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Effects of constant vs. fluctuating temperatures on performance and life history of the herbivorous pest Lymantria dispar (Lepidoptera: Eribidae)

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Effects of constant vs. fluctuating temperatures on performance and life history of the herbivorous pest *Lymantria dispar* (Lepidoptera: Eribidae)

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Studies at Virginia Commonwealth University

By

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ABSTRACT

Effects of constant vs. fluctuating temperatures on performance and life history of the herbivorous pest *Lymantria dispar* (Lepidoptera: Eribidae)

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The role of temperature variation in organismal performance is understudied, but is critically important for understanding the response of biodiversity to climate change. To address this issue in herbivorous insects, I studied the direct and interactive effects of thermal regime (constant vs. fluctuating temperatures) and nutrition (dietary nitrogen) on gypsy moth (*Lymantria dispar*) performance under laboratory conditions. Predictions for differences between constant and fluctuating thermal conditions were derived from Jensen’s inequality, and artificial diets of differing nutritional quality were made by modifying nitrogen (casein) content. Larvae were reared in the laboratory under four temperature regimes (22°C constant, 22°C fluctuating (±6°C), 28°C constant, and 28°C fluctuating (±6°C)) and two diet treatments (high N, and low N). Gravimetric analyses were also conducted to calculate nutritional indices and assess the short-term effects of temperature and diet quality on fourth instar larvae growth efficiencies. Consistent with predictions from Jensen’s inequality, fluctuating thermal conditions significantly
reduced larval performance in both sexes across ontogeny. Low quality diet also reduced performance, but interactions between diet and thermal regime were only found in early instars.
INTRODUCTION

Global climate change and its effect on biodiversity have generated growing concern among biologists and society at large. Biological responses to climate change will be largely determined both by the degree of warming, including changes in means and variances, and the inherent sensitivities of organisms to this warming (Deutsch et al. 2008; Estay et al. 2014). While global mean temperatures are expected to increase as a result of global climate change, so too is the range of thermal variance expected to increase (Stouffer & Wetherald 2007; Boer 2009). Therefore, it is important to understand how organisms will respond to environmental variance as seen in the field (Neuwald & Valenzuela 2011). A number of studies have investigated potential effects of climate change on biodiversity; however, many of these studies use constant temperatures to test predicted influences on organismal performance (Chown et al. 2010). In nature, organisms rarely experience constant temperatures; rather they are exposed to real-time variation in temperature down to the micro-scale (Stenseth et al. 2002).

Climate change ultimately results in a global thermal shift, which has the potential to disrupt biological performance from the scales of individual organisms to whole-ecosystems (Woodin et al. 2013). Growth and performance are limited by temperature in all organisms; therefore, any mismatch in development can have physiological implications for interacting species. As a result, any reliable predictions for impacts of climate change on biodiversity should consider the underlying effects of temperature on organisms, including physiological and performance shifts. Ectotherms are thought to be especially vulnerable to climate change because their body temperature depends directly on environmental temperature. Since nearly all organisms are ectotherms, understanding their thermal biology is important when discussing the potential effects of environmental variance. This includes the organism’s threshold of thermal tolerance.
via its direct physiological response, and the biologically limiting conditions that shaped the organism’s response to the environmental conditions in which it evolved (Terblanche et al. 2011).

**Temperature performance curves describe organismal performance as a function of temperature**

Temperature performance curves (TPCs) describe the response of an organismal performance trait (e.g. growth) to changes in environmental temperature (Angilletta 2009). TPCs are bounded by upper and lower thermal limits (UTL and LTL, respectively), at which whole-organism performance becomes compromised, leading to death. The optimum temperature for maximum organism performance ($T_{\text{opt}}$) falls on the TPC somewhere between the LTL and UTL, and typically reflects the environment in which the organism evolved (Angilletta 2009).

Organism TPCs are typically asymmetric (e.g. Logan et al. 1991; Chown et al. 2010). At low temperatures, a typical TPC gradually increases from the LTL towards $T_{\text{opt}}$. At high temperatures, the function decreases more rapidly from $T_{\text{opt}}$ towards the UTL. While the underlying mechanisms defining the asymmetry are unclear, the shape of TPCs is highly standardized across physiological traits and is represented in diverse organismal types (Angilletta 2009).

**Jensen’s inequality predicts performance changes from TPCs as a function of thermal variation**

Historically, studies of organism thermal performance have been conducted at constant temperatures, despite organisms naturally experiencing wide ranges of thermal variance on assorted time scales (Folguera et al. 2009). For this reason, ecologists have increasingly
recognized the role environmental variance plays in organism performance (Ruel and Ayres 1999; Mironidis & Savopoulou-Soultani 2008; Folguera et al. 2011; Ketola et al. 2013). This importance is emphasized through a mathematical property of nonlinear functions called Jensen’s inequality (Jensen 1906). Jensen’s inequality states that, “for a nonlinear function, \( f(x) \), and a set of \( x \) values with a mean of \( \bar{x} \) (and a variance greater than zero), the average result of \( f(x) \), \( \bar{f(x)} \), does not equal the result of the average \( x \), \( f(\bar{x}) \)” (Ruel & Ayres 1999). When applied to temperature-dependent development, the inequality is termed the ‘Kaufmann effect’ (Worner 1992), and predicts that temperature variability will have positive effects on development at low temperatures, but negative effects at high temperatures, with respect to constant conditions around the same mean temperature (Ruel & Ayres 1999; Ragland & Kingsolver 2008). This is due to the asymmetrical nature of the curve. As shown in Figure 1A, variability in the accelerating part of the curve results in higher performance rates than a constant temperature of the same mean. This trend is reversed where the curve is decelerating (Ragland & Kingsolver 2008).

Depending on the degree of nonlinearity of the developmental rate curve, fluctuation around a mean on the more central portion of the curve should result in similar rates under constant and fluctuating thermal regimes (Worner 1992). This central point represents the inflection point (See Figure 1A), or the point at which the development rate function ceases to accelerate and begins decelerating (Estay et al. 2014). Performance responses to fluctuating conditions therefore vary relative to temperatures above or below the inflection point. However, depending on the shape of the nonlinear function and the degree of fluctuation, disproportionate rates should result from fluctuating temperatures around different means on the same side of the inflection point (See Figure 1B). Jensen’s inequality thus highlights the importance of
considering variable temperature in time and space in models of organism performance (Estay et al. 2011).

**Diet in the context of performance variability**

Jensen’s inequality is applicable to any nonlinear response function for any environmental variance encountered by an organism (Ruel & Ayres 1999). Current climate models predict temperature shifts as a result of increased carbon dioxide concentration in the atmosphere. Such effects can have both direct and indirect consequences on organisms through thermal variation and host plant nutritional quality (Stenseth et al. 2002; DeLucia et al. 2012). For example, it is well known that under high concentrations of carbon dioxide, foliar nitrogen concentration becomes reduced (Traw et al. 1996; Lindroth et al. 1997). Therefore, global increases in carbon dioxide concentration can have large-scale effects on foliar nitrogen dilution.

Moreover, empirical data have shown that foliar chemistry can vary on much smaller spatial and temporal scales. Insect herbivores have the potential to encounter significant nutritional variation between plants, within plants, and even on individual leaves (Meyer & Montgomery 1987; Hemming & Lindroth 1995). It is assumed that climate change will likely affect many environmental parameters simultaneously (Chown et al. 2010), and insect performance is often limited by both nutritional availability in host plants and environmental temperature throughout development (Lindroth et al. 1997; Couret et al. 2014). Therefore, I was motivated to understand the ways that diet and temperature interact to alter performance traits across larval ontogeny.

The effects of larval diet on performance parameters in herbivorous insects are relatively well understood (Barbosa & Greenblatt 1979; Scriber & Slansky 1981; Timmins et al. 1988; Bernays & Chapman 1994; Coley et al. 2006). Dependence on nitrogen in all metabolic
processes suggests that nitrogen is a limiting factor in growth, development, and survival in all animals (Mattson 1980). This is true of all phytophagous insects, which depend on proteins and amino acids in leaf tissues that can vary over both time and spatial scales (Bernays & Chapman 1994). Typically, leaves provide some of the best nutritional quality in plants (Bernays & Chapman 1994), and phytophagous insects show the best performance while feeding on leaves with high amounts of nitrogen (Panizzi & Parra 2012).

Despite many studies of insect performance as a function of diet, most often the experiments have been conducted under static temperature conditions. For example, Couret et al. (2014) investigated temperature, larval diet, and density effects on development rate and survival, but used constant temperatures to do so. Because performance is influenced strongly by both diet and temperature (both of which vary in nature), it is important to understand how these factors may interact (Lindroth et al. 1997). Lindroth et al. (1997) demonstrated that these interactive effects were significant in the invasive North American gypsy moth, *Lymantria dispar*, particularly for growth rates, food processing efficiencies and nitrogen consumption rates.

**Goals and objectives**

Currently there is a growing awareness of the need to go beyond the mean and study the effects of temperature variance on organisms (Estay et al. 2011). The main objective of this study was to compare the effects of constant vs. fluctuating temperatures on the performance of gypsy moth larvae throughout ontogeny. This study differs from similar studies in that, in addition to thermal variability, I tested for a possible interaction with diet for multiple performance variables, and for males and females separately through the complex lepidopteran life cycle. First, I measured larval growth rate, development time, and survival over the life
history of the lab strain gypsy moth (see below), and used Jensen’s inequality to make predictions for changes in those performance measures between constant and fluctuating thermal regimes about the same mean temperatures. In addition to temperature variability, larvae were fed artificial diets representative of either a high quality or low quality, based on nitrogen content. Finally, assimilation of diet was analyzed using nutritional indices (Waldbauer 1968; Scriber & Slansky 1981; Stockhoff 1993) to evaluate the direct and interactive effects of temperature and dietary nitrogen on food use efficiency.

MATERIALS AND METHODS

Study system

While many phytophagous insects cause limited damage to their ecosystems, some populations can periodically outbreak, causing large-scale defoliation of host plants (Bjørnstad et al. 2010). Since their introduction in the United States around 1869, European gypsy moth (Lymantria dispar) outbreaks have become frequent, having partially or completely defoliated 34.6 million hectares of US forests since 1924 (Whitmire & Tobin 2006). As a spring-feeding generalist species, L. dispar has over 300 species of deciduous and coniferous hosts (Elkinton & Liebhold 1990).

In North America, the gypsy moth’s range currently includes southern Canada down to North Carolina. However, invasive territory can expand into uninfested habitat through transition zones, potentially resulting in new infestations (Whitmire & Tobin 2006). These transition zones can vary from year-to-year, and while they don’t always result in infestation of new territory, global climate change may result in their expansion to more northern territories (Vanhanen et al. 2007).
In 1967, the U.S. Department of Agriculture (USDA) started a laboratory colony of *L. dispar* (Otis strain) that has been in continuous cultivation (Keena et al. 1998; Grayson et al. 2015). The detailed and well-documented biology of the *L. dispar* Otis strain makes it an ideal study system for our experiment. Importantly, Logan et al. (1991) have developed a standard TPC for development of the Otis strain. According to this study, the thermal optimum for development time of *L. dispar* is ca. 29°C, dropping rapidly towards its UTL (Figure 2). This TPC allows us to make specific predictions for the effects of thermal variance using Jensen’s inequality, and to establish the conditions of the thermal performance experiment.

*Specific predictions from Jensen’s inequality*

From the data in Figure 2, I chose to study two mean temperatures representing suboptimal and near-optimal temperatures for development located above the inflection point on the gypsy moth’s developmental rate curve. Worner (1992) points out that the extent of nonlinear rate responses to fluctuation depends on the range of variation due to the Kaufmann or rate summation effect. Keeping this and the insect’s critical thermal limits in mind, I selected a moderate range of variation of 6°C in either direction around mean temperatures near (28°C) and below (22°C) the gypsy moth’s thermal optimum for development.

Based on the properties of Jensen’s inequality, having chosen two mean temperatures on the decelerating part of the TPC, I predict that larval growth rate, development rate, and survival will be significantly reduced under fluctuating thermal conditions, compared to constant conditions around both mean temperatures. Furthermore, I predict disproportionate effects of constant vs. fluctuating conditions at a mean of 28°C compared to a mean of 22°C.
**Experimental method**

Egg masses were obtained from the USDA Otis laboratory strain of gypsy moth \((Lymantria dispar)\). Six egg masses were mixed and left in a petri dish at room temperature until hatching. Upon hatching, larvae were separated into groups of ten and placed in individual 5 ½ oz. cups, which were randomly allocated to one of eight temperature-by-diet treatment combinations (see below). Twelve cups, with ten individuals each, were chosen at random and weighed to calculate an average starting weight per cup.

Four incubators (Percival Scientific, model I22VLC9) were used to represent four different thermal regimes, with each thermal regime containing two different diet sub-treatments (see below). Incubators were programmed around two mean temperatures and included temperature treatments of constant 22°C, constant 28°C, fluctuating 22°C (22°C, ±6°C) and fluctuating 28°C (28°C, ±6°C). Photoperiod was correlated with thermal variation on a 16 h light-8 h dark cycle, such that peak temperatures coincided with light periods. Relative humidity was maintained between 60% and 80% throughout the experiment.

Two artificial diets were prepared to test whether the effects of temperature and temperature variation interact with dietary nitrogen to alter performance and life history. The standard Otis diet (USDA, Hamden Formula Gypsy Moth Diet #F9630B, Bio-Serv, Flemington, NJ) was used to represent a ‘high nitrogen (N)’ diet. ‘Low N’ diet was prepared by modifying the Otis diet’s casein content, using Stockhoff’s (1991) ratio of reduction and compensating reduction in dry weight due to casein with cellulose.

Each diet treatment consisted of 7 replicate cups of 10 caterpillars each \((n = 70)\), for a total of 140 individuals per thermal regime. Food was provided ad libitum, and refreshed every 6
days. Larval weights were measured on an analytical balance to the nearest 0.01 grams at five different time intervals, from hatching to pupation, to study effects through ontogeny (Figure 3).

As described above, larval weight one (measured at time T1 in Figure 3) was determined at hatching prior to introduction to treatment regimes. Weight two (T2) was measured 5 days following the start of the experiment; individual weight was calculated as an average of the group weight per cup, and instar and number survived were recorded. Weight three (T3) was measured as each individual molted into the third instar. Upon molt into third instar, caterpillars were removed from group cups, weighed, and placed into individually labeled cups following hardening of the exoskeleton. Weight four (T4) was measured for all individuals seven days following individual molt into the third instar; number survived and instar were also recorded during T4 measurements.

Following T4 measurements, larvae were left to develop and fed ad libitum until pupation. When pupation was noted, pupal weight (T5) measurements were taken after 3 days to allow for hardening of the pupal case. Since pupal weights were highly correlated with adult weight at eclosion ($R^2= 0.925355$, p-value < 0.0001), I present pupal weight as a final measure of the size achieved by caterpillars in the experiment. However, pupae were monitored until eclosion to determine sex. Thus, overall, the entire suite of measured performance variables included multiple estimates of growth, development and survival through ontogeny and for males and females separately (Tables 1-6 and Figures 4-6 & 8).

Development was measured in two ways, including development times and development rates. While development rate is simply measured as the inverse of instar duration, transformation from development times to development rates can lead to complications due to the non-linearity of the function (Logan et al. 1991). Since I make specific predictions from
Jensen’s inequality using developmental rate data from Logan et al. (1991) (see above), and because development rates and times do not scale linearly, I felt it warranted to present the results for both measures of development from our experiment.

_Nutritional indices_

To better understand the role of diet in performance under different thermal regimes, nutritional indices were measured using classical gravimetric methods (Waldbauer 1968; Scriber & Slansky 1981; Stockhoff 1993). A total of ten individuals were selected at random following initial transition to the fourth instar, for each diet and temperature treatment (n = 80 total). Upon selection, larvae were separated from food for three hours to ensure that most gut contents had been voided, while avoiding error due to induced starvation. Individuals were then placed into new, 5 ½ oz. cups upon 4th instar molt. Larvae were provided with pre-weighed food, and individuals were weighed at the start of the trial. After 72 hours, individuals were weighed again to determine final wet weight. Food and frass produced were also weighed. Specimens were then frozen until death. Following death, caterpillars, remaining food, and frass were placed in a drying oven set to 60°C for 7 days to ensure complete dehydration. Following drying, caterpillars, food, and frass were weighed to collect dry weights. These data were used to derive the efficiency of conversion of ingested food (ECI), or net growth efficiency, for each diet by thermal regime treatment using the following equation: $\frac{\text{Biomass Gained (dry)}}{\text{Food Ingested (dry)}} \times 100$.

_Statistical analyses_

Development and growth data were analyzed using analysis of variance (ANOVA) with post-tests conducted using Tukey’s HSD. Prior to statistical analysis, all data were checked for
normality using normal quantile plots and tested for unequal variances using the Brown-Forsythe test. To determine if the sexes should be treated separately, each response variable was first tested for significant interactions involving sex in a 3-way ANOVA including sex, diet, and thermal regime as main effects. When significant interactions with sex were present, the sexes were analyzed separately. In the single case where an interaction with sex was not present (i.e. early instar growth rates), data were analyzed for the sexes combined. In cases where data were highly non-normally distributed or variances were significantly unequal, a log_{10} transformation was performed. Survivorship data were analyzed using nominal logistic regression for binary response variables. All statistical analyses were conducted in JMP Pro 11. All tests were considered significant at p < 0.05, and all means are reported with ± 1 SE.

RESULTS

Development

Development rate

For females, there was a significant interaction between thermal regime and diet in their effects on developmental rate to the third instar (T1-T3 in Figure 4), in addition to significant main effects of diet and thermal regime (Table 1). Post-tests detected 1.1 times slower development rate for larvae fed low N diet than high N diet at constant 22°C, but no differences between diets for the other thermal treatments (Figure 4E). Overall, the main effect of temperature resulted in 1.2 and 1.1 times faster development rates under constant versus fluctuating conditions at 22°C and 28°C, respectively (Figure 4E). There was a significant main effect of thermal regime on late instar (T3-T5) development rates, but no main effect of diet or interaction between diet and thermal regime (Table 1). Post-tests detected development rates 1.1 times faster under constant conditions than fluctuating conditions around both mean
temperatures (Figure 4C). There was also a significant main effect of thermal regime on development rates to pupation (T1-T5) (Table 1). Consistent with early instar development rates, constant thermal regime resulted in overall development rates 1.2 and 1.1 times faster than fluctuating conditions at 22°C and 28°C, respectively (Figure 4A).

For males, there were significant main effects of thermal regime and diet on development rates to the third instar (T1-T3), but no significant interaction (Table 1). Development rate to the third instar was 1.2 times faster on the high N diet than the low N diet. The main effect of temperature resulted in 1.1 times faster development rates to the third instar under constant versus fluctuating conditions around both mean temperatures (Figure 4F). For late instar (T3-T5) development rates, there was a significant main effect of thermal regime but no main effect of diet or interaction between diet and thermal regime (Table 1). Post-tests detected 1.1 times faster development rates under constant versus fluctuating conditions for both mean temperatures (Figure 4D). For development rate to pupation (T1-T5), there were significant main effects of both diet and thermal regime, but no interaction between diet and thermal regime (Table 1). Development rate to pupation was 1.1 times faster on the high N diet compared to the low N diet. The main effect of temperature resulted in 1.1 times faster overall development rates to pupation under constant versus fluctuating conditions at both mean temperatures (Figure 4B).

Overall, interactive effects of diet and thermal regime were only significant for females in early instars. In general, diet significantly affected male development rates across ontogeny, but only affected females in early instars. Moreover, fluctuating conditions generally reduced development rates for both males and females at both mean temperatures, across ontogeny.
Development Time

Results for development times differed slightly from development rates. For females, there was a significant interaction between thermal regime and diet in their effects on development time to the third instar (T1-T3), in addition to a significant main effect of thermal regime, but no main effect of diet (Table 2). Post-tests detected 1.1 times slower early instar development time for larvae fed low N diet than high N diet at constant 22°C, but no differences between diets for the remaining thermal treatments. Post-tests also detected no difference between constant and fluctuating conditions for either diet around a mean of 28°C in early instars. Around a mean of 22°C, development times under fluctuating conditions were 1.1 and 1.2 times slower than constant conditions for larvae fed high N and low N diet, respectively. The main effect of temperature resulted in 1.2 and 1.1 times slower early instar development times under fluctuating versus constant conditions at 22°C and 28°C, respectively. There was a significant main effect of thermal regime, but no main effect of diet or interaction between diet and thermal regime on late instar (T3-T5) development times (Table 2). Post-tests detected 1.2 and 1.1 times slower late instar development times under fluctuating conditions at 22°C and 28°C, respectively. There was also a significant main effect of thermal regime on development times to pupation (T1-T5) for females. Consistent with both early and late instar development times, fluctuating thermal regime resulted in overall development times 1.2 and 1.1 times slower than constant conditions at 22°C and 28°C, respectively.

For males, there were significant main effects of thermal regime and diet on development times to the third instar (T1-T3), but no significant interaction (Table 2). Post-tests detected 1.2 times slower development time on the low N diet than the high N diet for early instars. The main effect of temperature resulted in an average of 1.1 times slower early instar development time.
under fluctuating versus constant 22°C, but post-tests detected no significant difference between constant and fluctuating 28°C. There was a significant main effect of thermal regime on late instar (T3-T5) development times, but no main effect of diet or interaction between diet and thermal regime (Table 2). Post-tests detected an average of 1.1 times slower late instar development time under fluctuating versus constant conditions at 22°C, but no significant difference between constant and fluctuating 28°C. There were significant main effects of both thermal regime and diet on development times to pupation (T1-T5), however there was no significant interaction between diet and thermal regime (Table 2). Development time to pupation was 1.1 times slower on the low N diet compared to the high N diet. The main effect of temperature resulted in 1.1 times slower development times from hatch to pupation under fluctuating versus constant conditions around both mean temperatures.

Interactive effects of diet and thermal regime on development times were only significant for females in early instars. In general, diet only affected male development times, increasing development in early instars and from hatch to pupation. Overall, fluctuating conditions significantly extended development times in males at 22°C in early and late instars, and females at both mean temperatures across ontogeny.

**Growth**

*Growth rate*

Unlike all other analyzed response variables, there were no significant interactions with sex found in the initial 3-way ANOVA for early instar (T1-T3) growth rates, therefore, data for both sexes was combined for analysis of early instar growth rates. There was a significant interaction between thermal regime and diet in their effects on early instar growth rates, in addition to significant main effects of both diet and thermal regime (Table 3). At 22°C, early
instar growth rates were higher under constant conditions for both diet treatments, but post-tests detected no significant differences between constant and fluctuating conditions for either diet treatment at 28°C. The main effect of diet resulted in an average growth rate 1.4 times faster for high N diet than for low N diet in early instars. Overall, the main effect of temperature resulted in 1.7 times faster average early instar growth rate under constant versus fluctuating conditions at 22°C, but no significant differences at 28°C (Figure 5E). There were significant main effects of both diet and thermal regime on late instar (T3-T5) and overall (T1-T5) growth rates, but no significant interaction (Table 3). Post-tests detected 1.3 times faster late instar and overall growth rates on the high N than the low N diet. The main effect of temperature resulted in late instar growth rates 1.5 times faster under constant versus fluctuating conditions for both mean temperatures (Figure 5C). The main effect of temperature resulted in growth rates 1.5 and 1.4 times faster under constant versus fluctuating conditions from hatch to pupation at 22°C and 28°C, respectively (Figure 5A).

For males, there was a significant main effect of thermal regime on late instar (T3-T5) and overall (T1-T5) growth rates, but no main effect of diet or interaction between diet and thermal regime (Table 3). Post-tests detected 1.3 and 1.2 times faster late instar and overall growth rates under constant versus fluctuating conditions at 22°C and 28°C, respectively (Figure 5, D & B).

In sum, a significant interaction between diet and thermal regime was found for the early instar period only. Generally, low quality diet only reduced female growth rates across ontogeny but had little effect on males. Overall, fluctuating conditions reduced both male and female growth rates at both mean temperatures in late instars and from hatch to pupation, but only affected larvae in early instars at 22°C.
Pupal weight

For females, there were significant main effects of both diet and thermal regime on pupal weights (T5), but no significant interaction between the two (Table 4). Post-tests detected 1.2 times higher pupal weights on the high N than the low N diet. The main effect of temperature resulted in pupal weights 1.3 times higher under constant versus fluctuating conditions for both mean temperatures (Figure 6A).

There was also a main effect of thermal regime on male pupal weights (Table 4), resulting in pupal weights 1.2 times higher under constant versus fluctuating conditions at both mean temperatures (Figure 6B). However, no main effect of diet or interaction between diet and thermal regime was present.

Net growth efficiency

Results for the efficiency of conversion of ingested food (ECI) mirrored that of the long-term growth data. There was no significant main effect of diet, or interaction between thermal regime and diet on ECI. However, there was a significant main effect of thermal regime on ECI (Table 5). Constant thermal regime was found to significantly increase ECI by 29%, from roughly 16% to 26% at 22°C (Figure 7). While post-tests detected no significant differences at 28°C, a similar trend between constant and fluctuating conditions was observed.

Survivorship

Of the 560 experimental larvae at the start of the study, 122 died before the third instar for an early instar mortality rate of 22%, while 200 died before reaching adulthood for an overall mortality rate of 36% across all treatments. There was a significant interaction between thermal...
regime and diet on early instar and overall survivorship, while only thermal regime had a significant main effect on survivorship to the late instars (Table 6).

Generally, mortality was higher under low quality dietary nitrogen conditions for each thermal regime. Surprisingly, mortality rate was higher for larvae fed high quality diet under fluctuating conditions around a mean of 22°C, resulting in mortality rates 9 times higher in early instars and 3 times higher in overall mortality measures (Figure 8, D & B). Furthermore, mortality during pupation was roughly 10% under low quality diet conditions, while only 1% under high quality conditions (Figure 8A). Mortality rates were far reduced in late instars, except for under constant thermal conditions around a mean of 28°C, which increased by 42% from early instar mortality (Figure 8B).

DISCUSSION

Our experiment sought to evaluate performance changes (e.g. development, growth, survivorship) of L. dispar through comparisons of constant and fluctuating thermal regimes and differing diet quality. Using Jensen’s inequality and data from Logan et al. (1991), I predicted that fluctuating thermal conditions would reduce larval performance both near the optimal temperature and at a suboptimal temperature above the inflection point for developmental rate (see Figures 1 & 2). Furthermore, using Jensen’s inequality, I predicted a disproportionately greater reduction in performance near the optimum temperature for development at 28°C compared to the suboptimal temperature of 22°C (see Figure 1B). I also hypothesized that interactions with nutrition (dietary nitrogen levels) could mediate the effects of thermal regime on larval performance.

Overall, consistent with my predictions from Jensen’s inequality, constant temperatures resulted in increased performance compared to fluctuating temperatures for all response
variables, for both sexes, and across ontogeny. However, contrary to my prediction from Jensen’s inequality, disproportionate rate-differences between constant and fluctuating thermal regimes at optimal and suboptimal temperatures were not observed. While the main effect of diet was increased performance on a high quality diet in general, there were significant interactions between diet and thermal regime in the early instars for growth rates, female development rates, female development times, and for overall survivorship.

**Effects of thermal regime**

*Constant vs. fluctuating temperatures*

Numerous previous studies demonstrate that fluctuating thermal conditions tend to reduce larval performance relative to constant conditions (e.g., Logan et al. 1991; Lindroth et al. 1997; Kingsolver et al. 2004; Mironidis & Savopoulou-Soultani 2008; Arrighi et al. 2013). Nevertheless, previous empirical analyses can be improved upon by examining these performance differences between sexes and across ontogeny, and by more closely simulating field-realistic conditions by understanding interactions between thermal regimes and nutritional quality. Therefore, I chose to more thoroughly examine these performance differences resulting from fluctuating and constant thermal conditions around the same mean.

In general, development rates, development times, and growth rates showed similar responses to fluctuating conditions, especially for measurements from hatch to pupation. Constant thermal regimes resulted in significantly higher growth and development rates for both sexes from hatch to pupation and in late instars, at both mean temperatures. While early instar development rates showed significant increases under constant conditions at both mean temperatures, development times and growth rates for the same measurement period only showed significance around a mean of 22°C (Figure 4, E & F; Figure 5E). Interestingly, these
results suggest that fluctuation at cooler temperatures had a more significant effect on early instar growth and development than fluctuation at warmer temperatures; however, these effects disappeared in later instars and may have been a result of interactions with diet quality (see below).

Ontogenetic variation in thermal sensitivity has been documented previously (Bowler & Terblanche 2008; Arias et al. 2011). Bowler and Terblanche (2008) maintain that individual thermal tolerance is highly variable and largely age-dependent. Specifically, they concluded that tolerance to thermal variation at high temperatures is reduced in later life stages, and may be linked to behavioral thermoregulatory adaptation to warm temperatures through increased mobility (Bowler & Terblanche 2008). Similarly, Arias et al. (2011) found variable responses in performance throughout Tenebrio molitor life history. Specifically, they observed variation in developmental reaction norms across ontogeny that were likely due to variation in the energetic cost of physiological processes under stressful conditions (Arias et al. 2011).

Despite some evidence of variable responses to fluctuating conditions across ontogeny, the effects of fluctuating thermal environments on insect performance are generally consistent across empirical studies. For example, Kingsolver et al. (2009) measured the effect of temperature fluctuations on the thermal sensitivity of Manduca sexta growth, and found significantly reduced body sizes and increased development times under fluctuating conditions. Specifically testing predictions made through Jensen’s inequality, Paaijmans et al. (2013) demonstrated that thermal fluctuation reduced development under warm conditions, but increased development under cool conditions for a mosquito species. Similar methods have also been used to investigate effects of thermal variability on population dynamics. Estay et al. (2011) concluded that thermal variability reduces maximum reproductive rates and population
variability in *Tribolium confusum*, which demonstrates how the impacts of thermal variation at the level of individuals can scale up to the level of populations.

Like growth and development, fluctuating thermal regimes reduced survival at both mean temperatures during early instars (T1-T3) and from hatching to pupation (T1-T5). However, fluctuating conditions at 28°C actually increased late instar (T3-T5) survival compared to constant 28°C. Mortality was also higher at 28°C than 22°C for measurements from hatch to pupation. While survivorship was comparable between mean temperatures under constant conditions for early instars, similarities diminished in late instars, where mortality rates accelerated at high temperatures. These results imply that a constant temperature of 28°C is not acutely stressful, but may have long-term consequences for survival across developmental periods, particularly in late instars. Furthermore, despite increased performance at 28°C compared to 22°C in all other performance measures, survivorship appears to decrease at high temperatures across ontogeny.

*Specific predictions from Jensen’s Inequality*

The results were generally consistent with predictions derived from Jensen’s inequality (Figure 1) in that performance was reduced under fluctuating versus constant conditions at both mean temperatures. However, I did not find evidence for a disproportionately larger reduction in performance under fluctuating conditions at the near optimal compared to suboptimal temperature (Figure 1B). Across all performance measures, the proportional differences between constant and fluctuating thermal regimes were indiscriminate between the optimal and suboptimal temperatures selected for our experiment (Figures 4-6). As noted by Kingsolver et al. (2004), an increased range of fluctuation also increases the magnitude of performance response at differing mean temperatures. It is then possible that disproportionate effects between
temperatures were not found due to the extent of variability around the mean temperatures selected for our experiment. This is likely due to selection of a suboptimal mean temperature (22°C) above the inflection point, but with variability (± 6°C) either too low to produce disproportionate responses, or likely crossing the inflection point, resulting in a mixed effect on performance traits. However, further research specifically testing applications of Jensen’s inequality would be needed to test this hypothesis.

Interactions with diet

Not surprisingly, dietary nitrogen content had significant consequences for gypsy moth performance. Despite having equal access to food at unlimited quantities, main effects of diet resulted in reduced performance across all response measures for larvae reared on low quality diet. However, the effect of diet on performance differed between sexes. Low quality diet significantly increased development times only for males across all measurement intervals, while it reduced growth rates for females across ontogeny, and significantly reduced pupal weight in females. Reductions in pupal weight due to diet may be linked to sexual differences in growth rates (Gotthard et al. 1994). Decreases in pupal mass and increases in development time as a result of dietary nitrogen content are important to survival, as they indicate feeding costs and possible trade-offs associated with reduced diet quality (Stockhoff 1993). Increased development time in males in response to a low N diet may represent an acquisition tradeoff whereby slower development increases the likelihood of mortality in nature, but extends time dedicated to allocating resources towards a necessary final size (Angilletta et al. 2003). Similar effects have been noted in Manduca caterpillars, which may delay pupation until a certain weight threshold has been achieved (Scriber & Slansky 1981). Alternatively, our evidence suggests that females
choose to maintain consistent development times between diet treatments, at the cost of reduced growth rates and final size under low N diets. Interestingly, low N diet also resulted in mortality rates ten times higher over the period from pupation to adulthood, compared to high N diet. This result suggests that nitrogen acquired during the larval period is critical for pupation and metamorphosis into an adult.

Of the fifteen individual analyses conducted on performance measures (Tables 1-6), interactions between thermal regime and dietary nitrogen content were significant only for early instar female development, early instar growth rates for both sexes combined, and survivorship from hatch to pupation. Low N diet significantly increased early instar development time under constant conditions but increased survival from hatch to pupation under fluctuating conditions at 22°C. Low N diet also decreased early instar growth rates under fluctuating conditions at 28°C. However, there were no significant crossover interactions present, and the main effect of thermal regime was significant for all analyses.

While it is expected that low quality dietary nitrogen content would negatively impact performance, it is unclear why these significant effects were inconsistent with respect to temperature between early instar development time and growth rate (Figure 5E). Lindroth et al. (1997) found that high nitrogen diet significantly accelerated gypsy moth growth rates under high temperatures compared to low temperatures, but interactive effects disappeared for long-term feeding studies. Similarly, interactive effects disappeared over long-term performance measures in our study. Interactive effects on growth rates can possibly be attributed to either consumption rates or utilization efficiencies. Stamp and Bowers (1990) hypothesized that similar effects in Hemileuca lucina caterpillars are a result of utilization efficiencies so low at low temperatures that improved diet quality has no effect. Lindroth et al. (1997) reached similar
conclusions, attributing interactive effects of growth rate at high temperatures to utilization efficiencies, while conflicting studies relate interactive effects to differences in consumption rates (Stamp 1990). Our results for interactive effects are likely due to differences in utilization efficiencies, where reductions in both net growth efficiency (ECI) and early instar growth rates were significant under fluctuating conditions at 22°C only. Alternative explanations, however, state that interactions are more likely due to extended molting time at low temperatures (Lindroth et al. 1997; Stamp 1990; Stamp & Bowers 1990), or due to utilization of secondary compounds in nature that are not present in our study (Levesque et al. 2002).

Limitations

Laboratory studies impose many limitations when attempting to relate experimental findings to nature. In our experiment, a lab strain of gypsy moth was reared with artificial diet, using casein to supplement nitrogen content. However, the amino acid composition of foliar protein is different from the casein used in artificial diets, and Stockhoff (1991) found that survival time was shorter for wild gypsy moth larvae fed aspen leaves compared to artificial diet. Similarly, lab strains of *Lymantria dispar* are known to have greater overall performance on artificial diets than natural foliage (Grayson et al. 2015). Artificial diets also ignore the effect of secondary allelochemicals found in the field that can either hinder or benefit performance (Levesque et al. 2002). Hemming and Lindroth (1995) found that gypsy moths were strongly affected by phenolic glycosides present in quaking aspen leaves. Additionally, certain allelochemicals have been known to have a toxic effect under laboratory conditions but no effect under natural conditions (Stamp 1990). Furthermore, phenolics and nitrogen content are negatively correlated with leaf age (Meyer & Montgomery 1987) and may contribute to selective feeding through caterpillar ontogeny. Therefore, future studies would benefit from comparing
differences between artificial and natural diets using both natural and wild populations of gypsy moths.

Laboratory conditions also impose limitations on behavioral responses to stressful environmental conditions, including selective feeding and behavioral thermoregulation. Selective feeding highlights the tight synchrony between larval development and host plant ontogeny. Gypsy moth larvae typically hatch in the early spring and feed on plants with an early spring flush whose leaves are rich in nutrients, including nitrogen (Meyer & Montgomery 1987). Later instar gypsy moths are more motile, moving between feeding areas and protected areas below trees (Stockhoff 1993). Higher mobility may be an adaptive behavioral response to phenological variation in leaf flush through selective dietary variation (Hemming & Lindroth 1995), or response to unfavorable thermal conditions through behavioral thermoregulation (Stamp & Bowers 1990). Selective feeding exhibited by phytophagous insects imply an organism’s analysis of feeding costs and selective benefits throughout ontogeny, allowing insects opportunities to choose leaves satisfying nutritional requirements through host plant variability which is limited in our experiment (Stockhoff 1993). Limiting dietary nitrogen content was essential to our experiment, however, future studies investigating selective feeding would benefit from providing the option of both high and low quality diets to larvae.

Finally, and as exemplified by their expansive invasive range, wild gypsy moths experience a wide range of temperatures and thermal regimes (Elkinton & Liebhold 1990; Vanhanen et al. 2007; Bjørnstad et al. 2010). However, for our experiment, I used a laboratory strain of gypsy moth that has been reared at constant temperatures for over 40 years. Specifically, the Otis strain of gypsy moth has been selected for reduced larval maturation times and increased fecundity (Grayson et al. 2015). While Kingsolver et al. (2009) noted no
detectable divergence between wild populations of *Manduca sexta* and lab populations reared at constant temperatures over 250 generations in their response to fluctuating conditions, lab-reared populations have been known to have altered performance responses relative to wild counterparts in field conditions (Grayson et al. 2015). Therefore, it is important to consider these differences when applying conclusions of lab-reared populations to the field. Future studies would then benefit from including both wild and lab-reared populations in their experiments, and from simulating environmental conditions as accurately as possible.

Conclusions

As predicted, fluctuating temperatures generally reduced larval performance for both sexes through ontogeny, resulting in smaller adults. Although a high N diet contributed consistently to increased performance throughout development, interactions between diet and thermal regime were detected only in early instars. Observations of flexible performance responses in *L. dispers* to heterogeneous environmental conditions highlight the need to develop a mechanistic understanding of organismal response to environmental change. This and similar studies will play a critical role in monitoring the response of ecologically relevant species to a changing global climate. These results support the need to consider the complex interactions of naturally variable environmental conditions when applying lab-controlled results to natural environments.
LITERATURE CITED


Keena, M. A., T. M. Odell, and J. A. Tanner. 1998. Environmentally based maternal effects are the primary factor in determining the developmental response of gypsy moth (Lepidoptera: Lymantriidae) to dietary iron deficiency. Annals of the Entomological Society of America. 91: 710-718.


### Table 1. ANOVA results for the effects of thermal regime and diet on *L. dispers* development rate across ontogeny.

<table>
<thead>
<tr>
<th>Response</th>
<th>Interval</th>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>F Ratio</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
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1 measurement intervals from Figure 3
Table 2. ANOVA results for the effects of thermal regime and diet on *L. dispar* development time across ontogeny.

<table>
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<th>P Value</th>
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1 measurement intervals from Figure 3
Table 3. ANOVA results for the effects of thermal regime and diet on *L. dispar* growth rate across ontogeny.

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<th>Males</th>
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<td>Growth Rate</td>
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<td></td>
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</table>

1 measurement intervals from Figure 3
2 data for sexes combined
3 log-transformed data
Table 4. ANOVA results for the effects of thermal regime and diet on *L. dispar* pupal weight.

<table>
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<th>Response</th>
<th>Interval</th>
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<td></td>
<td></td>
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<tr>
<td>Pupal Weight</td>
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<td></td>
<td></td>
<td>Diet x Thermal Regime</td>
<td>Diet x Thermal Regime</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Error</td>
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1 measurement intervals from Figure 3
Table 5. ANOVA results for the effects of thermal regime and diet on *L. dispar* efficiency of conversion of ingested food (ECI), also known as *net growth efficiency*.

<table>
<thead>
<tr>
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<td>Error</td>
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Table 6. Logistic regression results for the effects of thermal regime and diet on the survival of *L. dispar* across ontogeny.

<table>
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<th>Interval</th>
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<th>Prob&gt;ChiSq</th>
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<td>Diet x Thermal Regime</td>
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1 measurement intervals from Figure 3
Figure 1. A graphical representation of predictions from Jensen’s inequality based on the shape of an organism’s thermal performance curve (TPC). For a typical TPC, performance increases from the lower thermal limit (LTL) as an accelerating function to the point of inflection, after which the function decelerates towards the optimum ($T_{opt}$). In panel (A), for a given mean temperature below the inflection point (solid blue line), average performance under fluctuating conditions (dashed blue lines) is predicted to be greater than performance under constant conditions. For a given mean temperature above the inflection point (solid red line), average performance under fluctuating conditions (dashed red lines) is predicted to be less than performance under constant conditions. In panel (B), for a mean temperature at $T_{opt}$ (solid red line), average performance under fluctuating conditions (dashed red lines) is predicted to be reduced even greater relative to sub-optimal temperatures (panel A) above the inflection point. See text for further details.
Figure 2. Thermal performance curve (TPC) for developmental rate of 3rd instar *L. dispar* redrawn from data in Logan et al. (1991). Vertical lines represent the near-optimal (28°C, dashed line) and suboptimal (22°C, dotted line) temperatures chosen for this experiment. Bracketed lines represent the range of thermal fluctuation (±6°C) experienced by larvae under fluctuating conditions at each temperature. See text for further details.
Figure 3. Larval measurement intervals (T) through *L. dispar* ontogeny. T1-T3 represent early instars and T3-T5 represent late instars. T1-T5 designates measurements from hatch to pupation.
Figure 4. Development rates (mean + 1SE) for both sexes of *L. dispar* larvae reared on low (light bars) and high (dark bars) levels of dietary nitrogen as a function of constant (22C, 28C) and fluctuating (22F, 28F) temperature regimes during (E, F) early instars, (C, D) late instars, and (A, B) from hatch to pupation. Bars with different letters are significantly different based on Tukey’s HSD post-tests. In cases with no significant interaction between diet and thermal regime, letters indicate differences among thermal regimes for the diets combined. In cases with a significant interaction, letters indicate differences among thermal regimes at each level of diet.
Figure 5. Growth rates (mean + 1SE) for both sexes of *L. dispar* larvae reared on low (light bars) and high (dark bars) levels of dietary nitrogen as a function of constant (22C, 28C) and fluctuating (22F, 28F) temperature regimes during (E) early instars (for males and females combined), (C, D) late instars, and (A, B) from hatch to pupation. Bars with different letters are significantly different based on Tukey’s HSD post-tests. In cases with no significant interaction between diet and thermal regime, letters indicate differences among thermal regimes for the diets combined. In cases with a significant interaction, letters indicate differences among thermal regimes at each level of diet.
Figure 6. Pupal weights (mean ± 1SE) for both sexes of *L. dispar* larvae reared on low (light bars) and high (dark bars) levels of dietary nitrogen as a function of constant (22C, 28C) and fluctuating (22F, 28F) temperature regimes. Bars with different letters are significantly different based on Tukey’s HSD post-tests. In cases with no significant interaction between diet and thermal regime, letters indicate differences among thermal regimes for the diets combined. In cases with a significant interaction, letters indicate differences among thermal regimes at each level of diet.
Figure 7. Effect of constant (22C, 28C) and fluctuating (22F, 28F) thermal regimes on *L. dispar* net growth efficiency (ECI).
Figure 8. Mortality rates (mean + 1SE) for *L. dispar* larvae reared on low (light bars) and high (dark bars) levels of dietary nitrogen as a function of constant (22C, 28C) and fluctuating (22F, 28F) temperature regimes during (D) early instars, (C) late instars, (B) from hatch to pupation, and (A) from pupation to eclosion.
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