consumption ratio and standardized temperature. B) Relationship between growth rates and the metabolism/consumption ratio. For simplicity, we show the quadratic model, though the linear model fit the data equally well (Table S2). In each panel, the solid black line is the overall trend and the dotted colored lines are the species-level trends.

CHAPTER III

WARMING DRIVES COMPENSATORY FEEDING IN AN ECTOTHERMIC HERBIVORE BY INCREASING METABOLIC NITROGEN DEMANDS

Abstract

Rising temperatures due to climate change will fundamentally reorganize food webs, community structure, and ecosystem function. These effects occur because rising temperatures alter basic physiological processes that dictate population growth rates and species' interactions in ectothermic organisms. Specifically, warming alters plant-herbivore interactions by increasing ectotherm metabolism and growth rates. Although growth rates, on average, increase exponentially with rising temperatures, many herbivores exhibit positive, negative, or no change in growth in response to rising temperature depending on diet quality. Here, we test whether increased metabolic N turnover determines how temperature affects growth rates and nitrogen-limitation of a generalist herbivore, *Spodoptera exigua*. We reared third-instar *Spodoptera exigua* (Lepidoptera: Noctuidae) on three artificial diets of varying nitrogen content at multiple temperatures and quantified caterpillar growth rates, metabolic nitrogen demand, and frass production as a proxy for consumption. We found that rising temperatures increased *S. exigua* growth rates on all artificial diets. However, warming forced *S. exigua* to greatly increase consumption of low-quality diets due to greater potential N-limitation. Thus, rising temperatures may alter the ways in which generalist ectothermic herbivores interact with plants. For example, under warming conditions fewer plant species may possess enough nitrogen to alleviate nitrogen-limitation, forcing herbivores to forage on a small subset of plant species to meet diet nitrogen requirements or causing herbivores to consume significantly more plant material to offset higher metabolic N demands. Understanding how temperature influences metabolic demands provides key information for predicting herbivore growth rates and foraging strategies in the future.

Introduction

Climate change will modify food web structure, community composition, ecosystem function, and population dynamics by initiating dramatic changes in basic physiological processes (Savage et al. 2004; Dell, Pawar & Savage 2011; DeLong, Hanley & Vassuer 2013). For example, rising temperatures trigger exponential increases in metabolism, growth, and reproduction rates in nearly all ectothermic species (Gillooly et al. 2001, but see Englund et al.

2011; Lemoine & Burkepile 2012). To fuel these rising costs of metabolism and growth, individuals must increase energy intake and therefore consumption rates, potentially strengthening interspecific interactions like predation or herbivory (Rall et al. 2010; Vucic-Pestic et al. 2011; Lemoine et al. 2013; Lemoine, Burkepile & Parker 2014). Theoretical and experimental examinations of how climate warming impacts herbivore feeding behavior frequently consider only a single food source of fixed nutritional quality, typically focusing on carbon as the limiting currency of metabolism (O'Connor, Gilbert & Brown 2011; Lemoine & Burkepile 2012). However, interactive effects of climate warming and other nutrients, like nitrogen (N) and phosphorus (P), on herbivore performance and feeding behavior remain an important, but understudied, research topic in ecology (Cross et al. 2015).

Temperature and diet quality can interact in numerous ways to determine herbivore consumption and growth rates (Kingsolver & Woods 1998; Kingsolver et al. 2006; Lee & Roh 2010). Basic cellular processes occur more rapidly as temperatures increase, stimulating demand for the fundamental nutrients necessary for these processes. For example, cellular division, which requires the production of phosphorus-rich RNA, occurs more rapidly at high temperatures (Elser et al. 2006). Indeed, high temperature switched rotifers from N- to P-limitation due to higher RNA:protein ratios caused by more rapid growth under warmed conditions (Wojewodzic et al. 2011). Higher temperatures also accelerate rates of protein denaturation, necessitating greater rates of protein synthesis and repair to maintain cellular function (Hachiya, Terashima & Noguchi 2007). Increased protein denaturation potentially increases consumer demand for N, a primary constituent of most proteins, and may induce consumer N-limitation at higher temperatures. However, the question of whether increased metabolic N respiration leads to temperature-induced N-limitation in herbivores has not yet been answered.

Preliminary evidence indicates that rising temperatures may lead to N-limitation of insect herbivores. Herbivorous insects have historically been considered N-limited even under ambient temperatures because plants tissues usually contain 2 – 5 times less than N in insect body tissues (Mattson 1980; Lemoine, Giery, & Burkepile 2014). Warming may exacerbate this

stoichiometric mismatch between plants and herbivores. For some herbivores, growth and consumption rates increase exponentially with temperature only on N-rich plants (Lemoine et al. 2013; Lemoine, Burkepile & Parker 2014). Other herbivores exhibit compensatory feeding by increasing consumption of low N diets (*i.e.*, compensatory feeding, *e.g.*, Williams, Lincoln & Thomas 1994). Still other, generalist herbivores decrease their diet breadth at high temperatures by preferentially feeding on a restricted set of host plants (Lemoine et al. 2013). Thus far, temperature-induced N limitation has only been assumed to be the driver of these patterns.

We used *Spodoptera exigua* (Lepidoptera: Noctuidae), a common agricultural pest that occurs throughout most of North America, as a model organism to address how increased metabolic N demand caused by increased temperatures affects the feeding behavior and performance of insect herbivores. *Spodoptera exigua* growth and performance are jointly controlled by both diet quality and temperature (Lee & Roh 2010). The physiological basis for such temperature-diet interactions remains unknown, despite the fact that metabolic N demand of its congener, *S. eridania*, has previously been described in detail (Karowe & Martin 1989). We used laboratory feeding assays to test three hypotheses. First, growth rates of *S. exigua* would be more strongly N-limited at high temperatures, which would manifest as a shallower positive relationship between growth and diet N under warmed conditions than under ambient conditions. Second, we predicted that total N excretion would increase at higher temperatures due to higher consumption rates, increased N throughput, and increased metabolic N turnover. Finally, a greater portion of frass N excretion at high temperatures would be comprised of N bound in uric acid at high temperatures. Increased production of uric acid, which contains metabolically processed N, often indicates more rapid higher N metabolism and turnover (Martin & Van't Hof 1988; Karowe & Martin 1989). Our results demonstrate that rising temperatures do induce N limitation in an ectothermic herbivore by increasing metabolic N demand, and that *S. exigua* offsets this increase metabolic demand by drastically increasing consumption rates of low-quality diets.

Methods

We conducted all feeding experiments at the Smithsonian Environmental Research Center (SERC) in Edgewater, MD. To test our predictions, we raised third instar *S. exigua* on four artificial diets containing differing levels of diet N. *Spodoptera exigua* eggs were purchased from a commercial supplier (Bio-Serv) and raised to the third instar at 25° C on a standard lepidopteran diet. We created three diets of varying quality by replacing casein in the standard lepidopteran diet with non-digestible cellulose to maintain constant water content. This is a standard method of generating artificial diets, and previous work demonstrated that N assimilation efficiency does not vary with casein concentrations in this species (Karowe & Martin 1989). We determined diet N concentrations of each diet via elemental analysis using 8 replicate subsamples from each diet (FlashEA 1112 Series CHN analyzer, Thermo Electron Corporation; Table 3.1). Our substitutions of casein resulted in diets of 1.8%, 2.8%, and 4.8% N. The 1.8% and 2.8% N diets approximate the N found in plant tissues (Lemoine, Giery & Burkepile 2014), whereas the 4.8% N diet provides non N-limited diet. Upon reaching the third instar, each *S. exigua* was weighed and randomly assigned to one of the three diet treatments and sealed in a plastic container with ~ 1 g of the respective diet.

We randomly assigned *S. exigua* caterpillars from each diet treatment to one of two temperature treatments: 25°/20° and 30°/25° C day/night temperatures ($n = 13$ per diet per temperature). Temperatures were maintained by climate-controlled growth chambers set to 14/10 hour day/night regimes with corresponding light cycles. We chose 25°/20° C as this temperature regime represents average conditions during the mid-late spring at SERC. The higher temperature treatment of 30°/25° represents a severe but likely increase in these spring temperatures because of climate change (IPCC 2007).

We allowed caterpillars to feed *ad libitum* for 36 hours. At the end of the feeding trials, we weighed caterpillars and frass from each container to calculate *S. exigua* relative growth rates ($\text{RGR}, \log(\text{Mass}_{\text{final}}/\text{Mass}_{\text{initial}})$) and frass production. We calculated consumption rates as the difference between pre- and post-trial food dry mass. Unfortunately, we were unable to obtain

reliable estimates of pre-trial diet wet weights as a consequence of variable and rapid evaporation during weighing. The inaccuracy in pre-trial diet wet weights made calculations of consumption rates (the difference in pre- and post-trial diet dry mass) unreliable. Therefore, we analyzed total frass production as a proxy for consumption. Frass production is a less than ideal proxy for consumption since assimilation efficiencies can vary by diet quality (Williams, Lincoln, & Thomas 1994). However, differences in frass production among diets and temperatures were so large (see *Results*) that they probably provide a conservative representation of relative consumption rates despite variation in assimilation efficiency (Bownes, Hill & Byrne 2013). Caterpillar frass was dried to a constant weight at 50° C, ground to a powder, and weighed to estimate dry frass production over the three-day trial period. To determine frass N content, we weighed 2-3 mg of dry frass for elemental analysis (FlashEA 1112 Series, Thermo Electron Corporation) and determined total frass N as $Frass_{dry\ weight} \times \frac{Frass\% N}{100}$.

We predicted that N metabolism would increase at higher temperatures. We therefore quantified the amount of respired N for each individual. Insects package metabolized N into uric acid and excrete it along with undigested material in a single frass pellet. We determined uric acid concentrations in the frass of each caterpillar using a modified spectrophotometric analysis (Bhattacharya & Waldbauer 1969; Martin & Van't Hof 1988; Karowe & Martin 1989). For each sample, we combined ~ 5 mg dry frass powder from each sample with 1 mL 0.6% lithium carbonate solution in 1.5 mL microcentrifuge tubes. Samples were vortexed and allowed to rock for ~ 1 hr. We then centrifuged samples for 5 minutes at 2200 RPM, separated the supernatant into a 15 mL falcon tube, and repeated the extraction. The procedure yielded ~ 2 mL of sample extract. We diluted each sample up to 3 mL using 0.6% lithium carbonate solution. We used a serum-based uric acid assay kit (Amplex Red Uric Acid Kit – Life Technologies) to spectrophotometrically determine the micromolar concentration of uric acid in each sample. We multiplied uric acid percentage by total frass weight to yield total uric acid production. We then multiplied total uric acid production by 0.33 to convert uric acid to metabolized N (uric acid is ~

33% N by mass). We corrected total metabolic N excretion and total N excretion by the initial caterpillar mass to yield estimates of N-excretion per gram body mass.

Statistical Analyses

To standardize initial starting mass, we excluded data from any caterpillars with initial sizes > 0.03 g. Caterpillar growth rates decline with increasing body size, such that the growth capacity of larger caterpillars with large initial sizes is reduced compared to small individuals. We removed 6 individuals from analysis. We used Bayesian ANCOVAs to assess the main and interactive effects of temperature and diet quality on RGR, metabolized N, and frass production. Temperature regime was treated as a categorical predictor with two levels. All predictors and responses were standardized prior to analyses. To guard against unlikely extreme effects, we placed weakly informative priors ($N(0, 1)$) on all parameters to ensure that extreme effects were not observed unless strongly supported by the data.

We modeled each response variable as coming from a normal distribution and log-transformed total N excretion to stabilize variances. Because of different variances among temperature treatments, this transformation still resulted in severe heteroscedasticity of metabolic N. Therefore, we analyzed untransformed metabolic N production but modeled different variances in each temperature treatment, which stabilized residual variances.

We ran each model for 25,000 burn-in iterations and saved every 10th sample from 25,000 posterior samples (2,500 posterior draws). For each model, we ran four MCMC chains simultaneously, resulting in a total of 10,000 independent posterior draws for each parameter (2,500 x four chains). All assumptions of distribution symmetry (*i.e.*, normality) and heteroscedasticity were examined using residual plots. Goodness-of-fit was assessed by plotting fitted group means against observed group means and by calculating R^2 (Gelman et al. 2013).

All models were run using STAN v2.5 accessed via PyStan. Data analyses were conducted in Python v2.7 using the *numpy*, *pandas*, and *scipy* modules (Jones et al. 2001; McKinney 2010; van der Walt, Colbert & Varoquaux 2011).

Results

Contrary to our initial expectations, we found little evidence that the strength of N-limitation of *S. exigua* RGR varied across temperatures. Relative growth rates increased with increasing diet N ($\text{Pr}(\text{Diet Effect}) = 1.00$) and were highest in the 30° C temperature ($\text{Pr}(\text{Temperature Effect}) = 1.00$) (Fig. 3.1). Indeed, RGR increased by $47 \pm 7.9\%$ at 30° C compared to caterpillars reared at 25° C. Although our data hinted that the relationship between diet N and RGR was shallower at 30° C than at 25° C, which would support our initial predictions of greater N limitation at higher temperatures, the effect was weak ($\text{Pr}(\text{Interaction}) = 0.817$). Therefore, *S. exigua* appear to maintain a fairly consistent increase in growth at high temperatures regardless of diet quality.

In support of our predictions, however, metabolic N respiration increased significantly at high temperatures ($\text{Pr}(\text{Temperature Effect}) = 1.0$) (Fig. 3.2). However, the effect of temperature on N respiration varied with diet quality ($\text{Pr}(\text{Interaction}) = 0.960$). At 25° C, metabolic N respiration did not vary with diet N. In contrast, metabolic N respiration at 30° C declined significantly as diet quality increased (Fig. 3.2). On the highest quality diet, N respiration at 30° C was nearly double that at 25° C ($196.8 \pm 39.2\%$ higher). On the lowest quality diet, which approximates natural plant tissues, N respiration was an astounding $245.8 \pm 37.5\%$ higher. Thus, *S. exigua* must overcome substantially increased metabolic N demands, especially on low-quality diets, in order to maintain consistent increases in growth at high temperatures.

Extremely high compensatory feeding at high temperatures allowed *S. exigua* to offset reductions in diet quality and maintain a high growth rate. Using frass as a proxy for consumption rates, *S. exigua* maintained relatively low food intake at 25° C, but increased intake substantially at 30° C ($\text{Pr}(\text{Temperature Effect}) = 1.00$) (Fig. 3.3). At both temperatures, *S. exigua* demonstrated compensatory intake of low-quality diets ($\text{Pr}(\text{Diet Effect}) = 0.996$). However, the rate of compensatory feeding was much higher at 30° C than at 25° C ($\text{Pr}(\text{Interaction}) = 1.00$) (Fig. 3.3). At 25° C, frass production was $167.6 \pm 39.6\%$ higher on the 1.8% N diet compared to the 4.8% N diet. At 30° C, frass production was $214.8 \pm 24.4\%$ higher on low-quality compared to high-quality

diets. Thus, compensatory food intake was 47.2% greater at 30° than at 25° C. The compensatory feeding enabled *S. exigua* to maintain increased growth on low-quality diets at 30° C despite metabolic N respiration rates 2.5 x higher than at 25° C.

Discussion

Confirming our initial predictions, we found that the potential strength of N-limitation for *S. exigua* varied with temperature as a result of increased metabolic N turnover. Increased metabolic N cycling at higher temperatures forced *S. exigua* to greatly increase consumption of low-quality diets to alleviate potential N-limitation. Our results therefore support the hypothesis posited by others that increased metabolic N demand at higher temperatures is a primary driver of herbivore feeding behavior (Lemoine et al. 2013). Herbivore feeding behavior exhibited the most severe changes on diets of similar quality to plant tissues, suggesting that climate change may drastically alter plant-herbivore interactions via changes in fundamental physiological processes that control these interactions.

We show that increased metabolic N demands may indeed be responsible for reduced herbivore growth on low-N plants at high temperatures. Warming increased metabolic N turnover in *S. exigua*, confirming our suspicion that warming can drastically alter herbivore N budgets by increasing metabolic N turnover rates (Fig. 3.2). Increased metabolic N demand arises from rapid protein denaturing at high temperatures, such that individuals require considerably more N to repair damage and synthesize new proteins (Hachiya, Terashima & Noguchi 2007). Protein repair and cellular maintenance occupy a larger share of the overall N budget at high temperatures, yielding less excess N available for growth after accounting for basic cellular processes (Somero 2011). Indeed, respiration and metabolic rates were highest for caterpillars reared on low-N diets arising from the greater difficulty of processing diets rich in non-labile carbohydrates (Martin & Van't Hof 1988; Karowe & Martin 1989). However, increased cellular respiration rates do not generally translate into increased metabolic N turnover. Uric acid production by *S. exigua* content was invariant with respect to diet N content at 24° C (Karowe & Martin 1989), similar to the lack of

a relationship between temperature and metabolic N at 25° C reported here (Fig. 3.2).

Interestingly, we demonstrate that rising temperatures completely alter this pattern by increasing the metabolic costs of processing low N foods, as indicated by the negative relationship between diet quality and metabolic N production at 30° C (Fig. 3.2). Given that our low-quality diets mimic plant nutritional quality, our results suggest that climate warming may increase metabolic N demands of insect herbivores by up to 250%.

Though metabolic N demands of *S. exigua* increased substantially at the higher temperatures we used, relative growth increased linearly with increasing diet N at both temperatures (Fig. 3.1). The absence of a strong temperature x diet interaction is surprising. Indeed, temperature x diet interactions on insect growth appear to be ubiquitous (Kingsolver et al. 2006; Lee & Roh 2010; Lemoine et al. 2013; Lemoine, Burkepile & Parker 2014). For example, temperature stimulated growth rates of adult *Popillia japonica* beetles only on high-N plants (Lemoine et al. 2013). Likewise, growth of *Epimecis hortaria* caterpillars increased exponentially with temperature on high-N *Lindera benzoin* and had no relationship with temperature on low-N *Liquidambar styraciflua* (Lemoine, Burkepile & Parker 2014). In both situations, the authors proposed that increased metabolic N demand caused reduced growth on low N plants at high temperatures. That our results contradict these patterns suggest that *S. exigua* in our study were operating differently than insects in these earlier experiments.

In our study, *S. exigua* offset rising N demands by increasing consumption of low-quality diets in order to maintain relatively high growth rates, whereas earlier work reported that many herbivores do not change feeding rates, suffering stunted growth, longer development times, and reduced fitness on low-N plants at higher temperatures (Lemoine et al. 2013; Lemoine, Burkepile & Parker 2014). One possible explanation for the differences between the results of this study and earlier work is that we used highly palatable artificial diets, whereas earlier studies often used natural plant tissue. Natural plant tissues contain secondary compounds that may prevent the compensatory feeding required to offset temperature-induced nitrogen limitation (Cruz-Rivera & Hay 2003). Concomitant increases in atmospheric CO₂ concentrations along with warming will

likely increase concentrations of secondary defenses in plant tissues, which can either limit an herbivore's ability to compensate for low-quality diets or reduce the assimilation efficiency of ingested foods (Williams, Lincoln & Thomas 1994). In either case, the combination of increased metabolic N due to warming and reduced food quality due to increased atmospheric CO₂ will have severe consequences for plant-herbivore interactions.

In conclusion, our study demonstrates that rising temperatures will increase the metabolic N demands of ectothermic herbivores, and we argue that such changes have profound implications for the nature of plant-herbivore interactions in the future. By stimulating metabolic N respiration, warming increases the potential for N-limitation of consumers, who will be forced to either alter their feeding behavior or suffer reduced growth, development, and fitness. When presented with host choices, mobile herbivores like *S. exigua* may concentrate feeding on a relatively select subset of species, particularly those characterized by high-N and rapid growth (Coley, Bryant & Chapin 1985; Merks-Jacques, Despland & Bede 2008). Otherwise, herbivores must either increase feeding rates or suffer stunted growth and developmental rates. Thus, potential N-limitation at higher temperatures will likely be a major determinant of herbivore feeding behavior in the future.

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Table 3.1 – Casein (protein) and cellulose components of each diet, and the corresponding nitrogen concentration in each diet.

Nitrogen %	Grams casein (% of diet)	Grams cellulose (% of diet)
1.8%	0.00 (0%)	12.5 (27.4%)
2.8%	2.48 (5.4%)	10 (21.9%)
4.8%	7.5 (16.4%)	5 (10.9%)

Figure 3.1 – Relative growth rate of *S. exigua* on each diet at both temperatures. Points represent the mean response in each group, bars denote ± 1 S.E. The regression line gives median posterior prediction and the shaded area represents the 95% CI of the regression line.

Figure 3.2 – Metabolic N concentrations in frass of *S. exigua* on each diet at both temperatures. Points represent the mean response in each group, bars denote ± 1 S.E. The regression line gives median posterior prediction and the shaded area represents the 95% CI of the regression line.

Figure 3.3 – Total frass production of *S. exigua* on each diet at both temperatures. Points represent the mean response in each group, bars denote ± 1 S.E. The regression line gives median posterior prediction and the shaded area represents the 95% CI of the regression line.

CHAPTER IV
INCREASED TEMPERATURE ALTERS FEEDING BEHAVIOR OF A GENERALIST
HERBIVORE

Abstract

Temperature can regulate a number of important biological processes and species interactions. For example, environmental temperature can alter insect herbivore consumption, growth, and survivorship, suggesting that temperature-driven impacts on herbivory could influence plant community composition or nutrient cycling. However, few studies to date have examined whether rising temperature influences herbivore preference and performance among multiple plant species, which often dictates their impact at the community level. Here, we assessed the effects of temperature on the performance and preference of the generalist herbivore *Popillia japonica* among nine plant species. We show that, on average, consumption rates and herbivore performance increased at higher temperatures. However, there was considerable variation among plant species with consumption and performance increasing on some plant species at higher temperatures but decreasing on others. Plant nutritional quality appeared to influence these patterns as beetles increased feeding on high-nitrogen plants with increasing temperature, suggesting stronger nitrogen limitation. In addition to changes in feeding rates, feeding preferences of *P. japonica* shifted among temperatures, a pattern that was largely explained by differential deterrence of plant chemical extracts at different temperatures. In fact, temperature-induced changes in the efficacy of plant chemical extracts led *P. japonica* to reduce its diet breadth at higher temperatures. Our results indicate that rising temperatures will influence herbivore feeding behavior by altering the importance of plant nutritional and chemical traits, suggesting that climate change will alter the strength and sign of plant-insect interactions.

Introduction

Environmental temperature regulates a number of critical ecological processes through its effects on the physiology of ectothermic organisms (Melillo et al. 2002). Temperature-driven shifts in metabolic demand can alter vital biological rates, including consumption and growth, of many organisms (Lemoine and Burkepile 2012). The effects of temperature on ectothermic metabolism can therefore potentially propagate throughout the entire biological hierarchy (Allen

2005). For example, temperature influences key ecological interactions, such as herbivory (O'Connor 2009), predation (Rall et al. 2010), or competition (Tilman 1981). By influencing such important interactions, environmental temperature can indirectly control food web structure (Chase 1996; Kratina et al. 2012) community composition (de Valpine and Harte 2001), and nutrient cycling (Melillo et al. 2002). However, the role of temperature in regulating herbivore feeding preferences, and therefore how top-down control is allocated among plant species, remains relatively understudied.

Insect herbivores are the most diverse group of metazoa on earth and can have large impacts on plant community composition (Carson and Root 1999, Maron and Crone 2006) and nutrient cycling (Weisser and Siemann 2004) by differentially consuming plant species. Insects are also ectotherms whose metabolic rates increase exponentially with temperature (Rall et al. 2010). Temperature can strongly affect insect consumption, growth, and developmental rates by changing basic metabolic processes (Kingsolver and Woods 1997, Briscoe et al. 2012). Further, previous studies suggest that temperature-driven increases in metabolic rates can induce stronger nutrient limitation (Elser et al. 2003), which may result in different food preference hierarchies at different temperatures depending on nutritional quality.

Herbivore performance, for example, is often limited by plant nutritional content, such that herbivores preferentially consume plants rich in limiting nutrients to satisfy nutritional demands (Sternner and Elser 2002). Higher temperatures, however, can increase the rate of protein denaturing, thereby necessitating higher rates of production and repair of nitrogen-rich proteins (Angilletta 2009). The synthesis of heat-shock proteins also occurs at temperatures well below an organism's upper thermal limit (Gehring and Wehner 1995). Additionally, high temperatures lead to increased protein turnover rates and significant respiratory and nitrogen-utilization costs (Hoffman and Somero 1995, Hachiya et al. 2007).. Thus, increasing temperatures might force herbivores to increase their intake of nitrogen-rich foods to provide sufficient materials to repair damaged proteins and synthesize new proteins (e.g. heat-shock proteins). Similarly, rising temperatures can also increase ectotherm growth rates (Gillooly et al. 2001), potentially

escalating the demand for phosphorus-rich materials like RNA and ATP, both of which are required for cellular growth (Elser et al. 2003, Wojewodzic et al. 2011). Thus, at higher temperatures herbivores may also shift feeding preferences towards phosphorus-rich plants. In addition to these nutrient-driven changes, temperature might also influence plant-insect interactions that are mediated by plant chemical defenses. For example, herbivores can become more or less sensitive to chemical defense compounds at higher temperatures (Stamp and Yang 1996, Stamp and Osier 1998), depending on the herbivore and plant species. Yet, the influence of both plant nutritional quality and chemical defenses on herbivore preferences among multiple plant species remains untested.

Here, we used the herbivorous beetle *Popillia japonica* to determine how temperature affects herbivore performance and preference among nine plant species. *P. japonica*, a known agricultural pest, is considered invasive in North America, and maintains a broad diet of > 300 plant species from 79 families (Potter and Held 2002). Therefore, *P. japonica* served as a model organism to ask three specific questions: 1) how does temperature affect insect herbivore performance among host plants?; 2) do herbivore feeding preferences vary with temperature?; and, 3) do plant traits (e.g. nutrient content, chemical defenses) explain variation in herbivore preference and performance at different temperatures? To address these questions, we used no-choice and choice feeding assays with nine species of plants at four temperatures (20°, 25°, 30°, and 35° C) to determine how *P. japonica* performance and preferences varied among host species. We coupled herbivore performance data with plant nutritional characteristics to assess how plant traits affected herbivore performance across temperatures. Further, we performed feeding assays incorporating chemical extracts of host plants into artificial diets to assess whether the effectiveness of chemical defenses varied with temperature. We expected that at high temperatures, nitrogen and phosphorus limitation would increase in strength, thereby increasing the preference of *P. japonica* for plants rich in these limiting nutrients, while strengthening the effects of plant chemical defenses.

Methods

All experiments were conducted at the Smithsonian Environmental Research Center (SERC), Edgewater, MD, USA from June – August 2012. Adult *P. japonica* (0.102 ± 0.018 g, mean $\pm$ s.d.) were collected by hand from various host plants in forest and edge habitats, and all individuals were used in experimental trials within two days of capture. While in captivity, individuals were held at room temperature and fed fresh *Platanus occidentalis* leaves daily. Using laboratory feeding assays, we evaluated the performance and preference of *P. japonica* on nine abundant plant species known to host *P. japonica*: *Acer negundo*, *Acer rubrum*, *Liquidambar styraciflua*, *Platanus occidentalis*, *Rosa multiflora*, *Rubus allegheniensis*, *Rubus phoenicolasius*, *Viburnum prunifolium*, and *Vitis vulpina* (see Fig. D1 for phylogenetic tree). Most of the plant species are native to the United States, except for *R. multiflora* and *R. phoenicolasius* which are introduced plant species with the same native range as *P. japonica* and are confamilial and congeneric, respectively, with North American *R. allegheniensis*. In particular, *P. japonica* strongly prefers plants from the genus *Rosa* as host plants (Held and Potter 2004). Thus, *R. multiflora* represents a preferred host plant from the native range of *P. japonica*.

Feeding Assays

We assessed the performance and feeding preferences of *P. japonica* on each of the nine plant species using both no-choice and choice feeding assays. To standardize leaf quality among replicate assays, fresh, mature leaves were collected from 2 – 3 neighboring individuals of similar size for each plant species. Thus, all plants from which leaves were harvested within a species were from identical microhabitats and leaves were of similar ages.

In no-choice assays, a single *P. japonica* individual was weighed and placed in a plastic rearing cup with a single, pre-weighed leaf from one of the nine plant species. Each rearing cup was randomly assigned to one of four temperatures (20°, 25°, 30°, and 35° C, see Table E1 for temperature and light data) maintained by growth chambers on a 14:10 light:dark cycle ($n = 8$ per plant species per temperature). Temperatures were selected to represent average temperatures

from late spring 20° C (average high temperature in April) to mid-summer months 32° C (average high temperature in July) in the study range, along with a 35° C treatment that represents a conservative 3° C increase beyond the typical peak temperature due to predicted climate change (IPCC 2007). During the month of June, when beetles were collected, daily temperatures in the area averaged $24.25 \pm 3.43^\circ \text{C}$ (NOAA Station APAM2). Thus, 20 – 30° C falls within two standard deviations of daily temperatures encountered prior to initiating our experiments. Further, *P. japonica* experience normal development and growth between 15 – 37° C (Ludwig 1928). Thus, our temperature regimes are within the thermal tolerance range of *P. japonica*. Similar experimental designs have been used to evaluate herbivore thermal response curves (Kingsolver and Woods 1997, 1998). Temperatures were held constant, as alternating day:night temperatures has no effect on the growth or development of *P. japonica* as long as the temperatures are within the thermal window of 15 – 35° C (Ludwig 1928). No individual *P. japonica* was used for more than one feeding assay.

Leaf petioles were placed in water-filled microcentrifuge tubes capped with cotton to prevent desiccation, and we observed no obvious differences in leaf turgor during the assays. After 24 hours, beetles and leaves were reweighed to estimate consumption rates and changes in herbivore mass. Feeding assays of this duration have been used to assess herbivore performance and dietary preferences in lepidopterans (Kingsolver and Woods 1997, Kingsolver and Woods 1998) and coleopterans (Gange et al. 2012, Kosonen et al. 2012). Furthermore, results from 4-6 h assays testing *P. japonica* feeding preferences among plant species generally mimic survival patterns on each species after 21 days (Held and Potter 2004), and body mass of adult coleopterans is positively correlated with reproductive fitness (e.g. number of eggs laid, Vamosi 2005). Thus, mass change may represent one possible metric of adult *P. japonica* fitness, although longer-term studies with direct counts of eggs produced would better capture the relationship between feeding behavior and adult fitness.

Control assays with no herbivores accounted for autogenic change in leaf weight over the 24 h period ($n = 5$ per plant species per temperature). Leaves of all plant species except *L.*

styraciflua gained mass over 24 h in the absence of herbivores. Larger leaves gained more mass than did smaller leaves except in *R. phoenicolasius*, where mass gain was constant across all leaf sizes. We therefore used species-specific equations to correct for autogenic change based on leaf mass (Table E2). Mass-specific autogenic changes, whether positive or negative, were added to consumption rates. Negative autogenic changes (*i.e.* plants lost mass in control assays) would therefore lower estimates of consumption and vice versa.

To gauge how herbivore feeding preferences varied with temperature among multiple host plants offered simultaneously, we conducted choice-feeding assays with 10 adult *P. japonica* placed in clear, 1.6 L plastic containers with a single leaf from all nine species simultaneously. Leaf petioles were placed in water-filled microcentrifuge tubes as described above. After 24 h, leaves were reweighed to estimate daily consumption rates. We replicated choice assays at 25° and 35° C ($n = 8$ per temperature) because these temperatures represent the average high temperatures at the study site in May, when adults first reach high abundances, and 3° C above the average high temperature in July. Consumption rates were corrected for autogenic changes in plant mass as described above.

Foliage used in this study was collected from plants under natural field conditions and not from plants grown at each temperature. Theoretically, rising temperatures could increase plant growth rates (Veteli et al. 2002) and alter nutritional content or concentrations of defensive compounds as plants shuttle more resources into growth (Coley et al. 1985). However, previous studies have found relatively small effects of temperature on plant phytochemistry, particularly % water, % nitrogen, and concentrations of tannins, phenols, and other chemical defenses (Veteli et al 2002, Richardson et al 2002, Williams et al. 2003, Zvereva and Kozlov 2006). This holds true for some species used in our study (Williams et al. 2000, Williams et al. 2003). Thus, growing the different plant species to use in feeding assays at the different temperatures described above likely would have had minimal effects on the patterns in our results.

Plant Traits

To assess the mechanism by which temperature affects herbivore performance among plant species, we quantified nutritional characteristics of undamaged leaves ($n = 3-5$) of each plant species. Leaf toughness was measured using a force gauge (BFG 1000N; Mecmesin, Sussex, UK). Prior to all nutrient content analyses, leaves were weighed, dried to a constant weight at 60° C, and re-weighed to estimate water content. Dried leaf material was ground to a fine powder for carbon (C), nitrogen (N), phosphorus (P), and protein analyses. Percent C and N were estimated using an EAI CE-440 elemental analyzer (Exeter Analytics, Coventry, UK). Phosphorus content was determined using dry oxidation-acid hydrolysis extraction followed by colorimetric analysis on a microplate spectrophotometer (PowerWave XS; Biotek, Winooski, VT). Protein content was measured using a modified Lowry kit optimized for a microplate spectrophotometer (Pierce, Rockford, IL).

Total Crude Extracts of Plant Chemical Defenses

In order to examine how plant defensive chemistry affected feeding by *P. japonica* and whether the effectiveness of plant defenses varied among temperatures, we conducted feeding assays with total crude extracts from plant leaves of each species used in the feeding assays described above. All chemical extract methods and feeding assays follow Lind and Parker (2010). Fresh leaves of each plant species (2 g equivalent dry weight) were ground in a coffee grinder and then extracted using both lipophilic and hydrophilic solvents (2:1 v/v dichloromethane (DCM): methanol (MeOH), 1:1 v/v DCM:MeOH, 2:1 v/v MeOH:H₂O) added to freshly chopped leaves to extract secondary chemical compounds. Extraction times were 2 hours, 2 hours, and 12 hours for each step, respectively. All plant species were subject to identical extraction times. Extracts were condensed using a vacuum centrifuge (Savant SPD131 SpeedVac, Thermo Scientific, Waltham, MA). These methods have successfully demonstrated that plant secondary metabolites affect herbivore feeding behavior (Stachowicz and Hay 1996; Cruz-Rivera and Hay 2003; Lind and Parker 2010).

Extracts were resuspended in 5 mL acetone and added to an artificial diet mixture of 1 g wheat germ, 1 g cellulose, and 0.025 g FABCO-1 antifungal agent. Thus, extracts from 2 g of dried leaves were added to 2 g of artificial diet preserving the natural ratio of secondary chemicals to food mass. 20 mL of boiling water were added to the diet and the mixture stirred until all acetone had evaporated. Agar powder (0.75 g) was added to the mixture to act as a solidifying agent and the mixture immediately poured into a 1.5 cm wide mold. Control foods lacking chemical extracts were prepared in an identical fashion using acetone without chemical extracts. Green food coloring was added to the control diets to mimic the color of the chemically treated foods (Pleau et al. 2002).

For each plant species, 1.5 x 1 cm strips of control and chemically treated agar foods were weighed to the nearest milligram and placed on opposite sides of an insect rearing cup (350 mL). A single, weighed adult *P. japonica* individual was then placed in the center of each cup. After 24 h, the agar strips were reweighed and mass-corrected consumption calculated as $(\text{Mass}_{\text{final}} - \text{Mass}_{\text{initial}}) / \text{Mass}_{\text{herbivore}}$. We replicated these chemical extract bioassays at 25° and 35° C ($n = 8$ per plant species per temperature).

Statistical Analyses

Consumption and herbivore mass change in no-choice assays were analyzed using a factorial ANOVA with plant species and temperature as fixed effects. The assumption of homogeneous variances could not be met, thus we used a weighted model where each observation was weighted by the inverse of the variance within its corresponding plant species x temperature treatment, allowing us to keep the data on the original, untransformed scale (Zuur et al. 2009). Plots of residuals versus fitted values showed that weighting observations adequately resolved issues with heterogeneous variances. In the presence of a significant interaction, *post-hoc* comparisons were made using pairwise *t*-tests with non-pooled variances. Since the main purpose of this study was to examine relative herbivore performance among plant species at different temperatures, we only conducted *post-hoc* comparisons among species within a

temperature. This reduced the number of unplanned comparisons from 630 (all possible comparisons) to 144. As a Bonferroni adjustment on 144 comparisons results in an extreme correction to the critical p -value ($\alpha = 0.0003$) and high likelihood of Type II errors, we used the False Discovery Rate (FDR) correction with 144 unplanned comparisons to assess the significance of *post-hoc* pairwise t -tests (García 2004). Choice assays were analyzed using MANOVA, with percent loss of each species as response variables and temperature as the predictor. Use of a MANOVA accounts for non-independence of the response variables in choice assays (Roa 1992). Pairwise t -tests with FDR corrections were used for *post-hoc* comparisons of species within each temperature as in the no-choice assays.

We were also interested in examining whether rising temperatures would increase the variance in consumption rates, herbivore mass changes, or feeding preferences among plant species. The assumption was that temperature stress would cause feeding to shift towards relatively fewer, more nutritious species. We thus used one-tailed F -tests to determine whether variance among mean consumption and relative growth rates was higher at 35° than at 25° C. To assess higher variance in the choice trials where all food options were offered simultaneously, we calculated 1-Simpson's diversity index (D) of consumption for each choice assay as $D = \sum p_i^2$, where p_i is the proportional consumption of each plant species, and 0 represents low diet diversity and 1 represents high diet diversity. We used a t -test with unequal variances to compare diversity indices between temperatures to determine whether temperature affected the diet breadth of *P. japonica*.

As some plant traits were highly correlated (% water:% protein $r = -0.75$, % water:%P $r = 0.59$), principle components analysis (PCA) was used to reduce the dimensionality of the plant trait matrix and account for potential collinearity among plant traits. Mean consumption and mass changes of *P. japonica* for each plant-temperature combination were used as the response variable in multiple regressions against the principle components. Each regression model included main effects for temperature, each principle component, and interaction effects for each principle component and temperature. Thus, we used two models, one for consumption and one

for mass changes, to assess if the effect of plant traits varied across temperatures. No traits exhibited a significant phylogenetic signal (Table E3), suggesting that phylogenetically controlled contrasts were unnecessary.

Chemical extract bioassays were analyzed by calculating the difference in consumption between chemically treated food and control food as the response variable. A positive value indicates preference for chemically-treated food, while a negative value indicates avoidance of chemically treated food. A linear model with no intercept term was used to assess whether feeding preferences varied among plant species. Removal of the intercept allowed us to include a dummy variable for each plant species. Thus, *t*-tests of each coefficient inherent in the regression determined whether the mean response for each species differed from zero. Two separate linear models were run for 25° and 35° C. Variances were heterogeneous among species, therefore we used a weighted model where each observation was weighted by the variance of its corresponding species (Zuur et al. 2009). We used a one-tailed *F*-test to determine whether the variance in mean effects of chemical extracts increased with temperature, as described above.

All statistical analyses were conducted in R v2.15 (R Development Core Team 2012). Weighted regressions were run using the '*nlme*' package (Pinheiro et al. 2012).

Results

Feeding Assays

In no-choice assays, average consumption rates increased with temperature ($F_{3,248} = 41.66$, $p < 0.001$), but this overall trend masked considerable variability in consumption rates of each plant species across temperatures (plant species x temperature interaction, $F_{24,248} = 4.18$, $p < 0.001$, Fig. 4.1A, see Table E4 for *post-hoc* results). As temperature increased, the rank order of consumption of each plant species changed. At 20° C, *P. japonica* consumed more *R. multiflora* and *V. vulpina* than all other species except *P. occidentalis*. At 25° C, *P. japonica* consumed more *R. multiflora* than any other plant. Moreover, the plant species consumed least by *P. japonica* changed with temperature, with *R. phoenicolasius* going from one of the least

consumed species at 25° C to being consumed at average rates at 35° C. Additionally, the variance of mean consumption rates of each plant species in no-choice assays was over 4x higher at 35° than at 20° ($F_{8,8} = 4.029$, $p = 0.033$, Fig. D2), indicating that rising temperatures increased the discrepancy between the greatest and least consumed plants.

Similar to consumption rates, *P. japonica* gained more mass at higher temperatures than at lower temperatures ($F_{3,248} = 81.77$, $p < 0.001$). However, the effect of temperature on *P. japonica* mass gains was contingent on plant species identity (plant x temperature interaction, $F_{24,248} = 7.59$, $p < 0.001$, Fig. 4.1B, see Table E4 for *post-hoc* results), although individuals gained the most mass on *R. multiflora* at all temperatures. At 20° C, *P. japonica* mass changes were uniformly low on all other plant species. But as temperature increased, *P. occidentalis*, *V. vulpina*, and *Rubus spp.*, became better host plants than others, chiefly *V. prunifolium* and *L. styraciflua*. Moreover, while there was little variation in *P. japonica* mass changes at low temperatures, variance in mass changes among plant species increased rapidly with rising temperatures (Fig. D3) and was over 3x higher at 35° than 20° C ($F_{8,8} = 3.36$, $p = 0.053$). Excluding the uniformly high growth rates on their native host-plant *R. multiflora* strengthened this pattern considerably, as variance in *P. japonica* mass changes among plant species was over 21x higher at 35° than 20° ($F_{8,8} = 21.38$, $p < 0.001$). Thus, the relative suitability of plants as host species changed dramatically as temperature increased, with a larger difference between hosts at higher temperatures.

In choice assays, differences in *P. japonica* feeding preferences varied with temperature (Wilk's $\Lambda = 0.153$, $\chi^2 = 17.82$, $p = 0.037$, see Table E5 for *post-hoc* results). At 25° C, *P. japonica* preferentially consumed more *R. multiflora* than all other species (Fig. 4.2). At 35° C, *P. japonica* feeding preferences shifted among plant species, preferentially consuming *R. multiflora* and *V. vulpina* over all other species. However, the increase in consumption of *V. vulpina* was offset by decreased consumption of all other plant species, leading to an overall decrease in consumption rates (Fig. 4.2). Accordingly, *P. japonica* had a significantly less diverse diet at 35° C ($1-D = 0.760$

± 0.031) than at 25° C ($1-D = 0.834 \pm 0.004$, $t = 2.96$, $p = 0.021$), indicating a reduction in dietary breadth at higher temperature for the species tested here.

Plant Traits

PCA of plant traits isolated three principle components with eigenvalues > 1 (Table E6). The first axis represented water content, %P, and % protein in plant leaves. The second axis was predominately a %C and toughness axis. The third axis represented leaf %C and %N. Together, these three axes explained 86% of the variation in nutritional characteristics among plant species.

Consumption rates were not significantly related to any principle component, nor did temperature interact significantly with any principle component. Although there was some indication of an effect of temperature and an interaction between PC 3 and temperature ($p = 0.073$ for both; Table E7), low power ($\beta = 0.321$, Monte Carlo power simulation, Bolker 2008) may have inhibited our ability to detect a statistically significant interaction at $\alpha = 0.05$.

Similarly, there was no effect of any principle component on herbivore mass change. However, disproportionately high growth rates on the native host-plant *R. multiflora* at all temperatures appears to have masked the influence of other plant traits in explaining impacts on *P. japonica* growth. Excluding *R. multiflora*, changes in *P. japonica* mass varied with PC 3, although a significant interaction with temperature indicated that the effect varied with temperature ($p = 0.003$, $R^2 = 0.30$, Fig. 4.3, Table E7). At 20° and 25° C, there was no relationship between *P. japonica* mass change and PC 3. However, at 30° and 35° C, there were strong negative relationships between *P. japonica* mass change and PC 3. As negative PC 3 values indicate plants with high %N and %C, this suggests that *P. japonica* performed best on plants with high %N and %C and performed poorly on plants with low %N and %C at high temperatures. In contrast, the absence of any PC 3 effect at low temperatures suggests that the measured components of plant nutritional quality were unimportant to *P. japonica* performance at low temperatures. Repeating these analyses excluding both *R. multiflora* and *R. phoenicolasius*,

leaving only native plant species that share no evolutionary history with *P. japonica*, led to qualitatively similar results.

Chemical Extracts

At 25° C, there was no overall effect of plant crude chemical extracts on *P. japonica* feeding (Table E8, Fig. 4.4), and only extracts from *A. negundo* were deterrent. At 35° C there was still no overall effect of plant crude chemical extracts, although three plant species exhibited shifts in plant chemical deterrence. Extracts of *R. multiflora* and *V. vulpina* became significantly stimulatory, whereas extracts from *R. phoenicolasius* became significantly deterrent (Table E8, Fig. 4.4). Moreover, variance in the effects of chemical extracts among species increased substantially with temperature and was nearly 17x higher at 35° C than 25° C ($F_{9,9} = 16.973$, $p < 0.001$).

Discussion

Metabolism of ectothermic organisms increases exponentially with temperature (Gillooly et al. 2001, Clarke 2004), suggesting that temperature can greatly alter individual nutritional requirements for either nitrogen or phosphorus by stimulating growth rates or protein synthesis and repair (Elser et al. 2003, Angilletta 2009, Wojewodzic et al. 2011). Here we demonstrate that higher temperatures increased variation in *P. japonica* consumption and growth rates among multiple plant species and that these changes were correlated with plant nutritional content. At low temperatures, herbivores performed equally well on all plant species. However, at higher temperatures, herbivores performed better on plant species with high nitrogen content compared to species with low nitrogen content. Furthermore, higher temperatures lead to changes in *P. japonica* feeding preferences and caused a reduction in diet breadth. Such changes were correlated with changes in the efficacy of plant chemical extracts at higher temperatures. Earlier studies addressing the interaction between diet quality and temperature on generalist herbivore performance have largely used artificial diets (Stamp and Yang 1996, Stamp and Osier 1998,

Kingsolver and Woods 1998, Kingsolver et al. 2006) or measured variation in nutritional quality within a single plant species (Himanen et al. 2008). To our knowledge, this is the first study demonstrating that temperature-driven changes in herbivore feeding preferences and performance correlate with natural variation in nutritional quality and secondary chemical composition across a range of plant species.

Averaged across all plant species, consumption rates of *P. japonica* increased with temperature while growth remained relatively constant (Fig. 4.1). These patterns are consistent with predictions that ectothermic herbivores must increase food intake at higher temperatures to offset increased metabolic or nutritional demands (O'Connor et al. 2011). One possible outcome of increased temperatures is increased repair and synthesis of nitrogen-rich proteins, which can limit growth rates (Somero 2011). Other generalist, aquatic insects show similar rapid increases in growth rate with increasing temperature only on high nitrogen diets (Gresens 1997). A second possible outcome is that thermal stress increases demand for carbon-rich materials such as carbohydrates or lipids, which are important components of insect diets (Raubenheimer and Simpson 2003, Thaler et al. 2012). Grasshoppers exposed to predation risk, for example, increase their demand for carbohydrate-rich foods and feed on plants with higher carbon:nitrogen ratios compared to unstressed grasshoppers (Hawlena and Schmitz 2010). Carbohydrates, particularly sugars, are thought to be an important feeding stimulant for *P. japonica* (Held and Potter 2002), and in our study nitrogen and carbon content were positively correlated (Fig. 4.3). Thus, the increased feeding on plants of higher nutritional quality that we observed could have been due to higher nitrogen, higher carbon content, or a combination of both.

To compensate for increased nutritional demands, generalist consumers can either increase uptake of low-quality diets (*i.e.* compensatory feeding) or feed only on high-quality hosts. For example, specialized lepidopteran herbivores often display compensatory consumption of low-quality diets at intermediate temperatures (Williams et al. 1994). In contrast, the generalist coleopteran herbivore *P. japonica* in our study showed no evidence for compensatory feeding. In fact, *P. japonica* exhibited widely differing thermal response curve shapes depending on host

plant species (Fig. 4.1). Interestingly, at low temperatures, beetles did not lose mass even on low quality plants. Accordingly, beetles performed worse on low quality plants at high temperatures than at low temperatures. Given that body size of adult coleopteran herbivores can be positively correlated with reproductive effort (Vamosi 2005), including within *P. japonica* (Saeki et al. 2005), these results suggest that temperature-driven impacts on diet choice may influence *P. japonica* fitness.

To date, the effects of temperature on chemically-mediated plant-insect interactions has received much less attention than the impact of plant nutrient content. Previous work has focused on either subsets of specific chemicals within a plant species (*i.e.* tomato, Stamp and Yang 1996), or the effects of temperature on the expression of chemical defenses within a plant (Zvereva and Kozlov 2006). We know of only one other study that has examined how bulk chemical extracts affect herbivore feeding behavior at multiple temperatures (Sotka et al. 2009). Overall, the effect of temperature on chemical defenses appears to be highly variable. Some chemicals become more or less effective (Stamp and Yang 1996, Stamp and Osier 1998, Sotka et al. 2009). Our data also support the premise that the efficacy of plant chemical defenses at higher temperatures will be highly variable among plant species. *Popillia japonica* did not select for or against extracts from any plant species at 25° C. But at 35° C, *P. japonica* preferred food incorporated with extracts from *R. multiflora* and *V. vulpina*, while avoiding foods incorporated with extracts from *R. phoenicolasius*. Interestingly, these patterns in chemical extract feeding assays matched patterns in herbivore choice assays. For example, at 25° C *P. japonica* displayed little preference among plant species (Fig. 4.2). At 35° C, *P. japonica* strongly preferred both *R. multiflora* and *V. vulpina* (Fig. 4.2) as assays with the chemical extracts would suggest. In our study, this occurs independent of any temperature-mediated changes in plant phytochemistry. These results suggest that temperature can affect herbivore feeding preferences among plant species by altering the efficacy of chemical defenses even without altering plant production of these defenses. The effects of temperature on chemically mediated plant-insect interactions

therefore require significantly more research to understand the interactions between temperature, plants, and herbivores.

Some patterns in the data were only apparent after excluding *R. multiflora* from the analyses. This might reflect the deep evolutionary relationship between *R. multiflora* and *P. japonica*. *Rosa multiflora* is an invasive plant with the same native host range as *P. japonica*, and *P. japonica* prefers *Rosa spp.* as host plants (Held and Potter 2004) in both its native and introduced range. Such an evolutionary relationship, coupled with uniformly high growth rates on *R. multiflora*, suggests that *P. japonica* may have developed a physiology attuned to *R. multiflora* chemical compounds independent of plant nutritional quality (e.g. Verhoeven et al. 2009). For example, some herbivores grow best when presented with chemicals from plants with strong co-evolutionary relationships even when nutritional content is held constant (Bowers and Puttick 1988). Likewise, seed beetles performed best on the host to which they have adapted, regardless of temperature and, most likely, nutritional content (Stillwell et al. 2007). In our study, individuals fed *R. multiflora* grew at least twice as fast as individuals fed any other plant at all temperatures, despite the relatively average nutritional value of *R. multiflora* (2.08 %N, 0.17 %P, 0.13 % protein) compared to the other plant species examined here (2.12 ± 0.36 %N, 0.21 ± 0.03 %P, 0.16 ± 0.09 % protein, mean $\pm$ S.D.). This may be because *P. japonica* has a close evolutionary history with *R. multiflora* that allows *P. japonica* to circumvent defenses of *R. multiflora* or efficiently digest chemical compounds produced by *R. multiflora*. Alternatively, high growth *P. japonica* on *R. multiflora* may be due to unmeasured nutritional compounds, such as carbohydrates, sugars, or sterols. *Rubus phoenicolasius* is also present in the home range of *P. japonica* and may also have a similar evolutionary history. This may explain why chemical compounds of *R. phoenicolasius* were effective deterrents despite its high nutritional quality. Excluding both *R. multiflora* and *R. phoenicolasius* from analyses did not change our results, suggesting that, in the absence of a shared evolutionary history, plant nutritional quality interacts significantly with temperature to alter herbivore growth patterns.

The exclusion of *R. multiflora* from data analysis therefore resulted in a set of plant species that are not preferred host plants from the native range of *P. japonica*, and, accordingly, consumption and growth rates of *P. japonica* on these plant species was solely a product of plant nutritional content and defenses, rather than a co-evolved interaction. The overall trend was that increasing plant nitrogen and carbon content and decreasing plant toughness had little effect on beetle growth at low temperatures but was a strongly correlated with beetle growth at high temperatures (Fig. 4.3).

We focused on examining herbivore response to increasing temperature while holding plant phytochemistry constant, but other studies have focused on examining plant response to rising temperatures. For example, plant growth rates increase with rising temperatures (Veteli et al. 2002), which could alter nutritional content or concentrations of defensive compounds as plants shuttle more resources into growth (Coley et al. 1985). However, studies have shown that the effects of temperature on plant secondary chemistry are highly idiosyncratic (Veteli et al. 2002, Richardson et al. 2002, Zvereva and Kozlov 2006). Temperature does not affect any measure of nutritional quality (e.g. water, nitrogen, tannins, phenols, sugars) of two *Acer* species, including *A. rubrum*, a species used in this study (Williams et al. 2000, 2003). Furthermore, although temperature can alter nutritional quality within a species, variation caused by temperature is substantially lower than inherent variation among plant species (Aerts et al. 2009). However, the effects of rising temperature on plant nutritional quality are likely highly idiosyncratic among species (Aerts et al. 2009), and the effects of temperature on plant chemistry must be considered more completely before applying results such as ours in a climate change context. Yet, our study does suggest that rising temperatures with climate change may reorder herbivore feeding preferences which may ultimately alter top-down forcing on plant communities.

Our short-term feeding assays indicate that herbivore feeding behavior and performance vary with temperature and among plant species within a single generation. However, long-term studies, encompassing a full feeding season or multiple generations, may result in different patterns. For example, it is possible that our results are due to short-term temperature changes

rather than a response to long-term temperature changes. However, the temperatures chosen for this experiment were within the range of temperatures experienced by *P. japonica* in the weeks preceding the experiment. Yet over longer time periods, physiological plasticity may allow individuals to increase nitrogen uptake efficiency or reduce metabolic demands at high temperatures (Terblanche et al. 2007). However, in some ectothermic species, individuals do not exhibit acclimation over long periods (Watts et al. 2011). Whether *P. japonica* successfully acclimates to high temperatures remains to be tested. Alternatively, developmental plasticity may allow future generations to increase performance at high temperatures (Donelson et al. 2011). Long-term physiological acclimation or adaptation that allows herbivores to increase nitrogen uptake efficiency in thermally stressful environments is a promising avenue of research.

There are few data regarding the role of temperature on plant-insect interactions via direct changes in herbivore physiology (Bouzat and Imeh-Nathaniel 2008). Here, we show that rising temperatures lead herbivores to perform better on plants that are higher in nitrogen, possibly due to altered herbivore physiology as a result of increased temperatures. Moreover, temperature altered the effect of plant chemical defenses, resulting in high variance in herbivore preferences among plant species and reduced diet breadth. In addition to driving changes in plant-insect interactions in a changing climate, such temperature-driven redistributions of herbivory might already be impacting contemporary plant community structure by releasing or subjugating competitively dominant plants to intense herbivory as temperature changes on a daily, seasonal, or yearly basis. To our knowledge this possibility has not been considered. Finally, our results suggest that herbivore feeding preferences and host-plant interactions are highly contingent upon environmental temperature, further supporting the notion that the abiotic template of an environment can dictate herbivore feeding behavior, with potential impacts on plant community structure, trophic dynamics, and ultimately ecosystem-level processes.

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Figure 4.1 – A) Mass-corrected consumption and B) mass changes of *P. japonica* on each of the nine plant species at each temperature during the no-choice assays. Overall means at each temperature are displayed adjacent to the species means. Data points represent mean $\pm$ S.E.

Figure 4.2 – Percent mass loss of all nine plant species at each temperature during the choice assays. Data points represent mean $\pm$ S.E.

Figure 4.3 – Relationship between *P. japonica* mass change and PC 3. Negative PC 3 values indicate high %N, high %C of leaf material. *Rosa multiflora* not shown, as it was excluded from regression analyses. Regression lines that did not differ from zero not shown.

Figure 4.4 – Difference in consumption rates between chemically-treated and control artificial diets. Positive values indicate preferential consumption of chemically-treated foods, negative values indicate avoidance of chemically-treated food. Asterisks denote species that differed significantly from 0. Data points represent mean $\pm$ S.E.

CHAPTER V
VARIABLE EFFECTS OF TEMPERATURE ON INSECT HERBIVORY

Abstract

Rising temperatures can influence the top-down control of plant biomass by increasing herbivore metabolic demands. Unfortunately, we know relatively little about the effects of temperature on herbivory rates for most insect herbivores in a given community. Evolutionary history, adaptation to local environments, and dietary factors may lead to variable thermal response curves across different species. Here we characterized the effect of temperature on herbivory rates for 21 herbivore-plant pairs, encompassing 14 herbivore and 12 plant species. We show that overall consumption rates increase with temperature between 20 and 30° C but do not increase further with increasing temperature. However, there is substantial variation in thermal responses among individual herbivore-plant pairs at the highest temperatures. Over one third of the herbivore-plant pairs showed declining consumption rates at high temperatures, while an approximately equal number showed increasing consumption rates. Such variation existed even within herbivore species, as some species exhibited idiosyncratic thermal response curves on different host plants. Thus, rising temperatures, particularly with respect to climate change, may have highly variable effects on plant-herbivore interactions and, ultimately, top-down control of plant biomass.

Introduction

Environmental temperature drives a number of important ecological interactions, including competition, predation, and herbivory, by determining the metabolic rates of ectothermic organisms (Vassuer and McCann 2005, O'Connor et al. 2009, Vucic-Pestic et al. 2011). As metabolic demands increase exponentially with rising temperatures, consumers generally either increase food intake or switch to higher quality diets to offset the rising costs of metabolism (O'Connor 2009, Lemoine et al. 2013). As a result, predation and herbivory rates tend to increase exponentially with increased temperature (Hillebrand et al. 2009, Vucic-Pestic et al. 2011). However, both consumption rates and fitness can decline precipitously once a species encounters temperatures beyond its thermal optimum (Lemoine and Burkepile 2012). A suite of

adaptive and evolutionary factors determine these thermal optima, such that a given consumer community may contain species with considerably different thermal response curves (Angilletta et al. 2004). To date, however, few (if any) studies have examined variation in thermal response curves for a community of co-occurring herbivores (Buckley et al. 2014).

Insect herbivores can be an important top-down force in terrestrial ecosystems, controlling plant biomass (Carson and Root 2000), maintaining species diversity (Bagchi et al. 2014), reorganizing competitive hierarchies (Kim et al. 2013), and enhancing nutrient cycling (Metcalf et al. 2014). Because insects are ectothermic, their physiological rates, including consumption and growth rates, are directly tied to environmental temperature. By extension, top-down control of plant biomass is also likely to be temperature dependent. Theoretical and experimental studies suggest that herbivory rates should increase exponentially with rising temperatures (O'Connor et al. 2011). One potential shortcoming of theoretical examinations of temperature-driven herbivore-plant interactions is that they do not often incorporate variation in thermal response curves within an assemblage of herbivore species. In part, this is because thermal response curves for multiple herbivore species on a single host plant remain mostly uncharacterized. Species are often examined singly, and ecologists have focused on a few readily available model species like *Spodoptera* spp. (Stamp and Yang 1996) and *Manduca sexta* (Kingsolver and Woods 1998). In contrast, the herbivore guild within a community, or even on a single host plant, can vary from 1 – 100s of species, each with different life histories, climatic niches, evolutionary histories, and dietary needs that may drive vastly different thermal response curves (Buckley et al. 2014).

Multiple factors aside from evolutionary history and local adaptation can determine the shape of a species' thermal response curve. For example, plant chemical defenses can become more or less effective at high temperatures depending on the identity of the herbivore, plant, and chemical compounds in question (Stamp and Osier 1998, Stamp et al. 1997). Similarly, different insect species can become more or less nutrient-limited at higher temperatures, which is also contingent on host plant quality (Kingsolver et al. 2006, Kingsolver and Woods 1998, Lemoine et

al. 2013). Thermal response curves therefore likely differ among herbivore species and within a single herbivore species utilizing different hosts. Given the interest in predicting the effects of climate change on trophic interactions and community structure (Singer et al. 2013, Urban et al. 2012), we sought to determine whether temperature influences herbivory in a predictable manner based on a few easily measured variables of plant nutritional quality.

Here, we report thermal response curves of consumption rates for 21 herbivore-plant pairs, encompassing 14 herbivores and 12 plant species (Table 5.1). We asked two specific questions: (1) what is the extent of within- and among-species variation in thermal response curves of consumption for insect herbivores? and (2) can plant nutritional quality explain variation in thermal response curves? By working with multiple species of both herbivores and plants, we demonstrate that thermal response curves vary substantially both among and within herbivore species. However, we were unable to detect any influence of plant nutritional quality on the overall shape of the thermal response curve across taxa, suggesting that thermal response curves are idiosyncratic and highly variable among plant-herbivore pairs.

Methods

All experiments were conducted at the Smithsonian Environmental Research Center (SERC), in Edgewater, MD, USA from June – August 2012. Using laboratory feeding assays, we evaluated the feeding performance of 14 herbivore species from three Orders (Lepidoptera, Coleoptera, Hymenoptera) on 12 plant species (Table 5.1). Herbivores were collected by hand from host plants in the forests and fields on the SERC premises throughout the summer. All herbivores were kept in a cage and fed leaves from the plant species on which they were collected. Individuals were used in feeding assays within 24 h of collection. No individual was used more than once. As herbivores were opportunistically collected, the number of replicates per host plant/temperature combination varied depending on the number of herbivores found (Table F1). Gregarious species (*e.g. Hyphantria cuneata*) have higher replicate numbers than do rare, non-gregarious species (*e.g. Danaus plexippus*). A single lepidopteran species could not be

identified beyond the Tortricidae family. We focused on folivorous individuals, using larvae from lepidopteran and hymenopteran species and adults only from a single coleopteran species (*Chrysomelid auratus*).

Feeding Assays

In no-choice assays, a single individual was weighed and placed in a single rearing cup with a single, pre-weighed leaf from a potential host plant (see Table 5.1). Each rearing cup was randomly assigned to one of four temperatures (20°, 25°, 30°, and 35° C, see Table F2 for temperature and light data from each growth chamber) maintained in growth chambers on a 14:10 light:dark cycle. Temperatures were selected to represent a realistic set of temperatures during the spring in summer months. Data from a nearby NOAA weather station (Annapolis, MD) indicate that temperatures can range from 20 – 35° during the summer months (June – July, Fig. G1). Leaf petioles were placed in water-filled microcentrifuge tubes capped with cotton to prevent desiccation, and we observed no obvious differences in leaf turgor during the assays. After 24 hours, herbivores and leaves were reweighed to estimate consumption rates. Feeding assays of this duration have been used to assess herbivore performance and dietary preferences in lepidopterans (Kingsolver and Woods 1998, Kingsolver and Woods 1997) and coleopterans (Gange et al. 2012, Lemoine et al. 2013). We divided all consumption rates by the initial mass of the individual used in the feeding assay to account for variation in body size among replicates. Consumption rates are reported as grams consumed per gram body mass.

Control assays with no herbivores accounted for autogenic change in leaf weight over the 24 h period ($n = 5$ per plant species per temperature). Leaves of all plant species except *L. styraciflua* gained mass over 24 h in the absence of herbivores. Larger leaves gained more mass than did smaller leaves. We therefore used species-specific equations to correct for autogenic change in leaf mass (Table F3) rather than using mean change in leaf mass across all autogenic controls. Mass-specific autogenic changes, whether positive or negative, were added to leaf final weights. Negative autogenic changes (*i.e.* plants lost mass in control assays) would therefore

lower estimates of consumption and vice versa. In total, we conducted 552 no-choice feeding assays, resulting in 496 observations after removing individuals that died or molted overnight (final replicate numbers for each herbivore/plant/temperature combination given in Table F1).

Plant Traits

To assess the mechanisms by which temperature affected herbivore performance among plant species, we quantified nutritional characteristics of undamaged leaves ($n = 3-5$) of each plant species, all collected from unique individuals. Prior to all nutrient content analyses, leaves were weighed, dried to a constant mass at 60° C, and re-weighed to estimate water content. Dried leaf material was ground to a fine powder for carbon (C), nitrogen (N), and phosphorus (P) analyses. Percent C and N were estimated using an EAI CE-440 elemental analyzer (Exeter Analytics, Coventry, UK). Phosphorus content was determined using dry oxidation-acid hydrolysis extraction followed by colorimetric analysis on a microplate spectrophotometer (PowerWave XS; Biotek, Winooski, VT).

Statistical Analyses

We used a Bayesian hierarchical model to determine thermal response curves of consumption for each herbivore-plant pairing. This allowed us to estimate parameters for the overall trend in consumption with increasing temperature, parameters for each herbivore-plant pairing, and the impact of plant nutritional quality on these parameters. A multilevel model is particularly appropriate for handling unbalanced data and small sample sizes for some herbivore-plant pairings, but some of the predicted responses for less well-sampled taxa will be pulled heavily towards the overall mean response (Gelman and Hill 2007). Although there could be a phylogenetic signal in the patterns of thermal curves of different insect herbivores, we did not have sufficient replication within genera or families to address this question. Most species were in unique families (only three families had more than one species represented) and all but three

species were lepidopterans (Table 5.1). Thus, we did not incorporate the possibility of a phylogenetic signal into our analyses.

Thermal reaction norms of consumption describe the influence of temperature on consumption rates. Regardless of the specific equation used to model a reaction norm, all thermal reaction norms are characterized by a thermal minimum below which consumption is zero, a thermal optimum where consumption rate is maximized and beyond which consumption declines, and a thermal maximum, above which consumption is zero. We modeled the thermal reaction norm of consumption rates for each herbivore-plant pairing using a quadratic exponential (*i.e.* Gaussian) curve because such curves often describe thermal reaction norms (Angilletta 2006):

$$y_{ij} = \exp(a_j + b_j \text{Temp}_{ij} + c_j \text{Temp}_{ij}^2) + \varepsilon_{ij}$$

where y_{ij} is consumption of the j^{th} observation in the j^{th} herbivore-plant pair and ε_{ij} is residual error. We assumed that errors were normally distributed with a constant variance, but the variance was allowed to differ for each curve due to differing numbers of replicates among herbivore-plant pairings. Hereafter, parameters will be referred to as the intercept (a), exponential (b), and Gaussian (c) terms. The intercept a denotes mean consumption rate (since all predictor variables were standardized, see below), the exponential term b denotes the rate at which consumption initially increases with temperature, and the Gaussian term c denotes the extent to which consumption rates level off or decline at high temperatures.

Plant nutritional quality can affect the shape of the thermal response curve by influencing any one of the three parameters that determine the shape of the Gaussian curve. Therefore, each parameter (intercept, exponential, and Gaussian) was modeled as function of nitrogen, phosphorus, and water content of the given plant for each herbivore-plant thermal response curve. For example, the exponential term of the j^{th} curve was a linear function of plant quality:

$$b_j = \mu_b + \gamma_1 \%N_j + \gamma_2 \%P_j + \gamma_3 \%H_2O_j + \delta_j$$

where μ_b is the overall, community-level linear parameter, and γ_1 , γ_2 , and γ_3 represent the influence of nitrogen (%N), phosphorus (%P), and water content (%H₂O) respectively on the

exponential parameter of the j^{th} thermal response curve. δ_j is a multivariate normal error term. Thus, mean consumption rate (a), the rate of increase with temperature (b), and the extent of curvature in the thermal response curve (c) were all modeled as linear functions of plant nutritional content. The random effects for each curve (*i.e* parameters a_j , b_j , c_j) were assumed to come from a multivariate normal distribution, allowing for covariance among parameter estimates.

All predictor variables were standardized prior to analysis to speed chain convergence. For all models, four MCMC chains were run for 5,000 ‘burn-in’ iterations to allow for chain convergence. Posterior distributions of each parameter were simulated by saving the 20th sample from an additional 5,000 posterior simulations, resulting in 1,000 independent estimates (250 per chain, with four chains). Chain convergence and autocorrelation were assessed using trace plots and density plots of posterior simulations. Each parameter was given a mildly uninformative prior normal distribution ($N(0, 1)$); variance parameters were given uninformative prior uniform distributions ($U(0, 100)$). Because predictors were standardized, the magnitude of parameter estimates will be small, such that a standard normal distribution is relatively uninformative. For each parameter, we calculated the 80% and 95% Bayesian credible interval (CI) from the posterior simulations. Parameters whose 95% CI excluded zero were considered highly significant, whereas parameters whose 80% CI excluded zero were considered marginally significant. If the 80% CI included zero, we assumed that the parameter had a low probability of being important. All assumptions of normality and homogenous variances were examined using residual plots. All analyses were conducted using Python v2.7. Bayesian models were evaluated using STAN v2.1 (Stan Development Team 2013), accessed via PySTAN. All code and raw data are available on the corresponding author’s website¹ and will be uploaded to the Dryad database².

¹ <http://www.natelemoine.com>

² <http://datadryad.org/>

Climate Change Simulations

We sought to understand how potential variability in thermal response curves among herbivores interacts with climate change to alter potential top-down control of plant biomass. Thus, we next built a simple model utilizing observed feeding rates and temperatures to estimate cumulative consumption first over one growing season, and then cumulative consumption given two climate change scenarios, +3° and a +5°C increases in temperature, a moderate and severe climate warming scenario, respectively (IPCC 2007). We obtained hourly temperature records for June - August 2013 from the NOAA weather station in Annapolis, MD (Fig. G2). We then used the 1000 posterior draws of observed feeding rates to estimate hourly consumption rates (including parameter uncertainty) for each herbivore-plant combination across the observed temperature range. Most hourly temperature readings were within the 20 – 35° C range used in our experiments (Fig. G2). This yielded 1000 estimates of cumulative consumption for every herbivore-plant pair. We then simulated climate change by adding 3° and 5° to hourly temperature records and repeating the above calculations.

This method makes several important assumptions: 1) thermal effects of climate change can be approximated by adding a constant increase in temperature to all hourly temperature records, 2) that an individual feeds at a constant rate for the entire season with no variation as the instar grows over time, and 3) that a single individual is responsible for feeding, or multiple non-overlapping individuals immediately replace one another upon dying to maintain a constant consumption rate across the growing season. These assumptions will often not hold true so our method of assessing climate change is a relatively coarse picture of how climate change may affect herbivory rates over the course of an entire season.

Results

The exponential parameter (b) of overall consumption rates was significantly greater than zero, indicating that overall consumption rates did increase exponentially with temperature (Figs.

5.1, 5.2). However, the increase was restricted to temperatures between 20° and 30° C (Fig. 5.1). The 95% CI of the Gaussian parameter (c) narrowly included zero, but the bulk of the posterior distribution for this parameter lay below zero, indicating that overall consumption rates began to level off at temperatures above 30° C ($\text{Pr}(<0) = 0.96$, Fig. 5.2). Accordingly, our model predicts relatively little change in overall consumption rates between 30° and 35° C (Fig. 5.1). Variance in consumption rates among herbivore-plant pairs also increased substantially with rising temperature. At 20°, variance among mean herbivore-plant consumption rates was 0.45, while at 35° this variance increased to 1.43. Thus, variability in consumption rates among herbivore-plant pairs increased by over 300%. As a result, at 20° C the predicted mean community-level consumption rates lie between 0.44 – 1.13 g per day (95% CI). Estimates of mean community-level consumption were more uncertain at higher temperatures, lying between 0.93 – 2.13 g per day (95% CI).

Uncertainty regarding overall consumption rates at higher temperatures stems from idiosyncratic thermal response curves among herbivore-plant pairs (Figs. 5.3, 5.4). Six herbivore-plant pairs (*Arge scapularis* – *Ulmus rubra*, *Chrysochus auratus* – *Apocynum cannibinum*, *Hyphantria cunea* – *Acer negundo*, *H. cunea* – *Liquidambar styraciflua*, *Melanophia canadaria* – *Lindera benzoin*, *Papilio troilus* – *S. albidum*) had Gaussian parameters (c) that were moderately or significantly different from zero, indicating decreasing consumption rates at higher temperatures (Figs. 5.3, 5.4). An additional eleven herbivore-plant pairs (*Atteva aurea* – *Ailanthus altissima*, *Danaus plexippus* – *Asclepias syriaca*, *Epimecis hortaria* – *L. benzoin*, *E. hortaria* – *S. albidum*, *Euchaetes egle* – *Asclepias syriaca*, *Malacosoma americanum* – *Prunus serotina*, *Melanophia canadaria* – *Acer negundo*, *Nematus tibialis* – *Robinia pseudoacacia*, *P. troilus* – *L. benzoin*, *Saucrobotys futilalis* – *A. cannibinum*, Unidentified Tortricid – *L. benzoin*) increased consumption with warming throughout the entire temperature range, where the exponential parameter (b) was significantly or moderately different from zero. In some cases the parameter value was small enough that the fit was approximately linear (e.g. *Epimecis hortaria* – *Sassafras albidum*, Figs. 5.3, 5.4). An additional four herbivore-plant combinations (*Danaus plexippus* – *A.*

syriaca, *E. hortaria* – *Liriodenron tulipifera*, *Euchaetes egle* – *Apocynum cannabinum*, *M. canadaria* – *S. albidum*, *Papilio polyxense* – *Foeniculum vulgare*) showed no detectable change in consumption rate with increasing temperature.

Even within herbivore species, thermal response curves varied considerably. For example, *E. hortaria* consumption of *L. benzoin*, and to a lesser extent *S. albidum*, increased exponentially with temperature (Fig. 5.3). However, *E. hortaria* consumption of *L. tulipifera* did not vary significantly over the observed temperatures (Figs. 5.3, 5.4). Likewise, *P. troilus* increased consumption of *L. benzoin* across temperatures, but consumption of *S. albidum* began to decline at 35° (Fig. 5.3). We were not able to detect any effect of plant nutritional content on the shape of thermal response curves among herbivore-plant pairs (Fig. 5.5).

High intra- and interspecific variability among thermal response curves for each herbivore-plant pair led to variable effects of increasing temperatures on potential top-down control of plant biomass (Fig. 5.6). For example, a 3° C increase in temperatures resulted in less than a 20% increase in cumulative consumption for twelve herbivore-plant pairs compared to what is predicted for current temperatures, while leading to a > 30% increase for four herbivore-plant pairs (Fig. 5.6). A 5° C increase in temperature exacerbated this variability, as five herbivore-plant pairs exhibited < 20% increase in cumulative consumption and four herbivore-plant pairs exhibited a > 50% increase (Fig. 5.6). Further, increased warming from 3° to 5° C had highly variable impacts on cumulative consumption rates among herbivore-plant pairs. For example, *Atteva aurea* nearly doubled its estimated consumption of *Ailanthus altissima* as warming increased from 3° to 5° C, whereas estimated consumption by *Melanophia canadaria* was unaffected by temperature increases beyond 3° C (Fig. 5.6). Thus, high intra- and interspecific variability in the herbivore-plant thermal response curves led to high variability in potential climate change effects on top-down control of plant biomass over an entire growing season.

Discussion

Temperature influences herbivory rates via direct effects on insect herbivore physiology. However, it is currently unclear how temperature affects top-down control of plant biomass at the community or species level. Our data suggest that the influence of rising temperature on potential top-down control of plant biomass via herbivory depends upon the identity of the herbivore-plant pair under consideration. Such variability in the relationship between consumption rate and temperature will make it difficult to predict the effects of temperature changes, *i.e.* climate change, on top-down control of plant biomass.

Theory predicts that herbivory rates should increase exponentially with rising temperature more quickly than primary production, reducing standing plant biomass at higher temperatures (Gillooly et al. 2001, O'Connor et al. 2009, O'Connor et al. 2011). However, meta-analyses of thermal response curves report substantial variability among species. Indeed, approximately 40% of the thermal response curves examined by Dell et al. (2011) exhibited curvature, wherein the thermal response curve began to decrease at high temperatures. In our study, 33% of the herbivore-plant pairs exhibited substantial curvature, reducing consumption of plant biomass at high temperatures, thereby contradicting theoretical predictions of exponential increases in top-down control of plant biomass at high temperatures. Often, reduced consumption rates at high temperatures result from metabolic demand exceeding energetic supply, such that energy available for tasks beyond cellular maintenance, such as movement, feeding, or digestion, decreases sharply at high temperature (Somero, 2011). This results in rapid decreases in consumer fitness at temperatures beyond an organism's thermal optimum (Lemoine and Burkepile 2012). We show that community-level herbivory rates display the same, albeit much less pronounced, curvature as do some individual species. The slow decline at higher temperatures, rather than a rapid drop-off beyond some threshold value, is a result of species-specific variation in thermal response curves. Almost half (43%) of plant-herbivore pairings did not show signs of decreased consumption at higher temperature, while one showed evidence of

declining consumption beyond 30° C, leading to no net change in overall consumption rates at higher temperatures.

Such variation in thermal response curves makes predicting the effects of temperature changes (*i.e.* microhabitat variation, seasonal effects, climate change) on herbivore-plant interactions challenging in the absence of species-specific information. Indeed, a generalist herbivore may have as many thermal response curves as host plants (Lemoine et al. 2013). We report similar patterns here. For example, *Epimecis hortaria*, the tulip tree beauty moth, rapidly increased consumption of both *Lindera benzoin* and *Sassafras albidum* with warming, but the increased consumption of *S. albidum* was much slower. In contrast, *E. hortaria* showed no relationship between consumption of *Liriodendron tulipifera* and temperature. Similarly, *Papilio troilus* increased consumption of both *L. benzoin* and *S. albidum* with increasing temperature, but consumption of *S. albidum* began to decrease at 35° C, and consumption of *L. benzoin* showed no curvature.

Given the high variation in thermal response curves among herbivore-plant combinations, predicting the effects of climate change on the top-down control of plant biomass remains challenging. Some studies have ascribed a single thermal response curve to herbivore species, demonstrating that plant biomass will decrease in a warming world as herbivory rates outpace primary production (O'Connor et al. 2011). Our results suggest that using a single consumption-temperature relationship for all herbivores can substantially overestimate the impact of climate change on plant biomass. For example, between 20° and 30° C, both *Chrysochus auratus* and *Saucrobotys futilalis* increased consumption of *Apocynum cannabinum*. However, at 35° C. *auratus* decreased consumption while *S. futilalis* continued to increase consumption, resulting in little change in overall consumption rates on *A. cannabinum* beyond 30° C.

When we integrated these changes in consumption over a full growing season, we showed that top-down control on plant biomass is likely to increase with increasing temperature but the magnitude of the increase depends on the herbivore-plant combination. Over the course of a summer, simulated warming resulted in > 20% increase in cumulative consumption for 10

herbivore-plant pairs, just under half of the pairings examined. Conversely, simulated climate change resulted in > 40% increase in cumulative consumption for five herbivore-plant pairs. Overall, the change in consumption ranged from no change to an increase of over 60%. This variability in consumption may explain why studies documenting significant effects of warming on top-down control of plant biomass typically examine one herbivore species (Chase 1996, Barton et al. 2009) while studies focusing on entire herbivore communities report weak or negligible effects of warming (Richardson et al. 2002).

Surprisingly, we were unable to detect any influence of plant nutritional quality on the shape of thermal response curves. Based on previous work (Lemoine et al. 2013), we expected consumption rate to increase more rapidly on plants of higher nutritional quality. Conversely, compensatory feeding predicts that consumption rates should increase more rapidly with temperature for plants of low nutritional quality as herbivores attempt to fuel rising metabolic demands (e.g. Williams et al. 1994). Our data suggest that plant nutritional content had little effect on thermal response curves among herbivore species. However, prior work has found that the relationship between temperature and consumption rates within a given species can vary with dietary quality. For example, the Japanese beetle *Popillia japonica* increased growth and consumption rates at high temperatures only on host plants with high nitrogen and carbon concentrations (Lemoine et al. 2013). This may also be the case in our data. Within an herbivore species, we can distinguish some patterns related to plant quality. For example, the generalist herbivore *Epimecis hortaria* increased consumption rapidly with warming only on higher nitrogen plant species. Within a plant species, however, patterns were less clear, as particular herbivore species were equally likely to have unimodal or exponential curves when feeding on the same plant. Thus, across all 21 plant-herbivore pairings, we were unable to detect an overall pattern relating plant quality to multiple thermal reaction norms. Thus, dietary quality may be more important for determining thermal response curves within herbivore species but cannot predict the shape of consumption thermal response curves among herbivore and plant species.

We focused on examining herbivore response to increasing temperature while holding plant phytochemistry constant, but rising temperatures might also affect plant phytochemistry. Plant growth rates increase with rising temperatures (Veteli et al. 2002), which could alter nutritional content or concentrations of defensive compounds as plants shuttle more resources into growth (Coley et al. 1985). However, studies have shown that the effects of temperature on plant secondary chemistry are highly idiosyncratic among species (Veteli et al. 2002, Zvereva and Kozlov 2006). Furthermore, although variable temperature can alter nutritional quality within a species, variation caused by temperature is substantially lower than inherent variation among plant species (Aerts et al. 2009). However, the effects of rising temperature on plant chemistry must be considered more completely before applying results such as ours in a climate change context.

One potential caveat of our study is small sample size at many herbivore-plant-temperature combinations. Given that we used field-collected organisms, sample size varied considerably depending on the rarity of the species. Common and/or gregarious species, like *E. hortaria*, *M. canadaria*, and *H. cunea*, have much higher sample sizes than rare or cryptic species, like *D. plexippus* and *A. scapularis*. Thus, some herbivore-plant pairs show considerable variability in the estimated thermal response curve and, in some cases, the prediction was heavily influenced by the overall response. However, most work regarding the influence of temperature on herbivory and its interaction with diet quality focus on a few readily available lepidopteran herbivores (Kingsolver and Woods 1998, Kingsolver et al. 2006). The influence of temperature on herbivory by the majority of the species reported here was heretofore unknown, and our research adds considerably to the body of work documenting the importance of temperature on rates of herbivory.

In summary, we show that herbivory, and therefore potential top-down control of plant biomass, is highly contingent upon environmental temperature. While theoretical predictions suggest that climate change might increase top-down control of plant biomass, our results indicate that the effects of temperature on herbivory rates are highly variable. A single plant

species might experience more or less herbivory at higher temperatures, depending on the identity of the herbivores present. Insects often control plant community structure (Carson and Root 2000) and dominance hierarchies among plant species (Kim et al. 2013). Thus, studies documenting the species-specific effects of temperature on insect herbivory levels will be crucial to understanding how climate change might affect community composition in the plant-herbivore assemblages of the future.

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Table 5.1 – Herbivore–plant pairings used in feeding assays. Species marked with (I) are introduced species; common names are given in parentheses. Below each species name, we have listed the order and family of each species.

Herbivore Species	Herbivore Diet	Plant Species
Unidentified tortricid (Lepidoptera, tortricidae)	Specialist	<i>Lindera benzoin</i> (northern spicebush)
<i>Arge scapularis</i> (elm argid sawfly) (Hymenoptera, argidae)	Specialist	<i>Ulmus rubra</i> (slippery elm)
<i>Atteva aurea</i> (ailanthus webworm) (Lepidoptera, yponomeutidae)	Specialist	<i>Ailanthus altissima</i> (tree-of-heaven) (I)
<i>Chrysochus auratus</i> (dogbane beetle) (Coleoptera, chrysomelidae)	Specialist	<i>Apocynum cannabinum</i> (dogbane)
<i>Danaus plexippus</i> (monarch butterfly) (Lepidoptera, nymphalidae)	Specialist	<i>Asclepias syriaca</i> (common milkweed)
<i>Epimecis hortaria</i> (tulip tree beauty) (Lepidoptera, geometridae)	Generalist	<i>Lindera benzoin</i> (northern spicebush) <i>Liriodendron tulipifera</i> (tulip poplar) <i>Sassafras albidum</i> (sassafras)
<i>Euchaetes egle</i> (milkweed tussock moth) (Lepidoptera, arctiidae)	Specialist	<i>Apocynum cannabinum</i> (dogbane) <i>Asclepias syriaca</i> (common milkweed)
<i>Hyphantria cunea</i> (fall webworm) (Lepidoptera, arctiidae)	Generalist	<i>Acer negundo</i> (box elder) <i>Liquidambar styraciflua</i> (sweetgum)
<i>Malacosoma americanum</i> (eastern tent caterpillar) (Lepidoptera, lasiocampidae)	Generalist	<i>Prunus serotina</i> (black cherry)
<i>Melanophia canadaria</i> (canadian melanophia) (Lepidoptera, geometridae)	Generalist	<i>Acer negundo</i> (box elder) <i>Lindera benzoin</i> (northern spicebush) <i>Sassafras albidum</i> (sassafras)
<i>Nematus tibialis</i> (locust sawfly) (Hymenoptera, tenthredinidae)	Specialist	<i>Robinia pseudoacacia</i> (black locust)
<i>Papilio polyxenes</i> (black swallowtail) (Lepidoptera, papilionidae)	Specialist	<i>Foeniculum vulgare</i> (fennel)
<i>Papilio troilus</i> (spicebush swallowtail) (Lepidoptera, papilionidae)	Specialist	<i>Lindera benzoin</i> (northern spicebush) <i>Sassafras albidum</i> (sassafras)
<i>Saucrobotys futilalis</i> (dogbane webworm) (Lepidoptera, crambidae)	Specialist	<i>Apocynum cannabinum</i> (dogbane)

Figure 5.1 – Boxplot and predictions of overall consumption rates. Boxes depict the mean consumption rate of each herbivore-plant pair at that temperature ($n = 21$ per box). Shaded area represents the 80% and 95% credible interval of the prediction. Line shows the median posterior prediction.

Figure 5.2 – Posterior estimates of the parameters of community-level consumption rates. Points represent the median estimate, while lines show the 80% (thick line) and 95% (thin line) CI.

Figure 5.3 – Thermal response curves for each herbivore-plant pair. Thick line shows the median posterior estimate, shaded area shows the 95% confidence interval. Solid lines are significant at the 95% level, while dotted lines are significant at the 80% level. In some cases, a dotted line has a highly significant linear term but a moderately significant quadratic term (e.g. *Hyphantria cunea* – *Liquidambar styraciflua*, see Figure 4). Points depict mean consumption rates (± 1 S.E.). Points are weighted by sample size, such that larger points contain more observations, to show how predictions for low sample sizes are pulled towards the overall response.

Figure 5.4 – Posterior estimates of the parameters of curve-level consumption rates. Points represent the median estimate, while lines show the 80% (thick line) and 95% (thin line) CI.

Figure 5.5 – Posterior estimates of the parameters of nutrient effects on the thermal response curves. Points represent the median estimate, while lines show the 80% (thick line) and 95% CI.

Figure 5.6 – Percent change (± 1 S.E.) in cumulative consumption resulting from a 3° and 5° C increase in warming for all herbivore-plant pairs.

CHAPTER VI

WARMING DISRUPTS FITNESS TRADE-OFFS IN *OENOTHERA BIENNIS* CAUSED BY
HERBIVORY

Abstract

Climate change will dramatically alter plant reproductive fitness. In particular, warming can reduce seed mass, increase seed output, and inhibit germination in numerous plant species. However, we know relatively little about the effects of warming on plant fitness outside of arctic and alpine habitats. Herbivory also influences plant fitness through indirect and direct effects on seed production. Although warming can increase herbivory pressure by increasing herbivore abundances or *per capita* herbivory rates, there is no information regarding the ways in which warming modifies how insect herbivores impact plant reproductive output. Here, we report the results of a study designed to assess the combined influence of climate warming and herbivory on reproductive fitness of a biannual herb, *Oenothera biennis*. We used *in situ* field warming combined with herbivore exclusions to factorially manipulate temperature and herbivory pressure over two growing seasons. We measured flowering phenology, fruit production, and seed mass at the end of the second growing season. At ambient temperatures, herbivores consumed a large proportion of flowers, reducing the number of viable fruits. Herbivory simultaneously reduced individual seed mass and increased seed production per fruit, which offset declines in fruit numbers, yielding little difference in total seed production between 'Herbivore' and 'No Herbivore' treatments at ambient temperatures. Warming stimulated flower production, reduced individual seed mass, and increased seed production per fruit in both the presence and absence of herbivores. Total lifetime seed production was highest in plants exposed to herbivores under warmed conditions, suggesting that warming enhanced the ability of *O. biennis* to compensate for herbivory. *Oenothera biennis* grown at ambient temperatures compensated for reduction in fruit numbers by produced fewer, bigger fruits packed with smaller seeds. Warming increased the number of fruits regardless of herbivore treatment, but herbivores still increased the number of seeds per fruit. Thus, warming led to increased reproductive output of plants exposed to herbivores compared to herbivore-free plants. Thus, climate warming may dramatically alter the ways in which insect herbivores influence plant community dynamics and evolution.

Introduction

Steadily rising temperatures associated with climate change have already dramatically altered numerous aspects of plant ecology and reproductive biology (Walck *et al.* 2011). A recent meta-analysis of numerous plant species found that earlier snowmelt and warmer spring temperatures have advanced flowering and reproduction by ~5 days on average compared to historical norms (Wolkovich *et al.* 2012). Warming may also directly reduce plant fitness (e.g. seed mass, seed number, germination rate, Hovenden *et al.* 2008, Liu *et al.* 2012), although few studies have examined the consequences of warming on plant fitness outside of arctic or alpine habitats (Walck *et al.* 2011). In addition to direct effects of rising temperatures on plant physiology, climate change can restructure the nature of plant-herbivore interactions, in particular those involving insect herbivores (Liu *et al.* 2011, Lemoine *et al.* 2013, Burnside *et al.* 2014, Rasmann *et al.* 2014). However, interactive effects of warming and herbivory on plant reproduction and fitness remain undetermined.

The impacts of herbivory on plant fitness under normal environmental conditions can be complex, with plants using multiple strategies to combat the negative effects of herbivory. Many plant species invest resources into induced defensive compounds in direct response to herbivory (Agrawal 1998). These induced defenses often increase plant survival, fruit production, fruit size, and seed number by deterring or mitigating subsequent herbivory (Mauricio 1998, Agrawal 1999). Plants can also alter resource allocation patterns to cope with herbivory, producing and increased number of lighter seeds when exposed to herbivores (Poveda *et al.* 2003). Finally, plants can delay flowering in response to foliar herbivory, either to shunt resources towards regenerating lost leaf tissue instead of flowering or to protect flowers and seeds from consumption by ephemeral florivores and granivores (Brody 1997, Freeman *et al.* 2003). Thus, the effect of herbivory on plant fitness and reproductive biology varies considerably, depending on the identity of interacting plant and herbivore species.

Although the sensitivity of plant-herbivore interactions to climate change has been well-documented, most experiments have not quantified the ultimate effects of warming and herbivory

on lifetime reproductive fitness (e.g., Lemoine *et al.* 2013, Lemoine *et al.* 2014). Warming often stimulates herbivory rates by increasing herbivore metabolic demands (O'Connor 2009, Lemoine & Burkepile 2012,). Warming may therefore strengthen the selective pressure of herbivores on plant fitness. However, herbivory rates decline rapidly once temperature exceeds a critical threshold that varies among and within herbivore species and also depends on host plant identity and quality (Kingsolver & Woods 1997, Kingsolver & Woods 1998, Lemoine *et al.* 2013, Lemoine *et al.* 2014). Thus, warming might weaken the influence of herbivores on plant fitness, especially if there is a mismatch in optimal temperature between plants and herbivores. Indeed, the numerous possible responses of plants and herbivores to rising temperatures make predicting the nature of plant-herbivore interactions in the future nearly impossible in the absence of detailed experiments.

Here, we report the results of a study designed to assess how climate warming alters herbivore control of plant reproductive fitness. To measure interactions between warming and herbivory, we used a factorial, split-plot experiment that manipulated both temperature and herbivore presence using the common evening primrose, *Oenothera biennis*, as a model species. To determine whether warming and herbivory jointly regulate *O. biennis* reproductive phenology, we monitored flower production weekly over four months. We expected that warming would advance flowering by several days in the absence of herbivores (Wolkovich *et al.* 2012) and that herbivores would delay flowering by up to a week at ambient temperatures (Brody 1997, Freeman *et al.* 2003), such that the combination of warming and herbivory would have little overall effect on flowering phenology. Importantly, we quantified multiple metrics of plant reproductive fitness to test whether warming altered the relationship between herbivores and plant fitness. We expected that both warming and herbivores would substantially decrease fruit set, fruit size, and seed size (Poveda *et al.* 2003, Hovenden *et al.* 2008), such that the deleterious effects of herbivores on plant fitness would be exacerbated at high temperatures.

Methods

Study Site and Experimental Design

To simulate climate warming, we installed *in situ* experimental warming infrastructure at the Smithsonian Environmental Research Center in Edgewater, Maryland. During the summer of 2013, we installed sixteen 2 x 2 m garden beds in an open, sunlit field. Plots were constructed of treated lumber, filled with commercial topsoil, and topped with a layer of compost. We initiated warming treatments by installing a single Kalglo MRM-1215 1500W (Kalglo Electronics Company, Bethlehem, PA) infrared heater ~1.5 m from the soil surface over eight randomly chosen plots. To control for shading by the heaters, we hung aluminum frames of the same size and shape as the heaters over the 'Ambient' temperature plots ($n = 8$ per treatment). In August, heaters were raised to a height of 2.5 m as the plants had outgrown the heaters. Heaters successfully raised leaf-surface temperatures by $\sim 3^{\circ}$ C during the day and $\sim 5^{\circ}$ C during the night (Fig. H1). This increase mimics projected climate change by 2100 and is consistent with observed patterns of more rapid increases in nighttime temperatures than daytime temperatures (Easterling *et al.* 1997, IPCC 2007).

In each of the 16 plots, two perpendicular 1 m long aluminum metal sheets driven into the soil divided the 2 x 2 m plot into four 1 x 1 m subplots. We only used two of the four sub-plots in this experiment. The two utilized subplots were randomly assigned as either 'No Herbivory' or 'Herbivory' treatments. 'No Herbivory' treatments were covered with translucent mosquito mesh tucked into the soil around the garden edges to prevent herbivore access. 'Herbivory' treatments consisted of the same mosquito net with all sides rolled up, mimicking shading effects of the net while still allowing herbivore access ($n = 16$ per herbivore treatment). This resulted in a split-plot experimental design, with warming as the whole plot factor and herbivory as the subplot factor. During the second week of August, nets were removed from all plots as the predominant herbivore *Popillia japonica* was now largely absent and plants had outgrown the nets.

Study Species

We used *Oenothera biennis* as a model organism to assess the joint effects of climate change and herbivory on plant fitness because it possesses numerous characteristics that make it ideal for manipulating herbivory and quantifying lifetime reproductive effort. *Oenothera biennis* is a bi-annual plant native to eastern North America that forms rosettes in its first year and bolts into a flowering stalk in its second year, although annual and triennial genotypes also exist (Johnson 2007). Although it hosts a number of insect and avian pollinators, including bees, moths, butterflies, and hummingbirds, *O. biennis* reproduces using self-pollination; pollinators are not required for fruit and seed production (Johnson 2001). Furthermore, *O. biennis* has a genetic system referred to as permanent translocation heterozygosity, such that outcrossing is extremely rare and almost all seeds are genetically identical to the parent plant (Johnson 2001, Agrawal *et al.* 2012). Therefore, rearing *O. biennis* under nets excluding insect pollinators likely had little effect on its reproductive biology.

In July 2013, *O. biennis* seeds were purchased from a commercial supplier (Ernst Conservation Seeds, Meadville, PA), sown into each subplot, and allowed to germinate and grow under 'Ambient' or 'Warmed' conditions. In October 2013, after the growing season, heaters were turned off and *O. biennis* rosettes overwintered under natural conditions. At the beginning of the 2014 growing season, heaters were turned on and nets placed over the 'No Herbivory' treatments. Plant density was relatively consistent among treatments (Table I2, 4.1 ± 1.6 plants per plot overall, mean $\pm$ 1 SE).

Herbivore Pressure

To estimate herbivory pressure in 'Ambient' and 'Warmed' conditions and to confirm that our herbivore exclusions successfully reduced damage, we quantified both the number of *P. japonica* in each plot and cumulative leaf damage once the life cycle of *P. japonica* had ended. At three points over the summer (July – August), we counted the total number of living *P. japonica* occurring in each plot and recorded whether they were on foliage or flowers. To estimate leaf

damage that occurred during peak herbivore loads, we visually estimated damage on August 18, just after *P. japonica* numbers declined. We assigned a value of 0%, 25%, 50%, 75%, or 100% damage to 25 randomly chosen leaves in each subplot. We averaged damage values over all leaves within each plot, yielding one damage estimate per plot. *Oenothera biennis* leaves are long-lived, such that our measurements capture cumulative herbivory rates from the beginning of the growing season up until the point of measurement.

Flowering Phenology

Our study sought to determine how warming and herbivory influence plant flowering phenology. To address this question, we counted the total number of flowers on every plant weekly for four months. *Oenothera biennis* flowered for the first time during the week of July 14 in 2014. Beginning that week, we counted the number of flowers on each plant every week. Only fully open and functional flowers were counted; we did not count buds or wilted flowers. Since *O. biennis* flowers only persist for 3-5 days (N. Lemoine and D. Doublet, *personal observation*), weekly flower counts did not result in recounting the same flowers. Indeed, flower counts changed dramatically on a week-to-week basis (Fig. 6.1). Flower counts ceased on the week of October 6, which represented the last flowering event at the end of the growing season. We averaged the total number of observed flowers per plant in each subplot to yield one estimate of flowers per plant for each subplot.

Fitness

We assessed the joint influence of climate warming and herbivory on *O. biennis* fitness by measuring five reproductive traits at the end the growing season in October. First, we summed total flower production per plant over all weeks. Second, we counted the number of fully developed fruits on each plant. Aborted fruits were rare, and therefore we did not quantify the number of undeveloped fruits. Third, we randomly harvested ten fruits across multiple plants in each subplot, which were weighed immediately to estimate average fresh fruit mass. Fourth, the

number of seeds per fruit was counted (for two fruits/subplot), and seed number was standardized by fruit mass prior to analysis to yield the number of seeds per gram fruit mass. Finally, the entire group of seeds was weighed to the nearest milligram, which was divided by the total number of seeds to estimate individual seed weight. Each of these estimates was then averaged at the subplot level to yield one estimate for each metric per subplot.

Data Analysis

We analyzed all response variables using a Bayesian split-plot models, with temperature as the whole-plot factor and herbivory and the herbivory:warming interaction as subplot factors. For phenology, we calculated the week of peak flower production for each plot as the integer week with the maximum number of flowers (with the week of July 14 being Week 1). We modeled week of peak flower production as a poisson-distributed response variable with a log link function. *Popillia japonica* counts and foliar damage were modeled as normally distributed variables. We analyzed the five fitness responses (total flowers, total fruits, fruit mass, seeds per fruit, and seed mass) as a single multivariate response (*i.e.* split-plot MANOVA), given correlations among these metrics (Fig. H2). Response variables were standardized prior to all analyses. Fruit mass and seed numbers were missing from two subplots due to lack of suitable fruits for measurement, so these subplots were excluded from MANOVA analyses.

All coefficients were given weakly informative prior distributions of $N(0, 4)$, which guards against unusually strong effects unless supported by the data. In the multivariate analysis, the correlation matrix of the response variables was given an uninformative inverse wishart prior with 5 degrees of freedom. All models were run for 25,000 burn-in iterations. Posterior distributions of each parameter were constructed using 25,000 additional samples from each chain. Chain convergence was assessed using traceplots and density plots of posterior samples. Furthermore, $\hat{R} = 1$ for all parameters, indicating model convergence. Traceplots did not indicate any autocorrelation of posterior estimates, so no thinning occurred. The posterior distribution for each parameter therefore contained 100,000 samples. We estimated treatment effects by adding

posterior distributions calculating relevant contrasts for each treatment or effect (e.g. Ambient:No-Herbivore - Ambient:Herbivore).

To visualize changes in fitness traits due to our treatments, we conducted principle components analysis (PCA) of the five fitness metrics. This enabled us to determine how herbivory and warming shift the location of *O. biennis* in overall trait-space.

All statistical analyses were conducted in Python v2.7 using the *numpy*, *scipy*, and *pandas* modules (Jones *et al.* 2001, McKinney 2010, Walt *et al.* 2011). Bayesian models were run using STAN v2.5 accessed via the PyStan module (Stan Development Team 2013). Results are reported as mean $\pm$ 1 S.E. unless otherwise specified. For fitness metrics, effects and effect sizes are reported from posterior distributions of contrasts, rather than of the raw data, as posterior effects correct for variation among plots. Goodness-of-fit plots showing a good fit of the model to the data can be found in Fig. H3. Histograms of contrast posterior distributions can be found in Figs. H4 and H5.

Results

Herbivore Pressure

Our herbivore exclusion experiments successfully inhibited herbivory while warming treatments had little influence on herbivore pressure. In the 'No Herbivores' treatment, foliar damage averaged $1.13 \pm 0.62\%$, compared to the 'Herbivores' treatment where foliar damage averaged $32.38 \pm 1.72\%$. Japanese beetles, *P. japonica*, caused the majority of damage to exposed plants. Adult beetles emerged in early June and remained at high abundances throughout July. *Popillia japonica* were slightly more abundant in 'Ambient' temperature plots over the growing season ($\text{Pr}(\text{Ambient} > \text{Warmed}) = 0.90$), but this effect was relatively small. 'Ambient' plots contained, on average, only 14 fewer adult beetles ($\text{CI}_{95} = 9 \text{ more} - 37 \text{ fewer}$). This small difference in *P. japonica* abundances translated to slightly less foliar damaged in 'Warmed' plots compared to 'Ambient' plots ($\text{Pr}(\text{Ambient} > \text{Warmed}) = 0.86$, $\text{CI}_{95} = 2.3\% \text{ less} - 6.9\% \text{ more damage}$) (Fig. 6.1a). Plants at 'Ambient' temperatures suffered $34.89 \pm 1.73\%$ leaf damage, while

'Warmed' plants lost $29.88 \pm 2.81\%$ of their leaf area (Fig. 6.1a). By early August, *P. japonica* numbers declined to < 10 individuals per plant. Although other herbivores were observed on *O. biennis* after this time (e.g. *Spilosoma virginica*), they occurred infrequently and we observed no significant change in foliar damage after net removal.

Flowering Phenology

Warming had surprisingly little effect of timing of flower production in *O. biennis*, whereas herbivory tended to delay date of peak flowering. A large rainfall event stimulated flower production early in the growing season (Fig. 6.1b). However, because *O. biennis* mainly blooms in early to mid fall, we restricted our analyses to the second, major bloom event by excluding data prior to August 18. During this time, flowering phenology was not related to temperature treatment ($\text{Pr}(\text{Warming Effect}) = 0.72$, Fig. 6.1b). Exposure to herbivores, however, slightly delayed flowering phenology ($\text{Pr}(\text{Herbivores} > \text{No Herbivores}) = 0.90$, Fig. 6.1b). Our model suggested that the most likely consequence of intense early-season herbivory was 1-3 week delay in peak of fall flowering ($\text{Pr}(1 \leq \text{weeks of delay} \leq 3) = 0.83$). Warming did not alter the influence of herbivores on timing of peak flower production in any way ($\text{Pr}(\text{Interaction}) = 0.62$).

Fitness

Under 'Ambient' temperatures, herbivores induced strong fitness trade-offs in *O. biennis*. However, by imposing severe constraints on reproductive characteristics, climate warming obviated any effect of herbivores on *O. biennis* fitness. Fitness metrics were highly correlated (Fig. H2), suggesting that *O. biennis* experiences fitness trade-offs. As expected, plants with high flower production also exhibited high fruit production ($r = 0.71$, $\text{CI}_{95} = 0.38 - 1.00$). Total fruit number, however, was negatively correlated with fruit mass, such that plants produced either few large or many smaller fruits ($r = -0.25$, $\text{CI}_{95} = -0.62 - -0.05$). *Oenothera biennis* fruits contained either numerous small seeds or few large seeds ($r = -0.28$, $\text{CI}_{95} = -0.62 - -0.12$). Strong warming

x herbivory interactions for nearly all fitness metrics suggested that these these fitness trade-offs were regulated by joint effects of climate change and insect herbivores (Fig. 6.2).

Fitness tradeoffs between flower, fruit, and seed characteristics were readily apparent under 'Ambient' conditions. Under normal temperature regimes, plants exposed to *P. japonica* produced an average of 39 fewer flowers, although this effect varied considerably ($CI_{95} = 29$ fewer – 106 more, $\Pr(\text{No Herbivores} > \text{Herbivores} \mid \text{Ambient Temperatures}) = 0.872$, Fig. 6.2a). Herbivores had similarly variable effects on fruit set at ambient temperatures, with plants exposed to herbivores producing 224 fewer fruits than plants protected from herbivores ($CI_{95} = 657$ fewer – 213 more fruits per plant, $\Pr(\text{No Herbivores} > \text{Herbivores} \mid \text{Ambient}) = 0.85$, Fig. 6.2b). Though damaged plants produced fewer fruits at ambient temperatures, each fruit was significantly heavier. Indeed, fruits in the 'Herbivores' treatment weighed 0.088 g more ($CI_{95} = 0.033$ g – 0.140 g) than in the 'No Herbivores' treatment ($\Pr(\text{Herbivores} > \text{No Herbivores} \mid \text{Ambient}) = 0.998$, Fig. 6.2c). Exposure to herbivory also increased seed production (248 more seeds, $CI_{95} = 114$ – 377, $\Pr(\text{Herbivores} > \text{No Herbivores} \mid \text{Ambient}) = 1.0$, Fig. 6.2d), but reduced seed mass by 0.2 mg ($CI_{95} = 0.15$ – 0.40, $\Pr(\text{Herbivores} < \text{No Herbivores} \mid \text{Ambient}) = 1.0$, Fig. 6.2e). Thus, at ambient temperatures, plants exposed to herbivores produced fewer, albeit bigger, fruits packed with more, smaller seeds.

Climate warming negated nearly all fitness tradeoffs imposed by herbivores on *O. biennis*. In the warmed plots, herbivores had little effect on total flower production ($\Pr(\text{Herbivores} > \text{No Herbivores} \mid \text{Warmed}) = 0.696$, Fig. 6.2a) and fruit set ($\Pr(\text{Herbivores} > \text{No Herbivores} \mid \text{Warmed}) = 0.673$, Fig. 6.2b). There were weak, positive effects of herbivores on fruit mass ($\Pr(\text{Herbivores} > \text{No Herbivores} \mid \text{Warmed}) = 0.852$, Fig. 6.2c), but these effects were small compared to those at ambient temperatures. Indeed, plants exposed to herbivores in 'Warmed' treatments weight only 0.027 g more ($CI_{95} = 0.030$ g lighter – 0.081 g heavier), an effect size only 31% as strong as that recorded in 'Ambient' plots. Herbivory had fairly strong effects on seed production in 'Warmed' plots ($\Pr(\text{Herbivores} > \text{No Herbivores} \mid \text{Warmed}) = 0.923$, Fig. 6.2d), but again this effect was weak compared to ambient plots. Damaged plants produced only 92 more

seeds (CI_{95} = 38 fewer – 224 more) than netted plants. Herbivory also weakly affected seed mass in warmed plots ($\Pr(\text{Herbivores} < \text{No Herbivores} \mid \text{Warmed}) = 0.866$, Fig. 6.2e). Seed mass was 0.07 mg lighter when exposed to herbivores (CI_{95} = 0.06 mg heavier – 0.10 mg lighter). Thus, warming greatly weakened all effects of herbivores on plant fitness characteristics.

Principle components analysis effectively demonstrated how warming disrupted herbivore control of *O. biennis* fitness characteristics. The first two principle components described more than 80% of the variance in plant traits, primarily representing variation in flower/fruit production and seed traits (Table 6.1). Under ambient temperatures, the two herbivory treatments occupied distinct spaces along these axes (Fig. 6.3). ‘No Herbivores’ plants were characterized by relatively high flower production, high seed mass, and low seed production (Fig. 6.3). ‘Herbivores’ plants, on the other hand, had low flower and fruit production, lower seed mass, and higher seed numbers (Fig. 6.3). In contrast, under warmed conditions, plants from both herbivory treatments occupied the same trait space: high fruit counts, low seed mass, and high seed production (Fig. 6.3).

Discussion

We found that warming disrupted herbivore control of *O. biennis* reproductive and fitness characteristics. Under ambient temperatures, exposure to herbivores caused *O. biennis* to invest in fewer, heavier fruits filled with more, but lighter, seeds (Figs. 6.2, 6.3). Warming disconnected the relationship between herbivores and plant fitness by shifting all plant towards phenotypes of numerous, small fruits containing many, small seeds (Figs. 6.2, 6.3). Thus, plant-herbivore interactions, specifically herbivore control of plant community composition and evolutionary processes, may be weakened in future climates.

Although warming had no effect on timing of flower production in *O. biennis*, herbivores delayed the timing of peak flower production by up to three weeks. Climate warming generally advances timing of flowering, specifically of spring- and summer-blooming species by stimulating plant germination, growth, and development (Wolkovich *et al.* 2012). However, phenological

responses to warming vary considerably among species (Sherry *et al.* 2007). By increasing the length of the growing season, climate warming may delay or extend flowering of plant species that bloom in the fall, like *O. biennis* (Sherry *et al.* 2007). Yet we found no indication that warming extended the fall flowering period of *O. biennis* after a single growing season. This is not surprising, as many species exhibit no phenological response to warming (Hoffmann *et al.* 2010). Herbivory, on the other hand, often delays flowering date as plants shunt resources away from reproduction towards regrowth of lost tissue (Brody 1997, Freeman *et al.* 2003, Poveda *et al.* 2003). In our study, *O. biennis* exhibited a similar response, delaying flowering when exposed to herbivores that persisted throughout the plant's life cycle well after the dominant herbivore had disappeared from the site.

Under ambient temperatures, herbivores reduced the number of flowers and fruits but increased fruit mass, as *O. biennis* produced substantially more, albeit smaller, seeds (Figs. 6.2, 6.3). Our results are consistent with those of earlier studies showing that herbivory reduces fruit set and seed mass (Ruohomäki *et al.* 1997, Maron 1998, Warner & Cushman 2002, Poveda *et al.* 2003). Previous studies demonstrated that foliar damage by *Popillia japonica*, the dominant herbivore in our study system, increased *O. biennis* fitness by stimulating production of seed defenses against seed predators, like *Schinia florida* and *Mompha spp.*, thereby increasing the number of viable seeds produced at the end of the growing season (McArt *et al.* 2013). However, *P. japonica* preferentially consumes flowers compared to leaves (Held & Potter 2004), leading to reduced fruit production. Additionally, seed predation by *S. florida* and *Mompha spp.* occurs primarily northern latitudes (Anstett *et al.* 2014) and these species were not present at our study site. Thus, induced defenses against seed predators would likely have little effect on overall fitness at our study site. Therefore, leaf herbivory under ambient conditions reorganized fitness phenotypes of *O. biennis*.

In contrast, experimental warming precluded any relationship between herbivores and reproductive characteristics. Relatively few studies examine the effects of warming on plant reproductive fitness, and the vast majority of these studies occur in in arctic or alpine habitats

(Walck *et al.* 2011). As a result, information regarding the effects of *in situ* warming and herbivores on reproductive fitness of temperate plant species is scarce. Overall, warming stimulated flower and production (Aerts *et al.* 2004), even in the presence of herbivores, although fruits of warmed plants tended to weigh less. Warming also reduced seed mass and increased seed number, similar responses to those reported by studies of arctic and alpine plants (Tøtland 1999, Prasad *et al.* 2002, Kudernatsch *et al.* 2008, Liu *et al.* 2012), although effects vary considerably among species (Williams *et al.* 2007). Our results reinforce the pattern of direct effects of warming on plant fitness, but importantly, demonstrate that these effects are invariant to the presence or absence of insect herbivores.

Of course, we report phenotypic characteristics of fitness but fitness itself is determined both by individual fecundity, post-dispersal germination rates, and post-germination survival. We calculated total lifetime seed production by multiplying the number of seeds per g fruit x fruit mass x the number of fruits per plant for all of our plots. Because the resulting estimate of lifetime seed production is a multiple of three random variables, the errors associated with this metric were inordinately high and made analyses difficult. However, interesting patterns emerged from these data. Under ambient temperatures, herbivory caused *O. biennis* to produce $82,286 \pm 65,799$ more seeds, which suggests that the effect of herbivores on overall seed production at ambient temperatures is not large (Fig. 6.4). In contrast, in warmed plots, herbivory caused *O. biennis* to produce $134,929 \pm 87,756$ more seeds, a much stronger effect than under ambient temperatures (Fig. 6.4). Although purely conjecture, these rough calculations suggest that fitness trade-offs might enable *O. biennis* to compensate for herbivory under ambient temperatures, while *O. biennis* may overcompensate for herbivory under warming but via different mechanisms.

Increased total seed production may not, however, lead to increased fitness if the corresponding reduction in seed mass reduces viability, germination, or post-germination survival. In *O. biennis*, reduced seed mass has little effect on germination rates and adult fecundity, although seedlings from heavier seeds grow more rapidly and may flower earlier (Gross & Kromer 1986, Kromer & Gross 1987). Thus, lifetime fitness of *O. biennis* may be determined

more by number of seeds than by seed size. However, air and soil warming strongly affects germination rates (Walck *et al.* 2011). In many cases, warming decreases germination rates (Yurkonis & Meiners 2004, Hovenden *et al.* 2008, Hoyle *et al.* 2013). *Oenothera biennis* may therefore be maximizing seed production to offset likely reductions in germination due to climate warming and herbivores. However, germination experiments in warmed and unwarmed conditions, or subject to other environmental stressors, remain a promising avenue of research.

Our study represents, to our knowledge, the first investigation of the interactive effects of warming and herbivory on plant fitness. We show that *O. biennis* compensated for floral and foliar herbivory by producing significantly more, but lighter, seeds per fruit. Lifetime reproductive fitness therefore did not vary between herbivore treatments. Warming stimulating flower production, reduced seed mass, and increased seed number across all herbivore treatments. Importantly, warmed plants exposed to herbivores produced substantially more seeds than warmed plants protected from herbivores. This suggests that warming increases the compensatory response of plants to herbivory. Thus, warming may dramatically shift the influence of herbivores on plant community structure and evolution in the future.

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Table 6.1 – Correlations (r) between fitness metrics and each principle component for the first two principle components from PCA of the fitness traits.

	PC1	PC2
Standard deviation	1.43	1.41
Percentage variance explained	41%	40%
Number of flowers	0.63	0.87
Number of fruits	0.70	0.94
Fruit mass (g)	0.37	0.39
Seeds per g fruit	0.16	0.92
Seed mass	0.19	0.91

Figure 6.1 – **a)** Foliar damage on *O. biennis* determined by visual leaf inspection on August 18. **b)** Number of flowers per plant over the course of the growing season for *Oenothera biennis*. Points and error bars denote means ± 1 S.E.

Figure 6.2 – Fitness metrics for *O. biennis* under each warming-herbivore combination. **a)** Total number of flowers observed over the course of the growing season. **b)** Total number of fruits counted at the end of the growing season. **c)** Average fruit mass per plant. **d)** Number of seeds per fruit mass. **e)** Average mass of an individual seed contained within fruits. Probabilities denote probability that each effect was important, ignoring sign. That is, we report $\max(\Pr(>0), \Pr(<0))$ for each effect. Higher values indicate a higher likelihood that the given effect was important (0.5 = effect just as likely to be positive or negative, 1.0 = effect was entirely positive or negative). Points and error bars denote mean ± 1 S.E.

Figure 6.3 – Principle components biplot (correlation scaling) of the five fitness metrics. To aid visual interpretation, we split warmed and ambient treatments into separate panels despite only using one analysis.

Figure 6.4 – Estimates of total lifetime seed production for *Oenothera biennis* under each of the four treatments. We calculated this estimate by multiplying seeds per g fruit by average fruit mass by the number of fruits per plant in each plot. Points and error bars denote mean $\pm$ 1 S.E.

CHAPTER VII
CONCLUSIONS

Climate change poses one of the most significant threats to natural ecosystem structure and function. Yet we know strikingly little about the ways in which climate warming will alter important ecosystem process, such as herbivory. Models such as the Metabolic Theory of Ecology (West et al. 1997) provide a simple method for incorporating temperature-dependence into theoretical models of population dynamics under climate change (O'Connor et al. 2011). Chapter II demonstrated that many of MTEs fundamental assumptions appear to have little biological basis and do not account for the unimodal relationship between fitness and temperature for most species. Thus, a more realistic model incorporating unimodal thermal response curves and multiple resource currencies is needed.

Indeed, diet nutritional quality, in particular nitrogen content, interacts with temperature to determine how many herbivores respond to warming (Kingsolver and Woods 1988). Indeed, nitrogen content appears to play an important role in determining how warming impacts plant-herbivore interactions. In Chapter III, I found that increased metabolic nitrogen demands at high temperatures drove *S. exigua* to increase consumption of low-quality diets by almost 300%. These results suggest that the rising costs of metabolic nitrogen demands due to climate warming demands impose considerable constraints on the feeding behavior of ectothermic herbivores.

However, plants also contain numerous secondary metabolites that can affect herbivore feeding behavior and performance (Agrawal 1998). Indeed, generalist herbivores must account for both nutrition and defensive compounds when choosing which plant species to consum (Cruz-Rivera and Hay 2003). In Chapter IV, I show that *P. japonica* growth and consumption rates both increased at high temperatures, but only on high nitrogen plants. Furthermore, *P. japonica* concentrated feeding on a restricted subset of plant species. This decision was based on the fact that secondary metabolites from these species became more effective feeding stimulants at higher temperatures. Thus, climate warming appears to alter the ways herbivores perceive potential host plants by altering the efficacy of plant chemical defenses.

In Chapter V, I showed that the effect of climate change on insect herbivory vary considerably both within and among species. Across 14 plant-herbivore combinations,

consumption showed a wide variety of thermal response curves, including exponential increase, unimodal curves, and no relationship. Among herbivore species, the shape of thermal response curves is likely determined by evolutionary history and adaptation to local environments (Angilletta 2009). Within herbivore species, the thermal response curve appears to have been determined by plant nutritional quality, as with *P. japonica* in Chapter IV.

Although a few studies have characterized the direct effects of warming on plant fitness, most examined arctic or alpine plants (Hovenden et al. 2008, Liu et al. 2012). Furthermore, no study has yet determined how warming alters the influence of insect herbivores on plant fitness. In Chapter VI, I showed that warming disrupted herbivore control of plant fitness. In particular, herbivore effects on flower production, fruit seed, and seed characteristics were greatly weakened in warmed conditions. This suggests that important ecological and evolutionary interactions between plants and herbivores will look dramatically different in the future.

In summary, this dissertation provides a physiological perspective on the potential effects of climate change on plant-herbivore interactions. By first examining herbivore physiology and then testing these patterns in natural settings, this dissertation provides a mechanistic understanding of the effects of rising temperatures on insect herbivore control of plant biomass and reproduction. Thus, this work considerably advances our knowledge with respect to the effects of temperature on important evolutionary and ecological interactions.

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Appendix A: Supplementary figures and model results for Chapter II.

Table A1 – Parameter estimates, information criteria values, and posterior probabilities for each model evaluated for respiration rates, consumption rates, assimilation efficiency, and ingestion efficiency. k is Boltzmann's constant, T is temperature ($^{\circ}\text{C}$ for Null, Linear, and Brière1 models, K for exponential and Gaussian models), M is urchin mass. The model in bold represents the model with the best fit for each measurement. The inclusion of a mass term in the model means that the 95% confidence interval for the mass scaling parameter did not include 0.

Model Name	Parameter Estimates	ΔAICc	Posterior Probability
Respiration			
Exponential	$R = 1.61 \times 10^6 e^{0.46 \frac{-1}{kT}} M^{0.33}$	0.0	0.91
Gaussian	$R = e^{567.15 + 28.84 \frac{-1}{T} + 0.37 (\frac{-1}{kT})^2}$	5.1	0.07
Linear	$R = -0.07 + 0.09T$	5.2	0.02
Null	$R = 0.15$	16.0	< 0.001
Consumption			
Brière1	$C = 0.03T(T - 9.17)\sqrt{(32.61 - T)}M^{0.25}$	0.0	0.70
Gaussian	$C = e^{-859 - 44.47 \frac{-1}{kT} - 0.57 (\frac{-1}{kT})^2} M^{0.25}$	1.8	0.28
Null	$C = 35.50M^{0.24}$	5.1	<0.001
Exponential	$C = 517e^{0.07 \frac{-1}{kT}} M^{0.24}$	6.8	0.01
Linear	$C = 47.04 + 1.3T + 0.21M$	9.2	<0.001
Assimilation Efficiency			
Exponential	$A = 21.21e^{0.09 \frac{-1}{kT}}$	0.0	0.46
Linear	$A = 0.49 + 0.01T$	0.0	0.45
Null	$A = 0.70$	1.3	0.09
Ingestion Efficiency			
Brière1	$I = 0.22T(T + 21.79)\sqrt{(32.01 - T)}$	0.0	0.72
Gaussian	$I = e^{1.10 + 0.46 \frac{-1}{kT} - 0.01 (\frac{-1}{kT})^2}$	2.0	0.26
Linear	$I = 1214.9 - 24.24T$	6.3	0.01
Exponential	$I = .009e^{-0.28 \frac{-1}{kT}}$	7.6	<0.001
Null	$I = 578.76$	15.2	<0.001

Table A2 – ΔAIC values for null, linear, and quadratic mixed effects models used in the meta-analysis.

Model	ΔAIC
M:C ~ Temperature	
Linear	0.0
Quadratic	7.5
Null	15.6
Growth ~ M:C	
Quadratic	0.0
Linear	0.0
Null	5.8

Figure A1 – Histogram of average daily temperatures at Virginia Key from 2005 – 2010.

Figure A2 – Metabolism of urchins held at 20°C for three days

Figure A3 – Comparison of the relationship between metabolism and consumption of *L. variegatus* on A) the original measurement scale and B) the standardized scale used in the meta-analysis. Note that the relationship remained constant after transformation.

Figure A4 – Relationship between temperature-corrected respiration rates and body mass for *L. variegatus*.

Figure A5 – Relationship between temperature-corrected consumption rates and body mass for *L. variegatus*. Consumption rates were temperature corrected by dividing consumption by the temperature term $0.3T(9.17 - T)\sqrt{(32.61 - T)}$ (Table S1). Note that, due to the temperature correction factor $0.3T(9.17 - T)\sqrt{(32.61 - T)}$, lower values indicate higher consumption rates. For example, at 26°C, a temperature-corrected consumption value of -0.8 corresponds to an actual consumption of 270 mg C, whereas a temperature-corrected consumption value of -0.1 corresponds to an actual consumption of 34 mg C. Thus, the decline in temperature-corrected consumption indicates an increase in actual consumption rates with increasing body mass.

Appendix B: Oxygen respiration methods for Chapter II.

After acclimating to the experimental temperatures for 24 hours, urchins were placed into an airtight, closed-system respirometry chamber (2 L volume) placed within the water bath. A YSI 550A dissolved oxygen probe was mounted in the respirometer to measure oxygen concentrations (mg/L). A pump maintained constant circulation within the respirometer. First, background oxygen consumption by bacteria and other microscopic organisms was estimated as the oxygen loss over a one-hour period in the empty respirometer. Then the urchin was placed in the respirometer and oxygen concentrations were recorded every 5 minutes for an hour. A one-hour time limit prevented urchins from experiencing hypoxic conditions.

The slope of the linear regression of oxygen concentrations against time represents the change in oxygen concentrations over a 1 h period. The difference between the baseline slope and the urchin slope is the oxygen consumption in mg/L of the urchin over a 1 h period. The oxygen concentration was multiplied by the volume of the respirometer (2 L) to obtain the mass of oxygen (mg) consumed by the urchin. Oxygen consumption was converted from mg O₂ to mg C respired using the stoichiometry of respiration.

To standardize with daily carbon intake, we multiplied the values by 24 to obtain daily carbon demand. The general equation for respiration is:

Thus, for every 6 moles of oxygen consumed, there are 6 moles of carbon dioxide produced.

To calculate how many mg of carbon is contained in 6 moles of carbon:

$$\frac{x \text{ g CO}_2}{6 \text{ mol CO}_2} = \frac{44 \text{ g CO}_2}{1 \text{ mol CO}_2} = 264 \text{ g CO}_2$$

$$\frac{x \text{ g C}}{264 \text{ g CO}_2} = \frac{12 \text{ g C}}{44 \text{ g CO}_2} = 72 \text{ g C} = 7.2 \times 10^4 \text{ mg C}$$

This shows that there are about 7.2×10^4 mg C in 6 moles of CO₂

To calculate how many mg of O₂ is contained in 6 moles of O₂:

$$\frac{x \text{ g O}_2}{6 \text{ mol O}_2} = \frac{32 \text{ g O}_2}{1 \text{ mol O}_2} = 192 \text{ g O}_2 = 1.92 \times 10^5 \text{ mg O}_2$$

showing that there are about 1.92×10^5 mg O_2 in 6 moles of O_2 . Thus, for every mg O_2 consumed, there are about $7.2 \times 10^4 / 1.92 \times 10^5 = 3.75 \times 10^{-3}$ mg C respired. We multiplied hourly carbon consumption by 24 to get an estimate of daily carbon consumption to standardize with consumption rates.

Appendix C: List of sources for the meta-analysis in Chapter II.

Table C1 – List of references from meta-analysis. X-marks denote whether study measured metabolism (Metab), consumption (Cons), and growth (Growth).

Ref	Experiment	Species	Range	Metab	Cons	Growth
1	1	<i>Manduca sexta</i>	14 – 42° C	X	X	X
2	2	<i>Paralichthys californicus</i> - adult	14 – 25° C	X	X	X
2	3	<i>Paralichthys californicus</i> - juvenile	14 – 28° C	X	X	X
3	4	<i>Acipenser oxyrinchus</i>	6 – 28° C	X	X	X
4	5	<i>Oncorhynchus mykiss</i> – Population 1	10 – 19° C	X	X	X
4	6	<i>Oncorhynchus mykiss</i> – Population 2	10 – 19° C	X	X	X
4	7	<i>Oncorhynchus mykiss</i> – Population 1	19 – 25° C	X	X	X
4	8	<i>Oncorhynchus mykiss</i> – Population 2	19 – 25° C	X	X	X
5	9	<i>Acroneuria californica</i>	16 – 28° C	X	X	X
5	10	<i>Acroneuria californica</i>	10 – 24° C	X	X	X
5	11	<i>Acroneuria californica</i>	6 – 20° C	X	X	X
5	12	<i>Acroneuria californica</i>	10 – 24° C	X	X	X
6	13	<i>Lytechinus variegatus</i>	20 – 31° C	X	X	
7	14	<i>Strongylocentrotus droebachiensis</i> - small	4 – 14° C	X	X	
7	15	<i>Strongylocentrotus droebachiensis</i> - medium	4 – 14° C	X	X	
7	16	<i>Strongylocentrotus droebachiensis</i> - large	4 – 14° C	X	X	
8	17	<i>Basilichthys australis</i> - juvenile	11.5 – 26° C	X	X	
8	18	<i>Basilichthys australis</i> - adult	11.5 – 26° C	X	X	
9	19	<i>Oncohyinchus nerka</i>	5 – 23° C	X	X	X

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Figure D2 – Relationship between temperature and variance of *P. japonica* consumption rates among plant species. Variance at each temperature was calculated as the variance of mean consumption of each plant species.

Figure D3 – Relationship between temperature and variance of *P. japonica* mass changes among plant species. Variance at each temperature was calculated as the variance of mean mass changes on each plant species.

Appendix E: Supplemental tables for Chapter IV.

Table E1 – Temperature data for each growth chamber, collected by HOBO pendant temperature loggers (HOBO UA-002 pendant loggers, Onset Computer Corporation, Bourne MA). Data are presented as mean $\pm$ 1 s.d.

Set Temperature (°C)	Mean Temperature (°C)	Mean Daytime Light Intensity (Lux)
20°	19.59 $\pm$ 0.53°	2115.5 $\pm$ 78.3
25°	26.14 $\pm$ 0.26°	3903.7 $\pm$ 429.7
30°	28.58 $\pm$ 1.89°	4776.3 $\pm$ 313.7
35°	34.63 $\pm$ 2.05°	2959.4 $\pm$ 499.9

Table E2 – Regression equations used to correct for autogenic change for each leaf species, where y is the correction factor and $Mass_i$ is the initial leaf mass. The response variable is change in leaf weight in the absence of herbivory.

Plant Species	Equation	R^2
<i>Acer negundo</i>	$y = -0.01 + 0.114 * Mass_i$	0.683
<i>Acer rubrum</i>	$y = -0.01 + 0.057 * Mass_i$	0.446
<i>Liquidambar styraciflua</i>	$y = 0$	
<i>Plantanus occidentalis</i>	$y = -0.002 + 0.075 * Mass_i$	0.847
<i>Rosa multiflora</i>	$y = 0.0002 + 0.104 * Mass_i$	0.787
<i>Rubus allegheniensis</i>	$y = 0.001 + 0.081 * Mass_i$	0.618
<i>Rubus phoenicolasius</i>	$y = 0.018$	
<i>Viburnum prunifolium</i>	$y = 0.001 + 0.042 * Mass_i$	0.161
<i>Vitis vulpina</i>	$y = -0.008 + 0.039 * Mass_i$	0.596

Table E3 – Significance of phylogenetic signal for each trait. Estimates of λ were compared to a null model of no phylogenetic signal ($\lambda = 0$) using a log-likelihood ratio test.

Trait	<i>p</i>-value
% Carbon	1.00
% Nitrogen	1.00
% Phosphorus	1.00
% Water	1.00
% Protein	1.00
Toughness	0.22

Table E4 – *Post-hoc* comparisons for consumption and mass change among plant species within each temperature during no-choice assays. Letters denote statistically similar groups. Letters are decreasing order of consumption and growth rates, with “A” representing plants with the highest consumption and growth.

Consumption				
Plant Species	20°	25°	30°	35°
<i>Acer negundo</i>	C	B	BCD	BC
<i>Acer rubrum</i>	BC	B	ABC	ABC
<i>Liquidambar styraciflua</i>	BC	B	CDE	C
<i>Plantanus occidentalis</i>	AB	B	AB	A
<i>Rosa multiflora</i>	A	A	A	AB
<i>Rubus allegheniensis</i>	C	C	E	BC
<i>Rubus phoenicolasius</i>	C	BC	E	BC
<i>Viburnum prunifolium</i>	C	BC	DE	C
<i>Vitis vulpina</i>	A	B	AB	AB

Mass Change				
Plant Species	20°	25°	30°	35°
<i>Acer negundo</i>	B	B	BC	CD
<i>Acer rubrum</i>	B	B	C	DE
<i>Liquidambar styraciflua</i>	B	BC	C	DE
<i>Plantanus occidentalis</i>	B	BC	B	BC
<i>Rosa multiflora</i>	A	A	A	A
<i>Rubus allegheniensis</i>	B	C	BC	B
<i>Rubus phoenicolasius</i>	B	BC	C	D
<i>Viburnum prunifolium</i>	B	BC	C	E
<i>Vitis vulpina</i>	B	BC	BC	DE

Table E5 – *Post-hoc* comparisons for consumption of plants during choice assays. Letters denote statistically similar groups. Letters are decreasing order of consumption and growth rates, with A representing plants with the highest consumption and growth.

Plant Species	25°	35°
<i>Acer negundo</i>	AB	BC
<i>Acer rubrum</i>	BC	C
<i>Liquidambar styraciflua</i>	AB	C
<i>Plantanus occidentalis</i>	BC	C
<i>Rosa multiflora</i>	A	A
<i>Rubus allegheniensis</i>	BC	C
<i>Rubus phoenicolasius</i>	AB	BC
<i>Viburnum prunifolium</i>	BC	C
<i>Vitis vulpina</i>	C	AB

Table E6 – Principle components analysis of plant traits for both consumption and growth analyses. Loadings with values near zero have been omitted from the table.

Principle Component Loadings			
	PC 1	PC 2	PC 3
Eigenvalue	1.56	1.21	1.12
Proportion of Variance	0.41	0.24	0.21
Cumulative Variance	0.41	0.65	0.86
<i>Loadings</i>			
% Carbon		0.625	-0.505
% Nitrogen	-0.237	-0.378	-0.643
% Phosphorus	-0.475	0.277	-0.357
% Water	-0.608	-0.102	0.222
% Protein	0.505	0.242	
Toughness	-0.302	0.567	0.383

Table E7 – Coefficient table of multiple regression of plant traits on consumption and RGR. Statistically significant relationships are in bold.

Consumption				
Parameter	Estimate	Std. Error	t-statistic	p-value
Intercept	-0.117	0.246	-0.475	0.639
PC 1	-0.035	0.119	-0.297	0.769
PC 2	0.124	0.186	0.667	0.510
PC 3	0.177	0.159	1.111	0.276
Temp	0.017	0.009	1.860	0.073
Temp x PC1	0.005	0.004	1.263	0.217
Temp x PC2	-0.007	0.007	-0.996	0.328
Temp x PC3	-0.010	0.006	-1.861	0.073
Growth				
Parameter	Estimate	Std. Error	t-statistic	p-value
Intercept	-0.0002	0.0044	-0.0409	0.9677
PC 1	-0.0019	0.0015	-1.2689	0.2166
PC 2	0.0043	0.0033	1.2851	0.2110
PC 3	0.0047	0.0023	2.0156	0.0552
Temp	0.0002	0.0002	1.0278	0.3143
Temp x PC1	0.0001	0.0001	1.9743	0.0600
Temp x PC2	-0.0001	0.0001	-1.2004	0.2417
Temp x PC3	-0.0003	0.0001	-3.3156	0.0029

Table E8 – Coefficient table of multiple regression of *P. japonica* feeding preferences during chemical extract bioassays. Statistically significant relationships are in bold.

25° C				
Plant Species	Estimate	Std. Error	t-value	p-value
<i>Acer negundo</i>	-0.094	0.042	-2.259	0.028
<i>Acer rubrum</i>	-0.186	0.181	-1.030	0.308
<i>Liquidambar styraciflua</i>	-0.115	0.448	-0.257	0.798
<i>Plantanus occidentalis</i>	0.177	0.217	0.816	0.418
<i>Rosa multiflora</i>	0.230	0.428	0.537	0.594
<i>Rubus allegheniensis</i>	0.160	0.113	1.421	0.161
<i>Rubus phoenicolasius</i>	0.204	0.200	1.020	0.312
<i>Viburnum prunifolium</i>	0.066	0.054	1.227	0.225
<i>Vitis vulpina</i>	0.287	0.251	1.145	0.257

35° C				
Plant Species	Estimate	Std. Error	t-value	p-value
<i>Acer negundo</i>	-0.532	0.352	-1.513	0.136
<i>Acer rubrum</i>	-0.329	0.259	-1.270	0.210
<i>Liquidambar styraciflua</i>	-0.089	0.421	-0.212	0.833
<i>Plantanus occidentalis</i>	-0.513	0.583	-0.880	0.383
<i>Rosa multiflora</i>	1.265	0.287	4.408	0.000
<i>Rubus allegheniensis</i>	-0.125	0.324	-0.386	0.701
<i>Rubus phoenicolasius</i>	-1.093	0.299	-3.656	0.001
<i>Viburnum prunifolium</i>	-0.043	0.449	-0.095	0.925
<i>Vitis vulpina</i>	0.766	0.349	2.198	0.032

Appendix F: Supplemental tables for Chapter V.

Table F1 – Number of replicates for each plant-herbivore pair used in this study.

Herbivore	Plant	20°	25°	30°	35°
Unidentified	<i>Lindera benzoin</i>	7	8	9	9
Lepidopteran Leafroller					
<i>Arge scapularis</i>	<i>Ulmus rubra</i>	3	2	3	2
<i>Atteva aurea</i>	<i>Ailanthus altissima</i>	3	4	5	4
<i>Chrysocys auratus</i>	<i>Apocynum cannibinum</i>	5	5	5	5
<i>Danaus plexippus</i>	<i>Asclepias syriaca</i>	3	3	3	3
<i>Epimecis hortaria</i>	<i>Lindera benzoin</i>	9	9	9	9
<i>Epimecis hortaria</i>	<i>Liriodendron tulipifera</i>	8	8	8	8
<i>Epimecis hortaria</i>	<i>Sassafras albidum</i>	4	4	4	3
<i>Euchaetes egle</i>	<i>Apocynum cannibinum</i>	4	5	5	4
<i>Euchaetes egle</i>	<i>Asclepias syriaca</i>	5	5	5	4
<i>Hyphantrea cuneata</i>	<i>Acer negundo</i>	8	8	8	7
<i>Hyphantrea cuneata</i>	<i>Liquidambar styraciflua</i>	8	8	6	8
<i>Malacosoma americanum</i>	<i>Prunus serotina</i>	4	7	6	6
<i>Melanophia canadaria</i>	<i>Acer negundo</i>	4	4	4	4
<i>Melanophia canadaria</i>	<i>Lindera benzoin</i>	9	9	9	9
<i>Melanophia canadaria</i>	<i>Sassafras albidum</i>	4	4	4	4
<i>Nematus tibialis</i>	<i>Robinia pseudoacacia</i>	6	5	9	9
<i>Papilio polyxenes</i>	<i>Foeniculum vulgare</i>	5	5	6	4
<i>Papilio troilus</i>	<i>Lindera benzoin</i>	5	6	6	6
<i>Papilio troilus</i>	<i>Sassafras albidum</i>	6	5	6	5
<i>Saucrobotys futilalis</i>	<i>Apocynum cannibinum</i>	9	12	9	9

Table F2 – Temperature data for each growth chamber, collected by HOBO pendant temperature loggers (HOBO UA-002 pendant loggers, Onset Computer Corporation, Bourne MA).

Set Temperature (°C)	Mean Temperature (°C)	Standard Deviation
20°	19.59°	0.53°
25°	26.14°	0.26°
30°	28.58°	1.89°
35°	34.63°	2.05°

Table F3 – Regression equations used to correct for autogenic change for each leaf species, where y is the correction factor and $Mass_i$ is the initial leaf mass. The response variable is change in leaf weight in the absence of herbivory.

Plant Species	Equation	R^2
<i>Acer negundo</i>	$y = -0.01 + 0.11Mass_i$	0.68
<i>Ailanthus altissima</i>	$y = -0.01 + 0.02Mass_i$	0.61
<i>Apocynum cannabinum</i>	$y = 0.00 + 0.09Mass_i$	0.70
<i>Asclepias syriaca</i>	$\log(y) = -2.19 + 0.29Mass_i$	0.28
<i>Foeniculum vulgare</i>	$y = 0.00 + 0.16Mass_i$	0.91
<i>Lindera benzoin</i>	$y = 0.00 + 0.03Mass_i$	0.41
<i>Liquidambar styraciflua</i>	$y = 0$	
<i>Liriodendron tulipifera</i>	$y = -0.02 + 0.07Mass_i$	0.44
<i>Prunus serotina</i>	$y = 0.01 + 0.17Mass_i$	0.95
<i>Robinia pseudoacacia</i>	$y = 0.00 + 0.07Mass_i$	0.65
<i>Sassafras albidum</i>	$y = -0.01 + 0.08Mass_i$	0.50
<i>Ulmus rubra</i>	$y = 0.00 + 0.06Mass_i$	0.57

Appendix G: Supplemental figures for Chapter V.

Figure G1 – Daily average, minimum, and maximum temperatures in Annapolis, MD from April – August 2013.

Figure G2 – Hourly temperature measurements from June – August 2013 from the NOAA weather station at the U.S. Naval Academy, Annapolis, MD.

Appendix H: Supplemental figures for Chapter VI.

Figure H1 – Hourly surface temperatures in ambient and control plots measured using an infrared thermometer gun. Each plot had five white plastic spheres (ping pong balls) mounted ~ 20 cm from the soil surface to provide a standard surface for temperature measurement. Spheres were placed at the center of each of the four subplots and directly in the center of the entire plot. Temperature measurements from the five spheres were averaged to yield a single measurement for each plot. Points and bars depict mean $\pm$ 1 SE.

Figure H2 – Pairwise scatterplots of all five response variables included in the multivariate models. The diagonal gives the density of the points for each variable. All variables were standardized prior to plotting.

Figure H3 – Goodness-of-fit for the multivariate model showing fitted vs. observed values. The dashed line indicates the 1:1 fit, such that points along the dashed line indicate perfect model fit.

Figure H4 – Histograms of posterior distributions for all relevant contrasts pertaining to total flower numbers, total fruit numbers, and fruit mass. Dashed line indicates 0 (*i.e.* no difference). All contrasts are No Herbivores – Herbivores, such that negative values indicate higher numbers or mass in the ‘Herbivory’ treatments and positive values indicate higher numbers or mass in the ‘No Herbivory’ treatment. For simplicity, the strength of the contrast is presented as $\text{Pr}(\text{No Herbivory} > \text{Herbivory})$, but $\text{Pr}(\text{Herbivory} > \text{No Herbivory})$ is obtained by subtracting this number from 1.

Figure H5 – Histograms of posterior distributions for all relevant contrasts pertaining to seed mass, seed numbers, and total seed numbers. Graphs are described in Fig. H4.

Appendix I: Supplemental tables for Chapter VI.

Table I1 – Initial seed mass sown into each subplot for each temperature:herbivory treatment combination.

Treatment	Initial Seed Mass ($\pm$ 1 SE)
Ambient : No Herbivory	0.501 $\pm$ 0.003 g
Ambient : Herbivory	0.499 $\pm$ 0.004 g
Warmed : No Herbivory	0.505 $\pm$ 0.009 g
Warmed : Herbivory	0.502 $\pm$ 0.004 g

Table I2 – Number of plants per subplot for each temperature:herbivory treatment combination.

Treatment	Number of Plants (± 1 SE)
Ambient : No Herbivory	4.4 $\pm$ 0.5
Ambient : Herbivory	3.6 $\pm$ 0.5
Warmed : No Herbivory	3.9 $\pm$ 0.6
Warmed : Herbivory	3.4 $\pm$ 0.5

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