The Younger Games:

Flies Compete for Oviposition Sites that Benefit Their Young

by

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ABSTRACT

I examined how competition affects the way animals use thermal resources to control their body temperature. Currently, biologists use a cost benefit analysis to predict how animals should regulate their body temperature. This current theory of thermoregulation does not adequately predict how animals thermoregulate in the wild. While the model works well for animals in low cost habitats, it does not work as well for animals in high cost habitats. For example, animals that are in habitats of low thermal quality thermoregulate more precisely than predicted by the current model. One reason these predictions may be wrong is that they do not account for interactions between animals. By including these interactions in future predictions, a more accurate model of thermoregulatory behavior can be created.

Before developing a theory for all animals, a model needs to be developed for a single model animal, such as fruit flies, that can be used to empirically examine how organisms thermoregulate under competition. My work examines how flies behave around other flies and develops a game theory model predicting how they should optimally behave. More specifically, my research accounts for competition among larvae by using game theory to predict how mothers should select sites when laying eggs. Although flies prefer to lay their eggs in places that will offer suitable temperatures for the development of their larvae, these sites become less suitable when crowded. Therefore, at some density of eggs, cooler sites should become equally beneficial to larvae when considering both temperature and competition. Given this tradeoff, an evolutionarily stable strategy (ESS) emerges where some flies should lay eggs in cooler sites while other flies should lay eggs at the warmer temperature. By looking at the

fitness of genotypes in habitats of differing quality (competition, temperature, food quality, space), I modeled the ESS for flies laying eggs in a heterogeneous environment. I then tested these predictions by observing how flies compete for patches with different temperatures.

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CHAPTER 1

INTRODUCTION TO THE DISSERTATION

Organisms use behavior and physiology to keep their body temperature within a tolerable range. One way that organisms can alter their thermoregulatory behavior is by changing how much time they spend in patches that differ in temperature. Other common, behavioral mechanisms include basking in the sun and flattening one's body against a warm rock to warm up.

Thermoregulation is important to animals because many physiological processes are dependent upon the body temperature of the animal. An animal that is closer to its optimal temperature may be able to run faster, digest food more quickly, or even increase their growth rate. While a colder animal may see a reduction in its performance – sometimes this reduction can be beneficial, such as a decreased metabolism, which can allow organisms to conserve energy and even enter a hibernation-like state.

It is especially important for us to know how animals will continue to thermoregulate as they experience changes in their environment due to climate change. As temperatures across the globe are rapidly changing, we need to understand how organisms will respond to these changes. Since many organismal performances depend on temperature, we can expect organisms to be impacted by these changing temperatures. Consequently, we need to be capable of predicting how these organisms will respond to changes in their environment.

I looked at how animals control their body temperature when they compete with other animals. Currently, what we know about how animals control their temperature is primarily based on studying isolated animals. Thus, when we attempt to predict how

animals should control their temperature, these predictions may use incorrect assumptions about animal behavior. One reason these predictions may be wrong is that they do not account for interactions between animals. By including these interactions in future predictions, we can more accurately predict animals' behavior towards temperature and how climate change will affect these behaviors.

My work looks at how flies behaviorally thermoregulate around other flies and develops a game theory model predicting how they should optimally behave. More specifically, my research accounts for biotic interactions by using game theory to predict how fruit flies should select sites when laying eggs. Although flies prefer to lay their eggs in places that offer suitable temperatures for the development of their larvae, these sites become less suitable as larvae become crowded. Therefore, at some density of eggs, sites that are sub-optimal in temperature should become equally beneficial to larvae when considering both temperature and competition. Given this tradeoff, an evolutionarily stable strategy (ESS) emerges where some flies should lay eggs in sites that are sub-optimal in temperature while other flies should lay eggs at sites that are the optimal temperature. By using the fitness of isofemale fly lines under different scenarios, I developed an ESS model predicting how flies should behave. I then tested these predictions by allowing flies from the same isofemale line to compete for patches that vary in thermal quality.

Additionally, I examined how other factors such as food quality, space availability, and flies' past thermal environments further affect this relationship. By examining these additional factors, I could see how flies from different types of habitats were impacted by these changes and whether different ecological factors compounded or

reduced the role that temperature played in oviposition site selection. Lastly, by examining how flies from different populations chose their oviposition site in relation to the thermal history that these flies were exposed, I was able to see how different fly populations might differ in their ability to thermoregulate.

Understanding how these additional abiotic factors influence thermoregulatory behavior under varying degrees of competition is very important as we attempt to predict how climate change and land use changes might affect organisms. This additional knowledge about how organisms thermoregulate is important because even a small change can have a drastic impact on predictions made by models. Mechanistic models are a common model that is used to predict how organisms respond to environmental change. To create a mechanistic model, we require physiological and ecological data to be inputted for each organism. Consequently, we need to know how different biological factors can have an integrative effect on each other in order to make accurate predictions.

An example of how changing the assumptions of mechanistic models affect their predictions can be seen with my work looking at how quickly animals' digest and assimilate energy at different temperatures. Animals have optimal temperatures at which they maximize the rate at which they digest and assimilate energy. However, if animals spend increased amounts of time at these optimal temperatures, they begin to see reduced energetic benefits from continuing to spend more time at this temperature. As a result, even if animals spend a long time at this optimal temperature, they may see little to no additional energetic benefit from this behavior after a set amount of time has passed. However, most mechanistic models that are used for predicting species ranges assume that this relationship between assimilation rate and temperature is linear. Therefore, we

parameterized the assimilation rates of lizards within our mechanistic model using both a linear function and an asymptotic, plateauing function to see how this changes the predictions of the model. By incorporating more realistic functions, such as an asymptotic function for assimilation rates, we can significantly alter predictions made by mechanistic models.

Making quantitative predictions for how temperature affects animals

Quantitative models enable us to make predictions about how organisms should respond to different environmental factors. Being able to make predictions about how organisms should respond to temperature is especially important both for our understanding of the natural world and for understanding how organisms will respond to a changing world. My dissertation examines how organisms' thermoregulatory strategies are altered as they experience temperature variation. Previous work at creating quantitative predictions of how organisms' thermoregulate has resulted in very useful, but simplistic, models that make general assumptions about how organisms should thermoregulate. While these models have been very useful and important, they also are not as accurate as they could be when applying them to animals that are found in nature (Blouin-Demers and Nadeau 2005, Levy et al. 2017). Consequently, we need to create more complex, quantitative models to help predict how organisms thermoregulate in nature. My dissertation attempts to do this by trying to understand how factors such as competition, space availability, food quality, and food assimilation are affected by temperature for organisms that have experienced different thermal backgrounds. I developed quantitative predictions for these ecological factors using natural populations of both flies (Chapters 2-4) and lizards

(Chapter 5) from different regions of the US, each population having experienced different temperature variations within their evolutionary histories. By creating quantitative models that account for variation in abiotic and biotic factors, we will be able to better understand and predict how temperature variation affects animals both currently and in the future.

Introduction to the attached chapter

My attached paper (Chapter 5) was originally published in the Journal of Ecology and examines how organisms see diminishing energetic returns from spending time at optimal temperatures, which ultimately affects the predictions of mechanistic models. If organisms spend a large amount of time at their optimal temperatures, they see reduced energetic benefits from continuing to spend time at this temperature. As a result, even if organisms continue to spend time at this temperature, they may see little to no additional energetic benefit from this behavior. However, most mechanistic models that are used for predicting species ranges assume that this relationship between assimilation rate and temperature is linear. Therefore, we parameterized our mechanistic model using both a linear function and our asymptotic function for assimilation rate to see how this changes the predictions of the model. By incorporating functions with diminishing returns, we can significantly alter predictions being made by mechanistic models.

This project had both a modeling and empirical component. I headed the empirical component, while Dr. Ofir Levy headed the modeling component. I worked as part of a team of five people. Dr. Travis Rusch helped to collect the study organisms and perform data collection. Dr. Mike Angilletta and Dr. Lauren Buckley oversaw the

completion of the project and provided funding. Ofir and Lauren created the mechanistic model. Ofir and Mike revised the final version of the manuscript along with feedback from Lauren, Travis, and me.

My duties included developing the experimental protocol for the project. Additionally, I trained/organized Travis and the undergraduates that were helping with the data collection. I led the work on collecting the study organisms as well as collecting the assimilation, consumption, and other physiological data for the study. I then performed the data analyses on the physiological data. I also wrote the first several drafts of the manuscript. Further, I presented the work at the Society of Integrative and Comparative Biology Conference.

This project originally started simply as a project to collect data for a mechanistic model. I was involved in the discussions and feedback as the data I was collecting was coming in and being analyzed. Because of these statistical analyses, the scope of the project changed. As the data began to show that it violated some of the basic assumptions of mechanistic models, we decided to change the type of question we were looking at. Rather than simply using the data to parameterize a mechanistic model, we decided to see if using a more realistic assimilation rate for the lizards altered the predictions being made by the model. We then brought Ofir on board with the project and began to test our new question about how non-linear assimilation rate affects the assumptions made by mechanistic models.

CHAPTER 2

THE HUNGER GAMES: FLIES CHOOSE OVIPOSITION SITES THAT BENEFIT THEIR YOUNG

CHAPTER 2: The hunger games: flies choose oviposition sites that benefit their young

Summary

I used game theory to predict how fruit flies, *Drosophila melanogaster*, should compete for oviposition sites. As both temperature and competition affect the fitness of an organism, the covariance between these variables should influence behavior. I modeled how flies can maximize fitness when choosing between patches that differ in surface temperature and intraspecific competition. Under low competition for food and space, flies should lay their eggs in a warm patch that promotes growth and development. However, as competition increases, flies benefit by laying some eggs in a cooler patch, which offers more food and space for their offspring. In other words, competition should cause mothers to choose less crowded patches despite the thermal cost. To look at this tradeoff, I observed where flies laid eggs given various densities of competitors. Flies at low-density laid eggs almost exclusively at 25°C. However, flies at high-density laid a greater proportion of eggs at 16°C than flies at low-density did. Surprisingly, flies did not avoid laying at 25°C when eggs were already present, suggesting that females responded to the presence of other females rather than the presence of eggs. By drawing on game theory to make quantitative predictions, this research builds on previous empirical studies of competition among thermoregulating animals.

Introduction

The current theory of thermoregulation predicts how an organism should thermoregulate within a heterogeneous environment. Mathematical models define the costs and benefits of a behavior given the frequency of thermal patches and the thermal sensitivity of performance (Huey and Slatkin 1976). However, researchers have noted that the theory does not adequately predict how organisms thermoregulate in nature. For example, Blouin-Demers and Nadeau (2005) concluded that lizards thermoregulate in low thermal quality environments much more accurately than predicted by an optimality model. This more precise thermoregulation may be due to costs ignored by the basic theory. Sears and Angilletta (2015) showed that the energetic cost of thermoregulation depends on the spatial distribution of preferred patches as much as their frequency. Still, non-energetic costs of thermoregulation, such as those imposed by competition, might also account for the unexpected behaviors of animals. Animals interact with each other during thermoregulation, and these interactions may influence the optimal behavior. Unfortunately, the role of competition has largely been ignored when modeling optimal thermoregulation (Angilletta 2009).

Game theory enables one to incorporate competition into a model of thermoregulation. Game theory describes how organisms allocate time to different patches when the preferences and frequencies of competitors determine the benefits of patch choice (Sih 1998, Brown et al. 1999). The ideal free distribution (IFD) describes how organisms should distribute themselves within their environment. Under the IFD, all organisms' strategies should be receiving an equal payoff within the game. In a density-independent environment, organisms are going to want to exploit resource rich patches.

However, if these resources are density-dependent, these patches are going to decrease in quality as more organisms move into the patch. Therefore, at some density, some percentage of organisms should begin to switch over to exploiting patches that are poorer in resources as the payoff from exploiting this patch equilibrates the payoff from the other patch (Křivan et al. 2008). For antagonistic competitors to coexist, the behaviors of these competitors must reach a stable equilibrium where the fitness of each competitor is equal to each other, referred to as an evolutionarily stable strategy, or ESS (Maynard Smith 1982, Nowak and Sigmund 2004). An ESS explains why multiple strategies coexist in a population when each strategy confers equal fitness and is not exploitable by a single mutant strategy. In these situations, where the fitness of a strategy depends on its frequency in the population, each organism's best strategy depends on what other organisms are doing (Nowak and Sigmund 2004).

A thermal game is a type of evolutionary game in which competitors choose habitat patches based on their temperatures (Angilletta 2009). Viewing a system in the context of a thermal game has delivered novel insight into how predators and prey interact in a thermal landscape, such as dragonfly larvae and tadpoles in a heterogeneous pond (Hammond et al. 2007). Studies have also begun to use game theory to examine how social dominance can cause subordinate crayfish to choose suboptimal thermoregulatory strategies (Tattersall et al. 2012) and how differences in food density can cause beetles to shift their thermoregulatory strategy based on food availability (Halliday and Blouin-Demers 2014). These empirical studies were partly inspired by models in which organisms must choose thermal patches in the presence of others.

An early model of a thermal game, developed by Hugh's and Grand (2000), defines the ideal free distribution of an ectothermic species that chooses thermal patches to maximize its growth rate. In this model, growth depends on the temperature of the patch, the availability of food, and the density of competing organisms. According to this model, organisms should prefer warm patches when there is high-food availability. However, when food is scarce, ectotherms should prefer cool patches due to the ectotherm having decreased metabolic functions. As the density of the population increases, the ESS includes a greater proportion of individuals that use the cooler patch, because the warm patch imposes d a certain threshold level of individuals. This model is a significant improvement over past models since most models tend to only consider the possible food consumption of an organism and do not examine how temperature and density levels may affect food rate intake, which will ultimately translate into growth and development. Here, I extend the model of Hughes and Grand to understand how abiotic and biotic factors interact to influence the behavior of fruit flies (*Drosophila melanogaster*). These flies must choose where to lay eggs given that temperature and competition affect the performance of offspring (Krebs and Loeschcke 1994, Gilchrist and Huey 2001). Flies prefer to lay eggs at select temperatures, that vary depending upon the developmental temperatures experienced by the flies (Dillon et al. 2009), choosing to avoid cooler or warmer sites (Schnebel and Grossfield 1986, Feder et al. 1997a). However, flies do not change their likelihood of oviposition on a necrotic fruit based on whether that same necrotic fruit has previously reached lethally high temperatures (Feder et al. 1997b) The fitness of fruit flies is also influenced by the densities of flies at a site due to an Allee effect at low-density followed by decreased survival at higher densities

(Wertheim et al. 2002). Consequently, fruit flies likely play a thermal game when deciding where to lay eggs in an environment with patches of food and temperature.

I will examine how fruit flies choose between microclimates when laying eggs under different levels of competition. I will develop a quantitative game theoretical model that will predict how I expect flies to alter the frequency in which they choose to lay their eggs in a patch. I will then empirically test the model by allowing flies to choose between two patches under varying levels of competition. By treating the behavior of flies as a thermal game, we should better predict their behavior in real environments, which vary in density, food, and temperatures. In this way, I hope to increase our understanding of how organisms thermoregulate when faced with competition for resources in a patchy environment.

A Game Theoretical Model of Oviposition Behavior

I found the ideal free distribution of the egg distribution using a method based on maximizing population growth between the two patches similar to the patch-choice model of Fretwell and Lucas (1969a). This model finds an ESS for these flies when choosing a thermal patch for their eggs in the presence of competing females. Flies choose to lay their eggs in either a warm or cool patch based on the growth rate, survivorship, and fecundity in each patch. As flies lay eggs in the patch that confers the greatest fitness, competition becomes more intense in that patch, which in turn, decreases the value of that patch. At a low-density, flies should choose the patch with the best temperature for their young. As the density of eggs increases in the patch, flies should lay some eggs in a cooler patch with fewer competitors (Figure 2.1). Thus the fitness of each

fly is represented by its fitness generating function, or G-function (Vincent and Brown 1988). This function accounts for a given fly's strategy and the strategies of all other flies.

To calculate the fitness of flies, I used the Euler equation:

$$1 = \sum_{x=1}^{\omega} \lambda^{-a} l(x) b(x), \quad (1)$$

where α is time it takes a fly to develop from an egg to an adult, x is age, l is survivorship from egg to adult, and b is the fecundity of the transferred eggs.

I used the ideal free distribution to model the ESS. First, I created a null strategy by dividing the number of eggs equally between patches. If the number of eggs was odd, the cold patch started with one additional egg. I then calculated the fitness of this null strategy using the Euler equation. I created mutant strategies by shifting a single egg between patches. Then, I compared the fitness of the mutant strategies with the fitness of the null strategy. If a mutant strategy was fitter than the null strategy, the mutant strategy became the null strategy. I continued to compare strategies until I could not find a fitter mutant strategy. I coded the model in Python Version 3.5 (code available upon request).

Since offspring develop in the area where their mother deposits them, flies should choose beneficial environments for their young. Therefore, although flies prefer to lay their eggs in places that will offer prefer microclimates that speed the development of their offspring, these sites will become less suitable as larvae become crowded and consequently have less food and space available to them. Consequently, there is an optimal temperature at which flies should choose to lay their eggs in a density-independent environment. However, in an environment where flies experience density-dependent effects, sites that differ in temperature from the density-independent optimal

temperature should become equally beneficial to larvae at some density when flies are considering both temperature and competition. Given this tradeoff, an evolutionarily stable strategy should emerge in which some flies lay eggs at the density independent optimal temperature sites while other flies will lay eggs at their eggs at an assortment of other temperatures when laying eggs in mass.

Methods

Maintenance of *Drosophila melanogaster*

I used flies descended from females collected in Beasley Orchid, Indiana, during the fall of 2011. Cooper and colleagues created twelve isofemale fly lines by mating a single adult virgin female fly with a male sibling for two generations (Cooper et al. 2014). The isofemale lines descended from these experimental populations capture aspects of the genetic variation of these populations. I maintained the isofemale lines on a standard cornmeal-yeast diet (recipe of the Bloomington Stock Center, Bloomington, IN) in 25 x 90 mm vials (Genesee Scientific, San Diego, CA) inside of an incubator (Percival Scientific, Perry, IA) at a temperature of 21°C and a light cycle of 12:12 L:D. Flies were transferred to new vials with fresh food approximately every three weeks.

Two males and two females from the same isofemale line were added to a vial for 48 hours to partially control for egg and larval density. When the next generation of flies emerged in these vials, I transferred two males and two females to a new vial for 48 hours. I repeated this protocol for three generations. Adult females from the third generation were used in the experiments described below.

Parameterizing the model

I used data collected from flies in the lab to parameterize of my model (Figure 2.2). To estimate the fitness of flies at each combination of density and temperature, I measured the survivorship, fecundity, and developmental time. To obtain flies for my experiment, I allowed females from 12 isofemale lines to lay eggs in petri dishes with a grape agar and yeast solution for 8 hours at 23°C. I then transferred either 1, 5, 15, or 50 eggs to new petri dishes (35mm diameter) containing the grape agar and 0.033g of yeast. These petri dishes were kept an incubator at 16 °, 20°, 25°, or 30°C with a 12:12 light cycle. I measured developmental time by checking the flies daily to record the number of days for adult flies to emerge. I measured survivorship by counting the proportion of adults that survived to adulthood.

To measure fecundity, I outbred adult female flies from my treatments with male flies from a control line (Cantonese, Bloomington Stock Center, Bloomington, IN) to capture only variation in fecundity because egg production, not sperm production, is typically the limiting factor for population growth. Additionally, since I was concerned about the effect of larval developmental temperature and adult flies would be able to freely move to new patches that varied in temperature, I moved all vials to a common temperature of 20°C for my fecundity experiments. I placed each pair of flies into a vial (25 x 95 cm) containing a standard cornmeal-yeast medium for four days in an incubator at 20°C with a 12:12 light cycle. I then removed the adult breeding pair and allowed any eggs laid by these fly pairs to develop to adulthood. From the adult flies that emerged within each of these vials, I again recorded developmental time (egg to adult) to the nearest day and counted the total number of flies emerged (fecundity).

I estimated how density and temperature related to fecundity and developmental time using a generalized linear modeling while I estimated survivorship using a logistic regression curve. I then used Akaike information criterion (AIC) to pick the best polynomial fit for each curve in R (Version 3.3.1, R-Core-Team 2016) (Zuur 2009). I then developed additional models based on predictions of how one might expect the fitness of flies to vary under different circumstances. I used the best fitting curve developed from the raw parameters to find an initial curve that describes how survivorship and fecundity change with increasing density. Furthermore, I hypothesized additional parameter data that was in line with findings on fitness data to create additional models regarding how you would expect flies to behave if their survival parameters were slightly altered. To create this additional parameter data, I also increased or decreased how quickly survivorship changed under increased competition since in nature you might have conditions changing within these patches causing survivorship to differ from what we measured. For example, resources might be replenished within these patches, which would cause survivorship to decline more slowly. Alternatively, predators might be attracted to higher densities of prey, which would cause survivorship to decline more quickly and possibly at an exponential rate.

Experimental tests of the model

To determine oviposition site preference in adults under different levels of competition, I created 2 identical thermal arenas in which flies chose between two petri dishes of agar medium (Figure 2.3). My thermal arena consisted of a Plexiglas container that consisted of six lanes by running copper tubing below small aluminum plates. Each lane 60mm

wide by 175mm long and had two aluminum plates on opposite ends of the lane where I could place an agar-filled petri dish with a drop of yeast. Flies could easily move between these patches when laying eggs. The temperature of each patch was controlled by conduction and convection between the agar medium and a metal plate. The plate was heated or cooled by water flowing through the copper tubing from a digital water bath (VWR, Radnor, PA).

I used the thermal arenas to test where flies from 15 isofemale lines chose to lay their eggs under different intensities of competition. In each arena, a fly could choose between a petri dish at a preferred temperature of 25°C and a petri dish at 20°C. For each isofemale line, I ran trials in which either 4 or 15 adult females were in an arena at a time. After 4 hours, I removed the flies and froze the petri dishes from each side of the arena. Later, these dishes were thawed, and the number of eggs was counted under a dissecting scope (Zeiss Stemi 2000-C) at each temperature.

I ran a second experiment in which eggs were added manually to the petri dish at the preferred temperature. In half of the trials, I added 100 eggs to the warm dish before placing females in the arena. In the other half, no eggs were added. In both cases, four females were allowed to lay eggs for a period of 4 hours. Eggs were counted as in the previous experiment.

I performed a linear mixed effects model to estimate the expected number of eggs laid under each combination of density and temperature. I assumed a Poisson distribution of error and adjusted the model for zero-inflated data (Zuur 2009). Models were fit using the glmmADMB package (Fournier et al. 2012) of R (Version 3.3.1, R-Core-Team 2016). The fixed factors in my model were temperature and density/treatment. I used

trial as a random factor to compare eggs laid in the two sides of the arena while accounting for variation in the number of eggs among trials. Using AIC values, the experimental date and isofemale line was dropped from my model due to poor fit.

Results

I modeled the fitness of flies using data on survivorship, development time, and fecundity. The survivorship (Figure 2.4) and fecundity (Figure 2.5) of flies depended on density and temperature during larval development. However, flies' development time only differed at different temperatures (Figure 2.6). Flies had slightly higher survival at 20°C than 25°C. Flies also had a slight Allee effect in 20°C, but not 25°C patches. Flies had higher fecundity at low densities in 25°C patches than in 20°C patches, but had higher fecundity at high densities in 20°C patches. The developmental time of flies decreased sharply with increased temperatures.

My game theoretical model (Figure 2.7) illustrates how competition affects the fitness of fruit flies. The model predicts that under scenarios where survivorship in hot patches falls quickly, fruit flies should lay eggs in the colder patch at lower density levels. In scenarios where survivorship in cold patches falls quickly, fruit flies should lay fewer total eggs in the cold patch. At low densities in the model, flies should lay their eggs in the hot patch. As the density of eggs in the hot patch increases, flies should then begin to also lay eggs in the cold patch.

I found that temperature and competition both affected patch choice in flies, but that pre-existing eggs did not alter their patch choice. Flies at low-density laid eggs almost exclusively at 25°C ($P < 0.001$), but those at high-density laid a significantly

greater proportion of eggs at 20°C than did flies at low-density ($P < 0.01$). At low densities, flies laid about 1.6x as many eggs in the warm patch (median=2.75) than in the cold patch (median=0.25). While at high densities, flies laid almost equal number of eggs in the warm patch (median=0.467) and in the cold patch (median=0.60). I found no differences in the proportion of eggs laid between sites when eggs were added pre-trial to their preferred temperature (P=0.43) (Figure 2.8, Table 2.1). When eggs were added pre-trial to the preferred, warm patch, flies still predominantly laid their eggs in the warm patch (median=1) as opposed to the cold patch (median=0). Flies laid the most total eggs per fly in the low-density treatment with no added eggs (median=3), while both the high-density treatment (median=1.07) and treatment with eggs added pre-trial (median=1) had about the same number of total eggs per fly.

In trials without any added eggs, flies laid their eggs in accordance with our model. Both our model predictions and empirical findings show flies primarily laying their eggs in the hot patch at low densities. At high densities, both the model and empirical findings show flies laying their eggs in both the hot and cold patch. However, in trials with pre-added eggs, flies did not behave in accordance with our model. Flies instead behaved as though no eggs were already present on the media and laid their eggs in a similar manner as the low-density treatment.

Discussion

Flies preferred to lay their eggs in the 25°C patch when there were few flies present. This finding is in line with where we would expect a fly to lay its eggs in the absence of competition. Under higher densities, however, flies began to spread their eggs between

patches, laying about 56% of their eggs in the 20°C patch. These finding accords with the model's prediction that flies should choose to lay some eggs in patches at suboptimal temperatures as density increases.

I found that female flies appear to respond to other adults in the thermal arena and not to the presence of eggs already laid in the patch. By comparing the number of eggs between trials with four females and trials with 15 females, I saw whether females responded to the level of competition. Furthermore, by comparing the number of eggs between trials that started with zero eggs and those that started with 100 eggs, I could see whether females responded to the presence of other adults or eggs. Flies did not avoid laying at 25°C when eggs were present, suggesting that flies responded to the presence of competing females rather than cues associated with the presence of eggs. This result is surprising given that flies in previous experiments have been shown to actively probe sites for food quality and substrate hardness before choosing where to oviposit (Yang et al. 2008a), to find carbohydrate (Young et al. 2018) or acetic acid (Joseph et al. 2009) rich media, and to avoid toxins such as ethanol (Miller et al. 2011). Given that flies did not respond to the eggs, this change in their behavior was probably due to the presence of other adult flies.

Game theory predicts that flies will alter their behavior and lay some eggs in both patches as density increases. In our experiments, the flies seem to shift their behavior and patch preferences as density increases, but a single isofemale line of flies does not appear to follow the patterns predicted by our model if it is a game where each player can perform a mixed strategy. Often when people think of players performing a game, they imagine that each player will change their strategy to optimize their fitness – this is a

game where each player can perform a mixed strategy. However, this scenario of each player being able to play a mixed strategy is not always the case.

Sometimes each player in the game has a pure strategy where they can only perform a single, fixed strategy and cannot or does not change their own strategy. If proportions of individuals within a population each exhibit a different fixed strategy, you should still see a mixed strategy across the population scale (Dawkins 1980). When different players each have a single, fixed strategy, like in our flies, you might find something that more closely resembles the 'shotgun blast' strategy that we see in our flies. Rather than each fly individually choosing to lay their eggs in different patches, you see some flies choose a single patch to primarily lay their eggs. But since each fly is choosing a different patch to primarily lay their eggs, we see a disbursement of eggs that is spread out between the patches as you would expect from our game model. This pattern is then maintained across the population because if one strategy starts to become rarer within the population, those flies will begin to have a higher fitness level and will increase their relative proportion in the population within the next generation. In this scenario, some flies will have different fixed preferred temperatures that they choose for their young. This variation in preferred temperatures across flies may explain some of the discrepancies we see when applying the Huey and Slatkin (1976) cost and benefit model of thermoregulation.

Flies may also want to lay their eggs where other larvae already exist. Flies rely on current environmental conditional when choosing oviposition sites (Levins 1969) and do not avoid sites that have previously experienced lethal temperatures (Feder et al. 1997b). While some studies which appear to show that flies in nature are choosing sites

based on temperature may actually only be picking up changes based on larval survival at different sites (Jones et al. 1987a, Huey 1991). Consequently, flies may not be able to rely solely on current environmental data if they want to maximize their fitness. Further, some species of *Drosophila* have been shown to preferentially lay their eggs at sites where larvae are already present (Solar and Palomino 1966) which lends credence to the idea that flies are gaining a benefit from choosing oviposition sites where eggs have already been laid. Additionally, fly fitness has been demonstrated to show an Allee effect with small increases in density causing increased fly survivorship as the presence of additional fly larvae can help break down and soften the substrate media (Wertheim et al. 2002). However, other insects, such as weevils, have demonstrated very strong avoidance and fitness effects of laying eggs near other eggs (Mitchell 1975), while some butterflies even use egg mimics to deter other eggs from being laid near their own eggs (Williams and Gilbert 1981).

However, this behavior may also be non-adaptive for survival of the fly's young. It is possible that flies avoid crowded sites to evade aggression from the other adult flies. In this scenario, flies are changing their patch choice not to maximize the fitness of their young, but simply to avoid any potential aggression from other flies present near the patch at the optimal temperature. Drosophila larvae are believed to use methods to exclude other larval competitors even when food resources remain constant in the environment (Gilpin 1974). Consequently, if fly larvae are using interference competition, it would make sense that the adult flies could also be engaging in interference competition when choosing their sites. Additionally, numerous other studies have found examples of interference competition in insects for oviposition sites

(reviewed in Denno et al. 1995). However, if flies are responding to aggression from other flies in the crowded treatment, you would expect each individual trial of flies to produce a near even split between the two patches. However, given that what you see at the individual trial level almost appears to be a 'shotgun blast', it suggests that it is not only due to the flies spreading themselves out to try to avoid aggression from other flies.

It is also possible that within these crowded situations that my flies are simply trying to make the best of a bad situation and just trying to reproduce while they still can. In the crowded thermal arena environment, the flies might be aware that there are far too many flies present relative to suitable areas for their young. Consequently, the flies may just be making an immediate decision to lay their eggs immediately wherever they can without attempting to use any decision-making process since they are worried that any delay in starting their reproduction would be more costly than waiting and trying to find a more suitable habitat. This problem can be seen in butterfly populations that choose to lay their eggs before they necessarily learn their surrounding environments and benefits of other patches (Papaj 1986). This problem could be further compounded by their previous existence living and reproducing within crowded vials in the lab. Granted even if they are engaging in this behavior and simply laying anywhere, in the crowded environment this still enables them to lay at their ESS where they should be spreading their effort between patches.

Previous studies that have applied game theoretical ideas to resource distribution give us further insight into our findings. In Hammond et al. (2007) study looking at predator-prey games in dragonflies and tadpoles, they found that the size of the individual relative to other predators and prey also affected how individuals distribute themselves

within space. Even though all of a fly's competitors were from the same isofemale line, I did not control directly for size across our competition treatments so I may also be seeing the artifacts of different sized competitors forcing individuals to choose sites that differ from the density independent optimal temperature. This finding is also further backed by another study that found they could predict how a pair of competing crayfish chose thermal habitats depending on whether the crayfish were previously habituated or naïve to each other. In this study, the subordinate crayfish chose suboptimal temperatures under the habituated treatment (Tattersall et al. 2012). A further study found that red flour beetles were more commonly found in cold patches that had higher food, but overall offered a lower fitness, than warmer patches that conferred a higher fitness. However, it is also possible that this pattern was a function of the beetles shuffling back and forth between the patches of varying thermal quality (Halliday and Blouin-Demers 2014). This finding thus further provides credence to the idea that the flies rely on their offspring to find and move to an optimal patch during the larval stage.

Flies change their thermoregulatory strategy in the presence of other flies which gives us a fundamentally different result than that predicted by a model that ignores biotic factors. By incorporating predictions from game theory, we can make better predictions about these flies' behavior than we could have made with classical models of thermoregulation. By taking into account how other competitors affect the benefits received in the environment, we can develop a better framework for predicting how organisms' thermoregulate in their environment and how these organisms will respond to climate change. Consequently, I believe we are a point where we need to start making more game theoretical models to predict how organisms thermoregulate by incorporating

in biotic factors such as competition. Behavioral thermoregulation is important for ectotherms since they live in thermally heterogeneous environments that differ in temperatures on a small spatial scale. Additionally, many researchers expect organisms counter climate change through behavioral thermoregulation (Clusella-Trullas and Chown 2011, Gvoždík 2012, Huey et al. 2012). While we know that many ectotherms attempt to maintain a certain temperatures (Cowles 1944, Dillon et al. 2009), we still do not know how important thermoregulation is when it is only one of many factors that an organism must consider in its environment.

Competition may alter the way an organism thermoregulates by causing organisms to move more frequently throughout patches or by causing the organism to spend more time in a thermally detrimental patch since resource extraction might be too great in thermally preferred microclimates due to large aggregations of competitors. It has previously been found that organisms shift their thermoregulatory strategy due to competitive interactions such as social dominance (Magnuson et al. 1979, Rusch and Angilletta 2017); however, this shift in thermoregulatory behavior has not been examined within a game theoretical context. Particularly given the impacts of climate change, we need to create integrative models that enable us to predict how organisms will respond to changes in their thermal environment, especially as organisms attempt to behaviorally thermoregulate and begin to shift their ranges or niches.

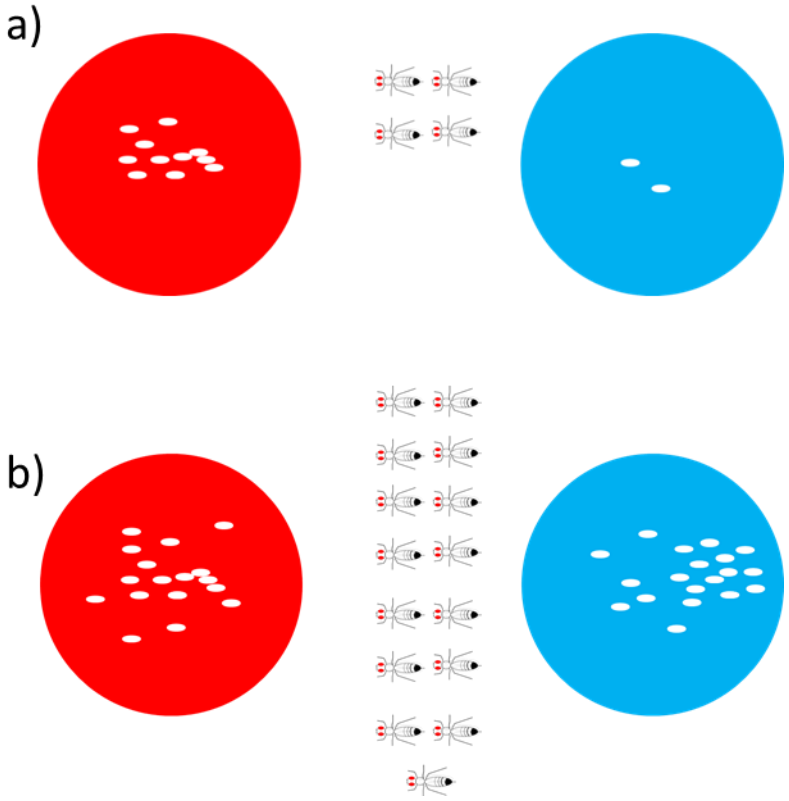

Figure 2.1. The amount of competition affects how flies should choose sites to oviposit their eggs. When there are low levels of competition, flies should prefer to lay their eggs in the patch that is most thermally beneficial to their young (a). When there is a high amount of competition, flies should begin to lay their eggs in less thermally beneficial patches to avoid high competition (b).

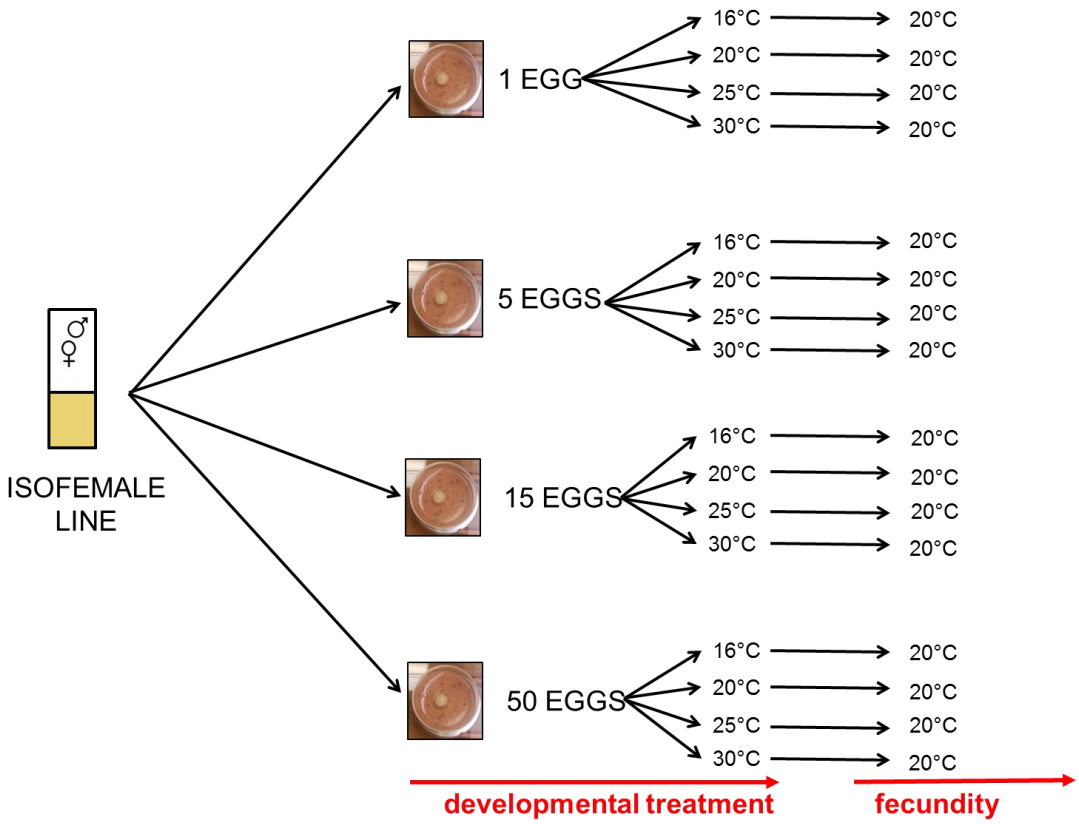

Figure 2.2. To estimate the fitness of flies at a variety of temperatures and densities, I transferred 1, 5, 15, or 50 eggs collected from a single isofemale line to 35mm petri dishes containing grape agar and 0.033g of yeast. These petri dishes were kept an incubator at 16 °, 20°, 25°, or 30°C with a 12:12 light cycle. I measured developmental time by checking the flies daily to record the number of days for adult flies to emerge. I measured survivorship by counting the proportion of adults that survived to adulthood. To measure fecundity, I mated dult female flies from my treatments with male flies from a control line (Cantonese) at a common temperature of 20°C for 4 days. I then removed the adult breeding pair and counted the number of adult flies emerged from any eggs laid by these fly pairs to estimate fecundity.

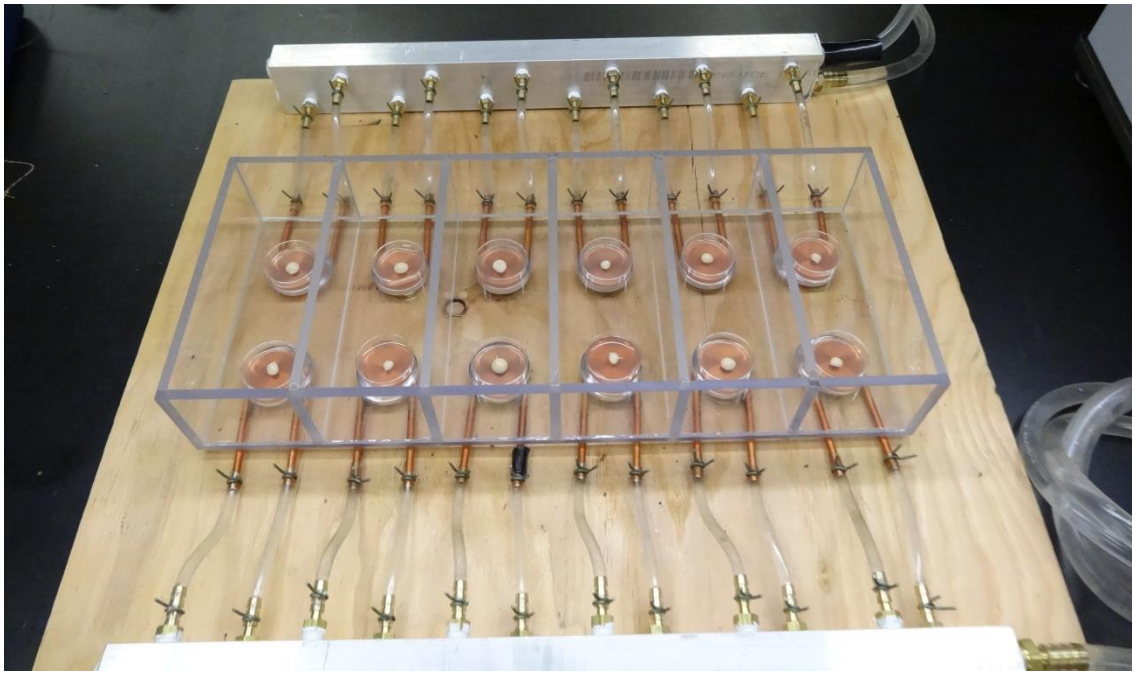

Figure Figure 2.3. My thermal arena consisted of a Plexiglas container that consisted of six independent lanes by running copper tubing below small aluminum plates. Each lane within the arena had two aluminum plates on opposite ends of the lane where I could place an agar-filled petri dish with a drop of yeast. Flies could fly between and choose to lay their eggs in either the 20°C petri dish or the 25°C petri dish.

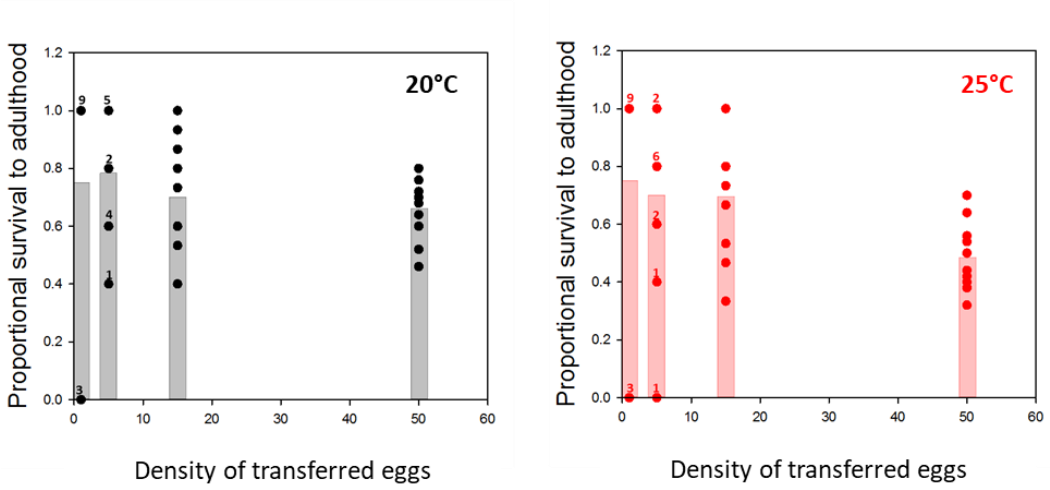

Figure 2.4. To parameterize my game theoretical model, I used survivorship data from 12 isofemale lines created from wild caught flies collected in Indiana. I transferred either 1, 5, 15, or 50 eggs to a petri dish and maintained the flies in an incubator kept at either 20°C (black) or 25°C (red) with a 12:12 light cycle. I measured survivorship by counting the proportion of adults that survived to adulthood. Each dot represents data from one isofemale line. For densities kept at either one or five individuals, the number of overlaid dots at each proportion is denoted with a number above the dot. Each bar represents the predicted model fit from the logistic regression curve. While survivorship was similar for both temperatures at low-density, survivorship dropped off much quicker as density increased for flies that were kept at 25°C.

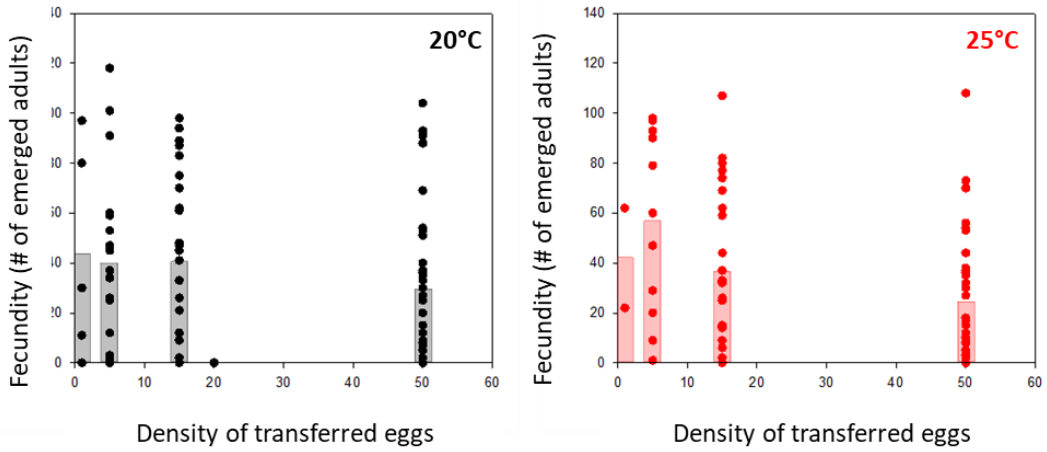

Figure 2.5 To parameterize my game theoretical model, I used fecundity data from 12 isofemale lines created from wild caught flies collected in Indiana. I transferred either 1, 5, 15, or 50 eggs to a petri dish and maintained the flies in an incubator kept at either 20°C (black) or 25°C (red) with a 12:12 light cycle. I measured fecundity, by outbreeding adult female flies from my treatment trays with a male fly from a control line (Cantonese) for four days at a common temperature of 20°C and counting the total number of emerged flies from the vial. Each dot represents data from one female fly. Each bar represents the predicted model fit from the generalized linear model. The fecundity of flies at 25°C was higher at low densities, but decreased faster than the fecundity of flies at 20°C as densities increased, causing flies at 20°C to have a higher fecundity at high densities.

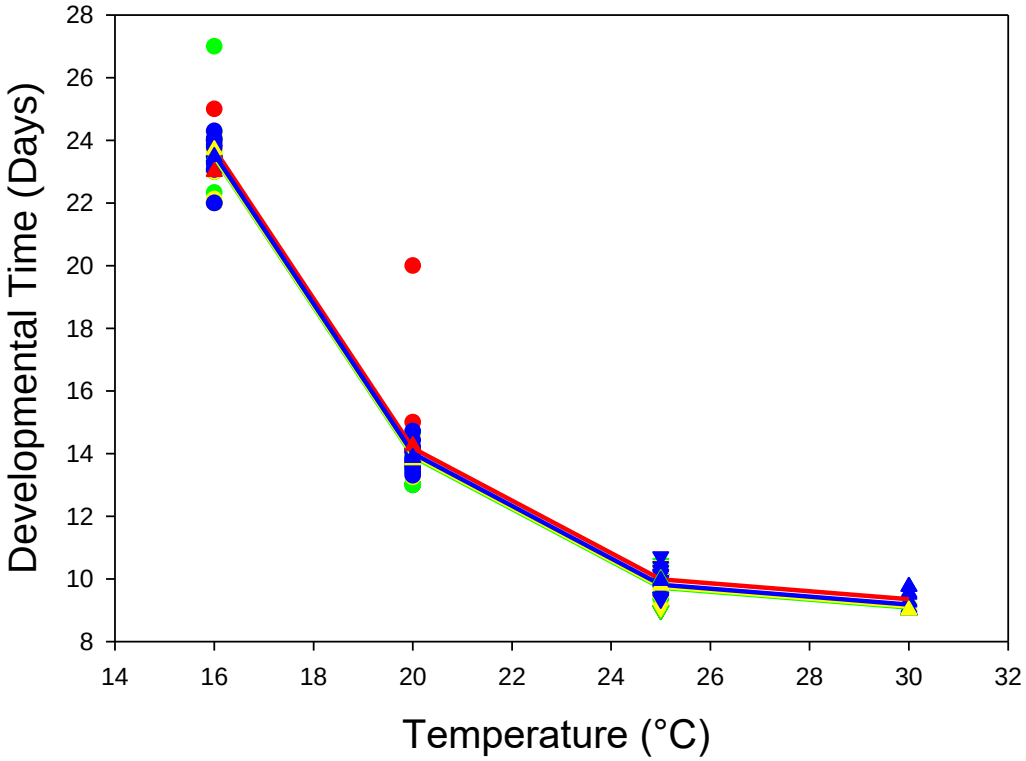

Figure 2.6. I used data on developmental time from egg to adult to set the parameter values for the game theoretical model. To parameterize my game theoretical model, I used 12 isofemale lines created from wild caught flies collected in Indiana. I transferred either 1 (red), 5 (green), 15 (yellow), or 50 (green) eggs to a petri dish and maintained the flies in an incubator kept at 16°C, 20°C, 25°C, or 30°C with a 12:12 light cycle. I then measured by checking the flies daily to determine time to adult stage. Each circular dot represents a data point, medians are represented by triangles and lines are the predicted values of the generalized linear model. Flies developed faster at warmer temperatures, but there was no effect of density.

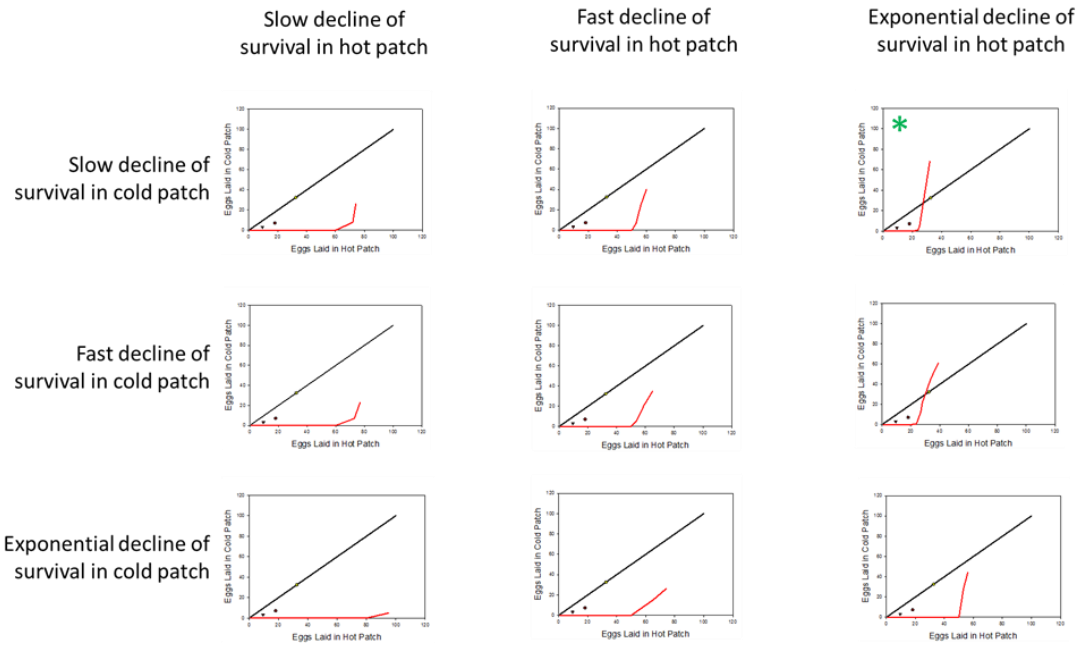

Figure 2.7. My models predict that flies should predominately lay their eggs in the warm patch as long as competition is low. As competition increases, flies should begin to lay more eggs in the cold patch especially if the rates or survival in the warm patch decline rapidly or if the flies in the cold patch experience only a slow decline in its survival rate under increased densities. The straight black line represents a trend line where the portion of eggs in each patch should be equal. The red line shows where my model predicts the flies to lay their eggs based on varying levels of survivorship if future fecundity and developmental time remain constant. The predicted model fit that most closely resembles the empirical data collected on fly fitness is denoted with a green apostrophe. The average egg density laid in the empirical, low-density fly treatment is represented with a burgundy circle and the average egg density laid in the empirical, high-density fly treatment is represented with an olive circle.

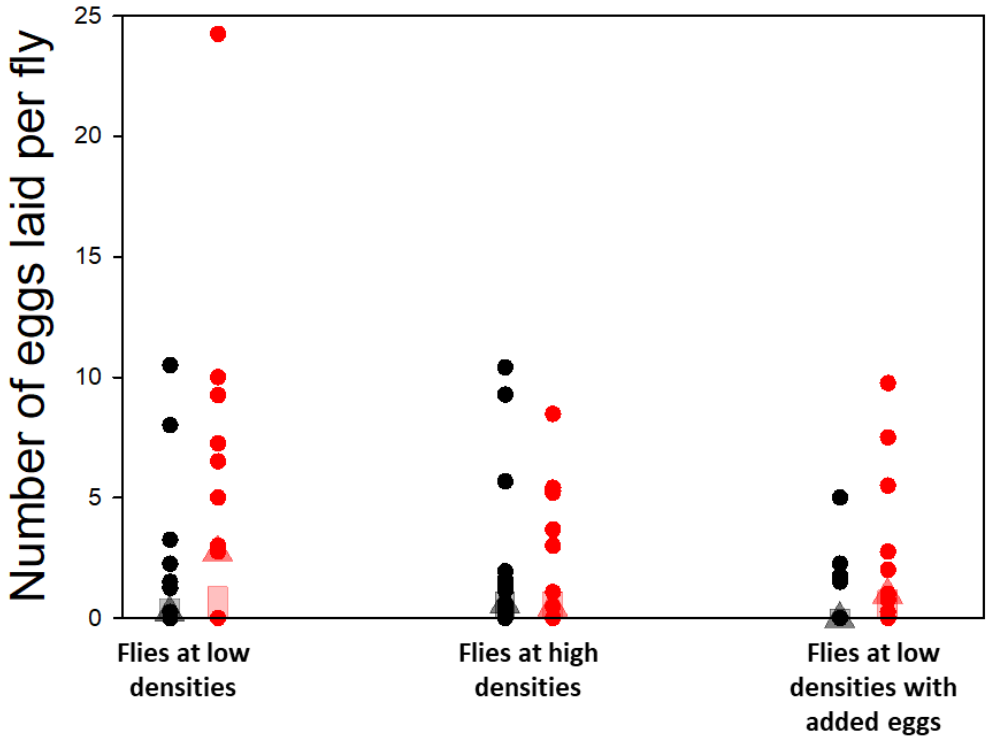

Figure 2.8. I added either 4 flies (low-density) or 15 flies (high-density) to each lane within a thermal arena. Flies then chose to oviposit their eggs in a 20°C patch (black) or a 25°C patch (red) within the thermal arena. For the treatment with added eggs, I added 100 eggs to the 25°C patch prior to adding flies to the thermal arena. Each dot in the graph represents the raw data for where flies chose to lay their eggs, while the triangles represents the median of where each fly laid their eggs, and each bar represents the predicted model fit for a linear mixed-effects model adjusted for zero-inflated data (Zuur 2009). Enclosed circles represent where flies in a low-density treatment laid their eggs after 100 eggs were placed in the warm patch. Flies laid eggs almost exclusively at 25°C when female density was low ($P < 0.001$). Flies laid more eggs at 20°C when female density was high than they did when female density was low ($P < 0.001$). Flies laid eggs almost exclusively at 25°C even when many eggs were already present at this site; laying behavior did not differ from that observed when no eggs were present at the start of the trial ($P = 0.43$).

Table 2.1: Results from a linear mixed-effects model adjusted for zero-inflated data. We found significant differences from our low-density treatment in relation to temperature and amount of competition from adult flies, but did not find a significant difference when eggs were already present at sites.

	Estimate	SE	*P*
Intercept	0.5767	0.5338	0.27994
High-Density	1.9040	0.7354	0.00963
Additional Eggs	-0.4305	0.7857	0.58376
25°C	0.9145	0.1135	$P < 0.001$
High-Density:25°C	-0.9206	0.1302	$P < 0.001$
Additional Eggs:25°C	0.1681	0.2118	0.42732

CHAPTER 3

HOW THERMAL HISTORY OF FLY POPULATIONS INFLUENCES WHERE THEY CHOOSE OVIPOSITION SITES FOR THEIR YOUNG

Chapter 3: How thermal history of fly populations influences where they choose oviposition sites for their young

Summary

Most studies of thermoregulation focus on the behavior of individuals without considering how these individuals compete for thermal resources. We need a theory of thermoregulation that accounts for variation in the local adaptation of organisms. Animals from different geographical regions and populations may thermoregulate differently due to having experienced different thermal conditions in the past. To test how thermal history influences thermoregulatory behavior, I examined how flies should oviposit their eggs when choosing between patches that vary in temperature and competition. To test this question, I used flies that were both experimentally evolved at different temperatures (16°C and 25°C) and natural populations of flies from different regions (Vermont and Southern Indiana). I created a model that looked at how each set of flies should optimally behave, given their genotype, when they must compete to lay their eggs in patches that vary in temperature and density of flies. I predicted that flies that had experienced colder temperatures, whether they evolved naturally or in the lab, would be more likely to shift their oviposition preference to colder patches as competition increased. We found that Vermont flies more readily shifted to colder patches than flies from Southern Indiana. Lab selected lines, however, did not behave the way we expected as neither the flies selected for 25°C or 16°C altered their oviposition preference as density increased.

Introduction

The Huey and Slatkin (1976) theory of thermoregulation predicts how an organism should thermoregulate within its environment by calculating the costs and benefits an organism experiences due to the availability and time spent in patches that differ in temperature from the organisms optimal temperature. In addition to the basic cost-benefit model of thermoregulation (Huey and Slatkin 1976), we have more complex thermoregulation models that predict how animals should choose different behavioral strategies based on variation in their environment (Sears and Angilletta 2015), predators (Polo et al. 2005, Angilletta and Mitchell 2009), and food (Hughes and Grand 2000). Further, organisms that have experienced different thermal histories are expected to thermoregulate differently under each of these models.

Organisms from different regions experience different thermal histories and consequently have varied thermal preferences and optimums (Bennett 1980, Huey 1982, Dillon et al. 2009). Consequently, animals from different geographical regions respond to temperature fluxes differently (Davis et al. 1998, Buckley 2008, Deutsch et al. 2008, Tewksbury et al. 2008, Kearney and Porter 2009). Organisms should attempt to maintain their thermal preferences to varying degrees based on how quickly their fitness changes with temperature. Organisms that are thermal generalists tend to have a wide thermal performance curve while organisms that are thermal specialists tend to have a high thermal optimum, but narrow thermal breadth (Levins 1968, Slatkin and Lande 1976, Huey and Hertz 1984, Angilletta et al. 2002b). Consequently, organisms whose fitness quickly changes with temperature can be thought of as thermal specialists, while organisms whose fitness changes more gradually along a thermal cline, can be thought of

as thermal generalists. As a result, it is important for us to understand how organisms with different performance curves fit into our current models of thermoregulation.

Thermal generalists can perform at a wider range of temperatures and often come from more thermally variable environments and higher latitudes (Janzen 1967, Ghalambor et al. 2006, Deutsch et al. 2008, Tewksbury et al. 2008) Further, the metabolic rates of organisms also vary predictably along with its thermal performance curve as many metabolic processes are thermally dependent (Gillooly et al. 2001, Gillooly et al. 2002, Savage et al. 2004). These variations in thermal performance influence growth rate (Berrigan and Charnov 1994, van der Have and de Jong 1996, Angilletta and Dunham 2003), sprint speed (Hertz et al. 1982, Angilletta et al. 2002a, Pinch and Claussen 2003, Zamora-Camacho et al. 2015), and many other physiological processes (Brett et al. 1969, Brett 1971, Huey 1982, Berrigan and Koella 1994, Sibly and Atkinson 1994, McCabe and Partridge 1997, Reeve et al. 2000, Robinson and Partridge 2001). Thermal specialists and generalists can be found in geographical regions that differ in their thermal heterogeneity. Consequently, in a thermal game, you expect organisms from thermally heterogeneous environments to more readily switch their strategy and choose a patch that is sub-optimal in temperature than a thermal specialist. Thermal specialists should be less likely to switch between patches that differ in temperature since specialists will see their benefits degrade faster as they move away from their thermal optima.

However, the importance of the interplay between the competitive interactions and their thermal environment should depend on the co-adaptation between the physiology and behavior of the organisms. One can use the g function to determine how 2

traits that covary can be used to predict a changing genotype (Houle 1991). When using the idea of a g function, to model co-adaptation of thermal physiology and behavior, organisms should change their thermal sensitivity based on variation in the temperatures they experience (Angilletta et al. 2006). Under ideal scenarios, organisms' behavior and thermal preferences should correlate with the temperatures at which they perform best, such as in some reptiles (Dawson 1975) and fish (Beitinger and Fitzpatrick 1979). However, some organisms do not perform the way that the theory would expect. Species of lacertid lizards that are thermal generalists thermoregulate more precisely than those species that are thermal specialists (Bauwens et al. 1995). This result is shocking because specialists are typically thought to be more precise thermoregulators than are thermal generalists. Other times behavior and physiology are only partially co-adapted. For example, skinks were found to prefer a temperatures that change in conjunction with their critical thermal maximum (Huey and Bennett 1987), but not with their critical thermal minimum or optimum (Garland et al. 1991). Consequently, even though we expect the genotypes that prefer certain temperatures to function best at the same temperatures, we do not always see this pattern. However, by bringing in evolutionary game models to look at how behavior and physiology are co-adapted, we might be able to see patterns that are not straightforward in a system where density is static.

By incorporating game theory into existing models of thermoregulation, we can better predict how animals will regulate their temperature (Angilletta 2009). Game theory describes how organisms allocate time to different patches when the preferences and frequencies of competitors determine the benefits of patch choice (Sih 1998, Brown et al. 1999). For antagonistic competitors to coexist, the behaviors of these competitors must

reach a stable equilibrium where the fitness of each competitor is equal to each other, referred to as an evolutionarily stable strategy (Nowak and Sigmund 2004). We know that biotic interactions can affect how animals disperse themselves in respect to temperature within their environment due to competition (Beitinger et al. 1975, Magnuson et al. 1979, Medvick et al. 1981, Seebacher and Grigg 2000, Stapley 2006, Rusch and Angilletta 2017) and predation (Lampert 1989, Downes 2001, Webb and Whiting 2005, Amo et al. 2007, Herczeg et al. 2008). Therefore, by using game theory, we can make novel predictions about how animals with different genotypes should optimally behave when they have to also worry biotic factors.

I tested these ideas using isofemale lines of fruit flies derived from both natural and experimental populations. The natural fly lines enabled me to test flies whose progenitors had experienced realistic thermal conditions while the experimental lines enabled me to test for a population that only diverged in regards to temperature. It has previously been found that flies along a latitudinal gradient vary in their fecundity (Peter et al. 2013), body size (James et al. 1997, Bochdanovits and de Jong 2003), and development (James and Partridge 1995, McCabe and Partridge 1997, James and Partridge 1998). Additionally, flies from selection lines that were experimentally evolved at different temperatures have also been shown to have variation in their fecundity (Partridge et al. 1995), body size (Reeve et al. 2000), development (James and Partridge 1995, Crill et al. 1996, McCabe and Partridge 1997), thermal limits (Hoffmann 2010), and specialist generalist tradeoffs (Latimer et al. 2011). The thermal physiology and membrane lipids have been found to differ across these specific isofemale lines for both the experimentally selected (Cooper et al. 2012) and natural populations (Cooper et al.

2014). Additionally, these specific fly selection lines show variation in their thermal breadth, as the fly lines that experienced varying thermal temperatures are better thermal generalists than those fly lines that experienced constant temperatures (Condon et al. 2014). Lastly, flies had better flight performance at the temperatures for which they were experimentally evolved (Le Vinh Thuy et al. 2016). Do these fly populations that have experienced different thermal conditions also exhibit differences in how they alter their thermoregulatory strategies for their young in response to competition?

I used my game theoretical model of oviposition behavior (Chapter 2), with data from both the natural and experimental fly lines, to predict how flies should optimally behave under varying degrees of competition. I then empirically tested this model by allowing flies, from both the natural populations and selection lines, to choose between two sites that varied in temperature. I hypothesized that flies will be more likely to utilize temperatures of which they had historically been exposed. I predicted that flies historically experiencing colder temperatures would alter their thermoregulatory strategy to begin choosing cooler oviposition sites under increased competition faster than flies, which have historically experienced warmer temperatures. Flies from populations that experienced a wide range of temperatures should also alter their thermoregulatory strategy to choose cooler oviposition sites than flies that have experienced more consistent warm temperatures. Therefore, I predicted that flies from higher latitudes would be more likely to use both warm and cold patches than flies from lower latitudes, while flies that were experimentally selected for cold temperatures would be more likely to use the cold patch than the flies experimentally selected for hot temperatures (Figure 3.1). Due to the known differences in thermal sensitivity and fitness in organisms from

different thermal backgrounds, it is important for us to determine how these principles affect the development of a more general theory of thermoregulation that is accurate for species and populations from different backgrounds.

Methods

Maintenance of *Drosophila melanogaster*

I used flies descended from females collected in Beasley Orchid, Indiana and East Calais, Vermont, during the fall of 2011. Twelve isofemale lines were created by mating a single adult virgin female fly with a male sibling for 2 generations (Cooper et al. 2014). Additionally, I also used ten isofemale lines that were evolved from five different populations of fly lines that were experimentally selected and allowed to evolve at either hot (25°C) or cold (16°C) temperatures. The experimental fly lines were collected by Yeaman *et al.* (2010) during September 2005 from an organic orchard near Cawston, BC. 400 virgin female flies were collected from an initial population of approximately 2000 adults to create the experimental lines. Each replicate population had two cages assigned to either a constant 16°C or 25°C and kept on a 12:12 L:D light cycle. Bottles located in each cage were transferred between cages from the same population and same temperature every four weeks to encourage random mating between cages. The lines were then allowed to evolve at their selected, constant temperature for over three years. During this time, the population size within each cage varied between 800 and 2000 flies. Detailed descriptions of how the experimental populations were developed is available in Yeaman *et al.* (2010). The isofemale lines descended from these experimental populations capture aspects of the genetic variation of these populations. I maintained the

isofemale lines on a standard diet (recipe of the Bloomington Stock Center, Bloomington, IN) in 25 x 90 mm vials (Genesee Scientific, San Diego, CA) inside of an incubator (Percival Scientific, Perry, IA) at a temperature of 21°C and a light cycle of 12:12 L:D. Flies were transferred to new vials with fresh food approximately every three weeks.

Two males and two females from the same isofemale line were added to a vial for 48 hours, to partially control for egg density. When the next generation of flies emerged in these vials, I transferred two males and two females to a new vial for 48 hours. I repeated this protocol for three generations. Adult females from the third generation were used in the experiments described below.

Parameterize the model

I used data collected from flies in the lab to parameterize of my model. To estimate the fitness of flies at each combination of density and temperature, I measured the survivorship and fecundity. To obtain flies for my experiment, I allowed females from 12 isofemale lines to lay eggs in petri dishes with a grape agar and yeast solution for 8 hours at 23°C. I then transferred either 1, 5, 15, or 50 eggs to new petri dishes (35mm diameter) containing the grape agar and 0.033g of yeast. These petri dishes were kept an incubator at 16°, 20°, 25°, or 30°C with a 12:12 light cycle. I measured survivorship by counting the number of adults that survived to adulthood.

To measure fecundity, I outbred adult female flies from my treatments with male flies from a control line (Cantonese, Bloomington Stock Center, Bloomington, IN) to capture only variation in fecundity because egg production, not sperm production, is typically the limiting factor for population growth. Additionally, since I was concerned

about the effect of larval developmental temperature and adult flies would be able to freely move to new patches that varied in temperature, I moved all vials to a common temperature of 20°C for my fecundity experiments. I placed each pair of flies into a vial (25 x 95 cm) containing a standard cornmeal-yeast medium for 4 days in an incubator at 20°C with a 12:12 light cycle. I then removed the adult breeding pair and allowed any eggs laid by these fly pairs to develop to adulthood. From the adult flies that emerged within each of these vials, I again recorded developmental time (egg to adult) to the nearest day and counted the total number of flies emerged (fecundity).

I estimated how density and temperature related to fecundity using a generalized linear model while I estimated survivorship using a logistic regression curve. I then used AIC to pick the best polynomial fit for each curve in R (Version 3.3.1 R-Core-Team 2016) (Zuur 2009). I then developed additional models based on predictions of how one might expect the fitness of flies to vary under different circumstances. I used the best-fitting curve developed from the raw parameters to find an initial curve, which could explain how survivorship and fecundity change with increasing density. Furthermore, I hypothesized additional parameter data that was in line with findings on fitness data to create additional models regarding how you would expect flies to behave if their survival parameters were slightly altered. To create this additional parameter data, I also increased or decreased how quickly survivorship changed under increased competition since in nature you might have conditions changing within these patches causing survivorship to differ from what we measured. For example, resources might be replenished within these patches, which would cause survivorship to decline more slowly. Alternatively, predators

might be attracted to higher densities of prey, which would cause survivorship to decline more quickly and possibly at an exponential rate.

Experimental tests of the model

I used the same Plexiglas thermal arena for the experimental tests of the model as in Chapter 2 (Figure 2.3). I used the thermal arenas to test where flies chose to lay their eggs under different intensities of competition. In each arena, a fly could choose between a petri dish at a preferred temperature of 25°C and a petri dish at 20°C. For each isofemale line, I ran trials in which either 4 or 15 adult females were in an arena at a time. Additionally, I concurrently ran equal number of cross-comparison treatment groups of flies in the arenas. During each trial, I would run either (i) an equal number of low latitude and high latitude flies of each density type or (ii) an equal number of cold selected and hot selected flies of each density type simultaneously within the arena. After 4 hours, I removed the flies and froze the petri dishes from each side of the arena. Later, these dishes were thawed, and the number of eggs was counted under a dissecting scope (Zeiss Stemi 2000-C) at each temperature.

I performed a linear mixed-effects model adjusted for zero-inflated data (Zuur 2009) using the glmmADMB package (Fournier et al. 2012) in R (Version 3.2.3, R-Core-Team 2016) to determine whether the number of eggs laid in each patch in each treatment type differed. The fixed factors in my model were temperature and density/treatment. I used trial as a random factor to compare eggs laid in the two sides of the arena while accounting for variation in the number of eggs among trials. The experimental date and isofemale line was dropped from my model due to poor fit using AIC values.

Results

I modeled the fitness of different genotypes of flies using data on survivorship and fecundity. Survival of flies at different densities and temperatures depended on the genotype of the fly (see Figures 3.2-3.5). For isofemale lines derived from low latitude populations, the survivorship of flies was similar at both 20°C and 25°C. For isofemale lines derived from high latitude populations, survivorship was higher in the 20°C patch than the 25°C at low densities, but similar for both the 20°C and 25°C patches at high densities. Survivorship of isofemale lines from cold selected lines was similar at both temperatures except that when there was either 1 or 50 flies, 25°C patches had slightly higher survivorship when there were 5 flies, 20°C patches had slightly higher survivorship. Hot selected flies had slightly higher survivorship at 20°C than 25°C and followed a very consistent pattern across density treatment. The fecundity of flies also changed at different densities and temperatures depending upon the genotype of the fly (see Figures 3.6-3.9). Low latitude flies' fecundity was highest in 25°C patches at low-density. While flies from high latitude flies were most fecund at intermediate densities in 25°C patches. Cold selected flies had much higher fecundity in the 20°C patch than the 25°C at all densities. Hot selected flies had similar fecundity at low densities in both the 20°C and 25°C patches, but at higher densities had higher fecundity in the 20°C patch.

My game theoretical models illustrate how competition affects the fitness of fruit flies. The models for both the low latitude (Figure 3.10) and high latitude (Figure 3.11) flies predicts that flies will initially lay eggs in the hot patch then shift to the cold patch as the density of eggs increases. Additionally, the model predicts that high latitude flies will begin shifting their effort to the cold patch at lower densities of eggs than the flies at low

latitudes. The models for the cold selected (Figure 3.12) and hot selected (Figure 3.13) flies both predict that flies should initially lay in one patch and then begin to spread out their effort, however, they differ between which temperature they should initially lay their eggs in at low densities. Hot selected flies are also expected to initially lay their eggs in the warm patch and then begin to spread their effort to the cold patch as the density of eggs in the hot patch continues to increase. However, due to having higher rates of fecundity when developed at cold temperatures, cold selected flies are expected to initially lay their eggs in the cold patch. In models where there is a slow decline of survival in the cold patch than in the hot patch, cold selected lines should continue to predominantly lay their eggs in the cold patch. Only in high-density scenarios, where there is also an exponential or relatively rapid decline of survival in the warm patch, should cold selected flies start laying eggs predominantly in the hot patch.

I found that temperature and competition both affected patch choice in flies from a high latitude, but only temperature affected patch choice in flies from a low latitude. Low latitude flies, at low-density, laid eggs almost exclusively at 25°C ($P = 0.004$) while flies at high-density did not significantly differ in laying their eggs at 20°C than flies at low-density ($P = 0.21$). When comparing across the high-density treatments, I found a significant difference between the number of eggs laid by low and high latitude flies for both the 20°C ($P=0.003$) and 25°C patches ($P=0.01$) (Table 3.1). High latitude flies at low-density laid eggs almost exclusively at 25°C ($P < 0.001$) and at high-density significantly increased the number of eggs laid at 20°C ($P=0.004$) (Figure, 3.13, Table 3.2). Isofemale lines derived from the high latitude populations behaved differently when laying eggs at low vs high densities. At low-density, high latitude flies laid a median of

five eggs at 25°C but zero eggs at 20°C. At high-density, high latitude flies laid a median of one egg at 20°C and three eggs at 25°C. Genotypes from the low latitude did not behave differently when laying eggs at low vs high densities. At low-density, low latitude flies laid a median of 1.5 eggs at 25°C and 0 eggs at 20°C. At high-density, low latitude flies laid a median of two eggs at 25°C and still zero eggs at 20°C.

The natural populations of flies behaved in partially in accordance with the model. Both the low and high latitude flies primarily laid their eggs in the hot patch at low densities in accordance with the model. At high densities, the high latitude flies starting spreading their effort to the cold patches as predicted by the model. However, the low latitude flies did not begin to lay their eggs in the cold patch as predicted by the model.

I found that flies experimentally evolved at either hot or cold temperatures, changed their egg laying behavior based on temperature, but not competition. Cold selected flies at low-density laid eggs almost exclusively at 25°C ($P < 0.001$) and increased competition did not alter this relationship ($P = 0.68$) (Table 3.3). Hot selected flies at low-density laid eggs almost exclusively at 25°C ($P < 0.001$) and did not change their behavior under increased competition ($P = 0.99$) (Figure 3.14, Table 3.4). Genotypes from flies that were selected for hot temperatures behaved differently when laying eggs at low vs high densities. At low-density, hot selected flies laid a median of four eggs at 25°C but zero eggs at 20°C. At high-density, hot selected flies laid a median of one egg at 20°C and four eggs at 25°C. Genotypes from flies that were selected for cold temperatures did not behave differently when laying eggs at low vs high densities. At low-density, cold selected flies laid a median of 7.5 eggs at 25°C and 0 eggs at 20°C. At

high-density, cold selected flies laid a median of 8eggs at 25°C and still zero eggs at 20°C.

The experimentally selected flies did not behave in accordance with the model. Both the cold and hot selected flies primarily laid eggs in the hot patch regardless of density type. The model predicted that the cold selection lines should have primarily laid eggs in the cold patch and switched to the hot patch at high densities; instead, the isofemale lines derived from cold-selected lines preferred to lay at 25°C regardless of density. The model predicted that the hot selected lines should prefer to lay at 25°C but allocate some eggs to 20°C at high densities; however, the flies in the choice experiment did not actually alter their behavior under increased competition.

Discussion

Flies from high latitudes behaved in accordance with our model. However, flies from low latitude did not change their behavior as predicted by the model. High latitude flies were predicted to see a competition effect at lower densities and to thus begin laying eggs at colder temperatures at lower densities than low latitude flies though so it is possible that the densities were not high enough in the low latitude experiment. Flies, from both the hot and cold selection lines, preferred the warmer patch at both low and high densities despite our model predicting that both should see an effect from competition. Additionally, flies from cold patches were predicted to prefer the cold patch to the warm patch at low densities.

High latitude organisms experience greater thermal variation as well as lower temperatures than low latitude organisms (Janzen 1967, Stearns 1976, Stearns 1992,

Ghalambor et al. 2006). Consequently, based on life history, my model predicted that high latitude flies would more readily switch to colder patches as competition increases relative to low latitude flies. This prediction of the model was observed; the high latitude fly lines did switch more readily to cold patches than low latitude lines. Further supporting the idea that flies prefer to lay eggs in sites similar to temperatures they have previously experienced, in an experiment by Nevo and colleagues (1998), flies collected from a warm, dry site were allowed to lay eggs in vials that varied in their temperature and moisture content and found that warm, dry vials that were most similar to their natural site had the most flies emerge. However, there was not a control to test whether this was due to oviposition selection or survival within the vials. My findings further support the idea that high latitude organisms are thermal generalists (Levins 1968, Slatkin and Lande 1976, Huey and Hertz 1984, Angilletta et al. 2002b).

Additionally, it has been previously found that along a latitudinal gradient that the critical thermal minimum and maximum are both positively correlated with highest and lowest temperatures experienced by a fly population (Hoffmann et al. 2002). This finding is in accordance, with how survivorship is the primary driver of the fitness function in my game theoretical model. While there was not a strong relationship between the fecundity of my high and low latitude flies, there was a difference in survivorship at different temperatures. Based on the empirical data used to parameterize my model, high latitude flies had a higher survivorship at 20°C than low latitude flies and low latitude flies had a higher survivorship at 25°C than high latitude flies.

Surprisingly, cold selected flies did not prefer the cold patch in either the low- or high-density treatment, which was in direct contrast to my model. It has previously been

found that cold-adapted flies have a smaller decrease in fitness when switching to colder patches (Huey et al. 1991). Due to this previous work, plus the fitness data that I collected, I expected to see an effect from increased competition with the cold selected flies even if I did not see it in the hot selected flies. An additional factor that could explain why we are not seeing differences could be due to both fecundity and survivorship of the selection line flies having antagonistic effects upon each other. The cold selection lines have slightly better survivorship at both temperatures while hot selected lines have greater overall fecundity at both temperatures. However, hot selected flies have much higher fecundity at 25°C than 20°C. Cold selected flies have a higher fecundity at 20°C than 25°C, but have a faster development time at 25°C. These differences in survivorship and fecundity thus could explain the pattern we are seeing at the fitness level.

These results for fecundity are similar to those previously found at cooler temperatures, but not at warmer temperatures. Condon and colleagues (2014) previously found an inverse relationship between the temperatures the flies were evolved at and the temperatures at which they had maximal fecundity. They found that cold selected flies had greater fecundity than hot selected flies at high temperatures while hot selected flies had greater fecundity at low temperatures than cold selected flies. While my results are similar for low temperatures, I found hot selected flies still had greater fecundity than cold selected flies at high temperatures. This difference could be due to intra-population variation in the hot selected isofemale fly lines that we used. More importantly though, this difference could be due to how we both measured fecundity. While we both developed each population at either colder or warmer temperatures, in my study the flies

were then able to lay their eggs at a common temperature of 20°C, while the flies in Condon's study (2014) remained at their developmental temperature. Therefore, we may have simply found different rates of fecundity given that we used different methods to estimate fecundity in the lab raised flies.

This greater variation within natural populations of flies as opposed to lab raised flies has been previously found when looking at size differences due to temperature (James et al. 1997). While thermal sensitivity has been demonstrated to evolve rapidly in artificial fly populations in the lab (Huey and Bennett 1990), thermal preferences of oviposition site preferences have been shown to have a very low heritability (Fogleman 1979). Consequently, my selection lines may have not had enough time pass to see an alteration in their oviposition site preference at the population level even if they had a change in their fitness. Additionally, these lines were created by keeping a breeding population of flies in cages at constant temperatures for 3 years. It is possible that selection was primarily taking place within these populations due to other factors such as being in a crowded cage and access to food rather than temperature. Since fly larvae are believed to competitively exclude other larvae from food (Gilpin 1974), it is possible that selection was primarily occurring in regards to their ability to access food and not due to oviposition site selection for preferred tempered temperatures.

The flies from the selection lines may not have experienced any selection for behaviors that changed their oviposition choice preferences. In other words, these flies may have only experienced selection for changes in their fitness at different temperatures, but not for behavioral selection of oviposition sites. The flies were allowed to evolve at different temperatures; however, their environment was uniform in temperature.

Therefore, flies did not have had the option to choose between sites that differed in temperature and therefore may not have experienced selection for choosing oviposition sites that offered the greatest fitness to their young. Flies in these cages received the most benefit from laying their eggs anywhere there was food since the entire environment was one temperature and there was no choice to be made regarding sites that differed in temperature. Consequently, there might not have been any selection for choosing oviposition sites that reflected optimal temperatures for fitness in the new environment.

Organisms from different populations may also depend on different food sources or preferences that are naturally variable within their environment. For example, insects populations can incorporate different plants (Thomas et al. 1987) and derive different benefits from certain foods (Chew 1977). Further, different diets can affect thermoregulation strategies (Underwood 1991, Pulgar et al. 2003) and insects are known to see various fitness benefits and costs associated with the type and timing of their diet (Raubenheimer and Simpson 1999, David et al. 2009, Meunier et al. 2017). Therefore, even though these flies are feeding on the same diet in the experiment, if they are adapted to different diets that they are used to feeding on in nature, they may have a different thermoregulatory strategy when feeding on foods that differ in food quality or have a specific carbohydrate to protein ratio. A population level difference in food preference could also explain why we only saw a difference between the natural populations of flies and not the experimentally selected flies. Consequently, we may also be seeing differences in these flies due to variation in local thermoregulatory strategies in regards to food preference or food quality.

Perhaps the most surprising thing about my results is that I found different oviposition preferences of flies that were from the same population (Chapter 2 and Chapter 3 low latitude flies). The flies in the low latitude treatment did not experience a density effect despite flies from that same population displaying a density effect in Chapter 2. In the first experiment, the flies changed their behavior as competition increased while the flies in the second experiment did not change their behavior under increased competition. These differences in results could be due to a couple of reasons. The two experiments were performed almost two years apart from each other and had to use different isofemale lines. These isofemale lines may have differed from the earlier isofemale lines that I previously used in how they respond to temperature or competition differences. Further, these isofemale lines might have just differed in their food or humidity preferences, which would have introduced a confounding factor. As a result, I may not have been able to pick up some of the same differences that I could with the earlier experiment.

It is possible that the flies are not behaving the way I expect them too because the fly genotypes used in my model were not an accurate representation of the natural genetic variation of these flies. For example, it has been found that for lab raised flies kept at 18°C and 25°C, larger females lived longer and had greater reproductive success, while small flies delay reproduction until later in life (McCabe and Partridge 1997). Since I only allowed flies to lay eggs for the first 4 days of their adult life cycle and flies develop smaller at hotter temperatures, I may not have captured the full scope of these flies' fecundity in particular smaller flies that delay their reproduction until later in life. Further, studies found only minor differences in fecundity due to population and that

fecundity was mostly a byproduct of developmental temperature with thermal reaction norms primarily similar across populations (Peter et al. 2013). Since all the flies were kept at a constant temperature prior to analyzing their oviposition site preferences, I may not have been able to see the expected variation due to developmental temperature being a primary driver of oviposition site selection (Fogleman 1979).

By adding in local thermal adaptation to the models from chapter 2, we can better understand how different populations of organisms might shift their thermoregulatory strategy. I found that flies from high latitude regions are more likely to switch their thermal strategy than flies from low latitude populations. However, I found no differences in oviposition site selection between populations that were experimentally selected for either hot or cold temperatures. In conclusion, we need to reassess how local adaptation and environmental stochasticity influence how organisms respond to temperature changes. We need to develop a localized theory of thermoregulation that account for regional climate. Ultimately, by incorporating these patterns into our thermoregulatory models we can better predict how these organisms should be thermoregulating presently in the environment as well as in the future as temperatures continue to change.

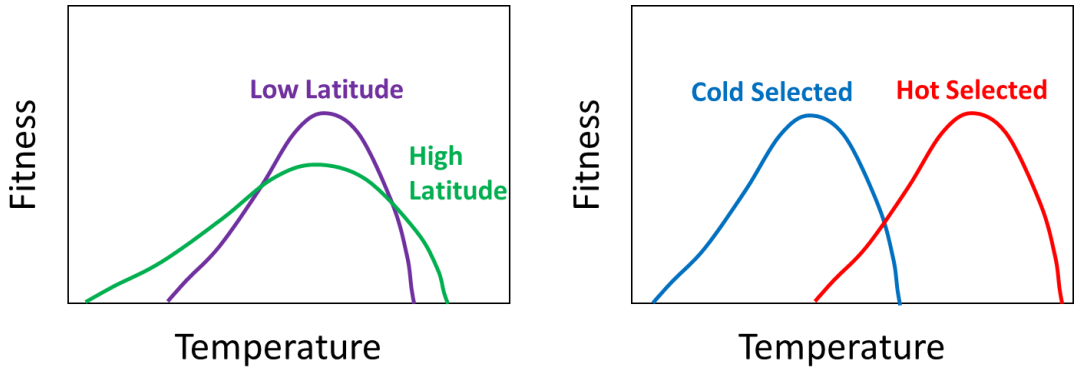

Figure 3.1. Generic predicted performance curves for flies from different thermal backgrounds. Flies from high latitude regions should have a wider performance curve than flies from lower latitudes since flies from high latitudes experience a wider range of temperatures than flies at lower latitudes. Flies that were experimentally evolved at cold temperatures should prefer colder temperatures than flies that were experimentally evolved at hot temperatures.

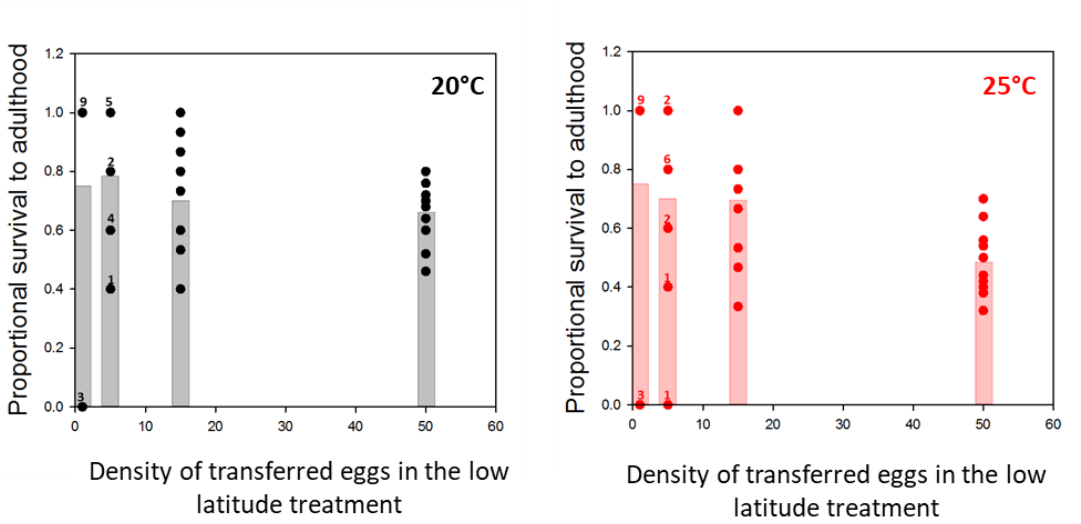

Figure 3.2. To parameterize my game theoretical model, I used survivorship data from 12 isofemale lines created from wild caught flies collected in Indiana. I transferred either 1, 5, 15, or 50 eggs to a petri dish and maintained the flies in an incubator kept at either 20°C (black) or 25°C (red) with a 12:12 light cycle. I measured survivorship by counting the proportion of adults that survived to adulthood. Each dot represents data from one isofemale line. For densities kept at either one or five individuals, the number of overlaid dots at each proportion is denoted with a number above the dot. Each bar represents the predicted model fit from the logistic regression curve. While survivorship was similar for both temperatures at low-density, survivorship dropped off much quicker as density increased for flies that were kept at 25°C.

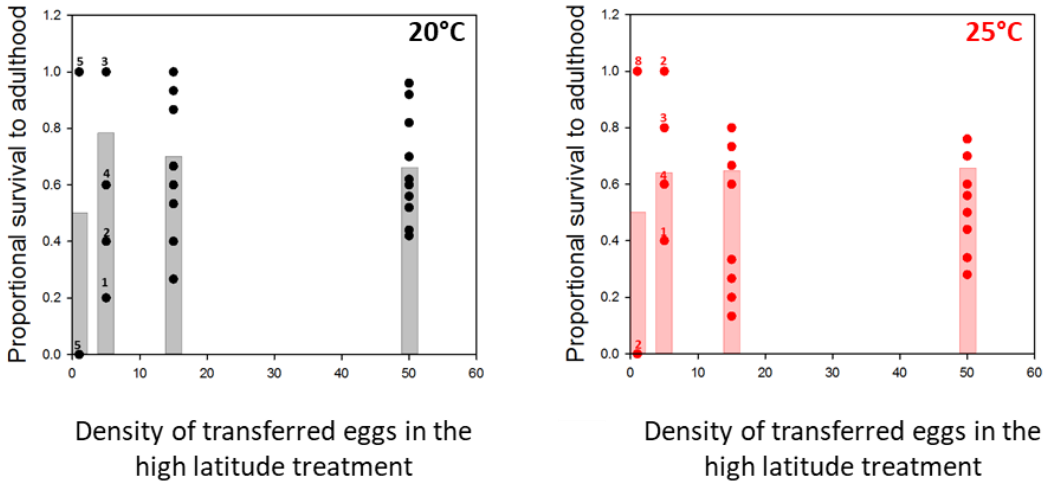

Figure 3.3. To parameterize my game theoretical model, I used survivorship data from 10 isofemale lines created from wild caught flies collected in Vermont (high latitude). I transferred either 1, 5, 15, or 50 eggs to a petri dish and maintained the flies in an incubator kept at either 20°C (black) or 25°C (red) with a 12:12 light cycle. I measured survivorship by counting the proportion of adults that survived to adulthood. Each dot represents data from one isofemale line. For densities kept at either one or five individuals, the number of overlaid dots at each proportion is denoted with a number above the dot. Each bar represents the predicted model fit from the logistic regression curve. Flies kept at 20°C had higher survivorship at low densities and had a slight increase in their survivorship as density increased while flies kept at 25°C saw a decline in their survivorship as density increased.

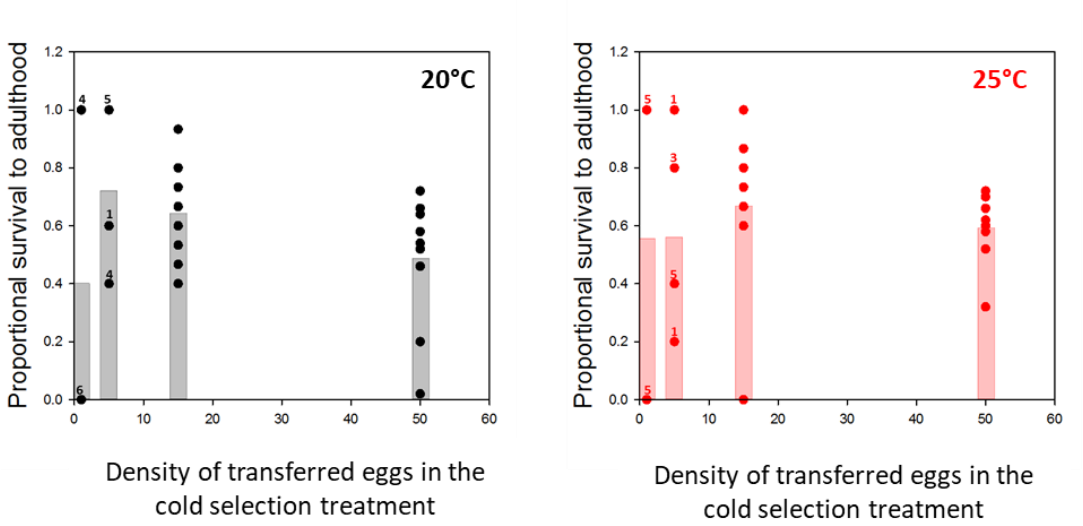

Figure 3.4. To parameterize my game theoretical model, I used survivorship data from 10 isofemale lines created from fly lines that were experimentally selected and allowed to evolve at cold (16°) temperatures for multiple years. Detailed descriptions of how the experimental populations were developed is available in Yeaman *et al.* (2010). I transferred either 1, 5, 15, or 50 eggs to a petri dish and maintained the flies in an incubator kept at either 20°C (black) or 25°C (red) with a 12:12 light cycle. I then measured survivorship by counting the proportion of adults that survived to adulthood. Each dot represents data from one isofemale line. For densities kept at either one or five individuals, the number of overlaid dots at each proportion is denoted with a number above the dot. Each bar represents the predicted model fit from the logistic regression curve. Flies at 20°C had higher survivorship at low densities than flies from 25°C. However, flies at 25°C saw a more gradual decline of their survivorship as density increased than flies at 20°C resulting in flies at 25°C to have higher survivorship at high densities than flies at 20°C.

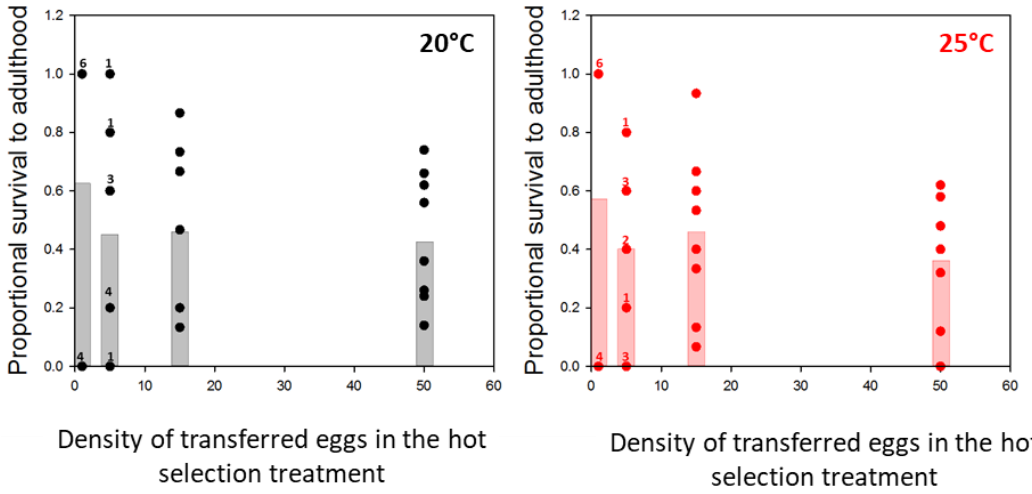

Figure 3.5. To parameterize my game theoretical model, I used survivorship data from 10 isofemale lines created from fly lines experimentally selected and allowed to evolve at hot (25°C) for multiple years. Detailed descriptions of how the experimental populations were developed is available in Yeaman *et al.* (2010). I transferred either 1, 5, 15, or 50 eggs to a petri dish and maintained the flies in an incubator kept at either 20°C (black) or 25°C (red) with a 12:12 light cycle. I measured survivorship by counting the proportion of adults that survived to adulthood. Each dot represents data from one isofemale line. For densities kept at either one or five individuals, the number of overlaid dots at each proportion is denoted with a number above the dot. Each bar represents the predicted model fit from the logistic regression curve. While survivorship was similar for both temperatures at low-density, survivorship dropped off much quicker as density increased for flies that were kept at 25°C.

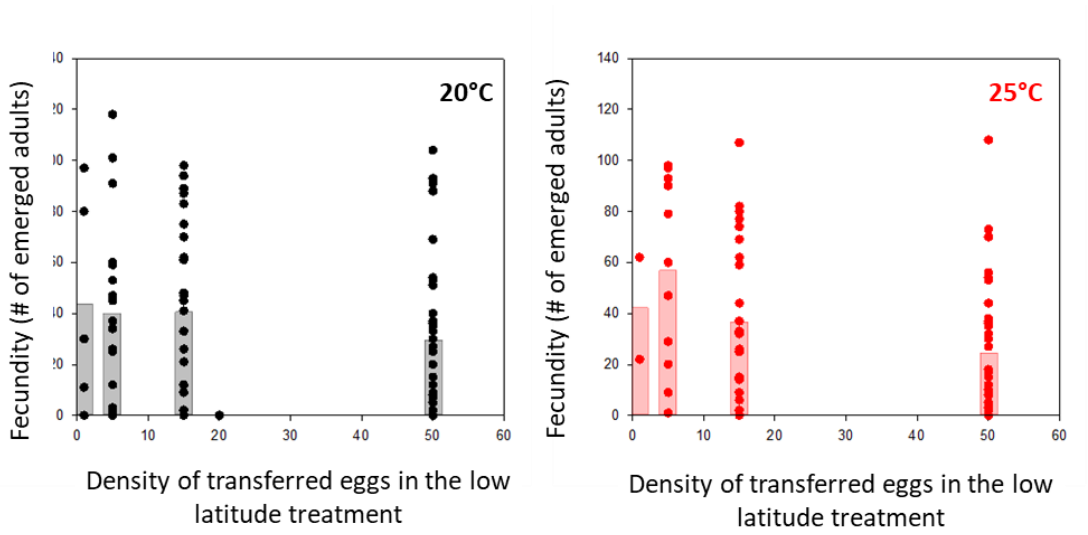

Figure 3.6 To parameterize my game theoretical model, I used fecundity data from 12 isofemale lines created from wild caught flies collected in Indiana. I transferred either 1, 5, 15, or 50 eggs to a petri dish and maintained the flies in an incubator kept at either 20°C (black) or 25°C (red) with a 12:12 light cycle. I measured fecundity, by outbreeding adult female flies from my treatment trays with a male fly from a control line (Cantonese) for four days at a common temperature of 20°C and counting the total number of emerged flies from the vial. Each dot represents data from one female fly. Each bar represents the predicted model fit from the generalized linear model. The fecundity of flies at 25°C was higher at low densities, but decreased faster than the fecundity of flies at 20°C as densities increased, causing flies at 20°C to have a higher fecundity at high densities.

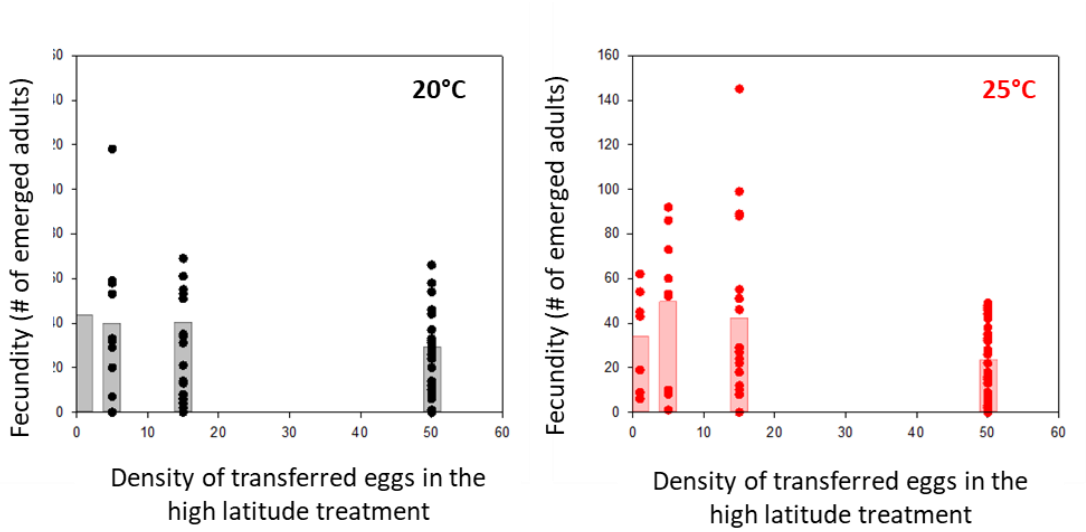

Figure 3.7. To parameterize my game theoretical model, I used fecundity data from 10 isofemale lines created from wild caught flies collected in Vermont (high latitude). I transferred either 1, 5, 15, or 50 eggs to a petri dish and maintained the flies in an incubator kept at either 20°C (black) or 25°C (red) with a 12:12 light cycle. I measured fecundity, by outbreeding adult female flies from my treatment trays with a male fly from a control line (Cantonese) for four days at a common temperature of 20°C and counting the total number of emerged flies from the vial. Each dot represents data from one female fly. Each bar represents the predicted model fit from the generalized linear model. While fecundity was similar for both temperatures at high densities, fecundity of flies at 25°C was higher at lower densities.

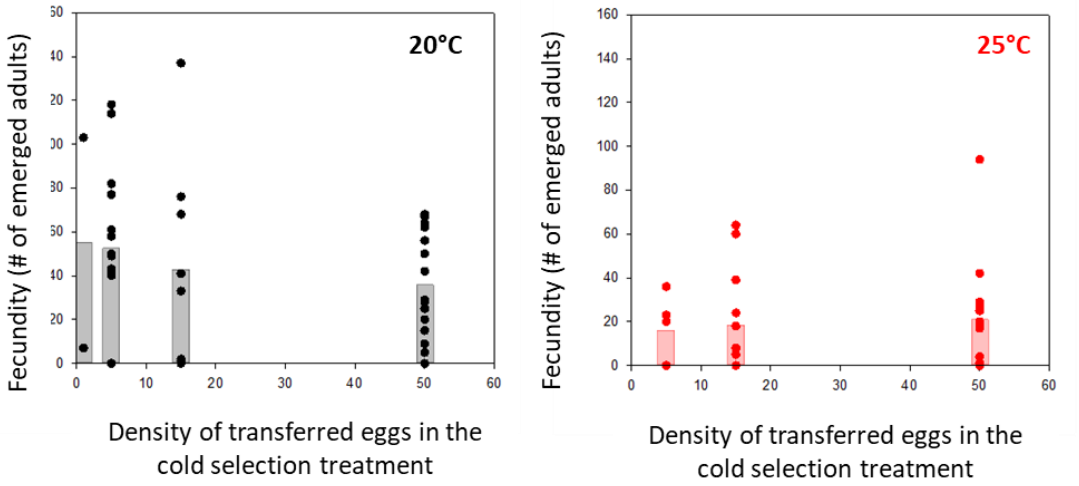

Figure 3.8. To parameterize my game theoretical model, I used fecundity data from 10 isofemale lines created from fly lines that have been experimentally selected and allowed to evolve at cold (16°) temperatures for multiple years. Detailed descriptions of how the experimental populations were developed is available in Yeaman *et al.* (2010). I transferred either 1, 5, 15, or 50 eggs to a petri dish and maintained the flies in an incubator kept at either 20°C (black) or 25°C (red) with a 12:12 light cycle. I measured fecundity, by outbreeding adult female flies from my treatment trays with a male fly from a control line (Cantonese) for four days at a common temperature of 20°C and counting the total number of emerged flies from the vial. Each dot represents data from one female fly. Each bar represents the predicted model fit from the generalized linear model. Flies kept at 20°C had higher fecundity at low densities than flies kept at 25°C. The flies kept at 25°C surprisingly saw a slight increase in their fecundity as density increased, while flies kept at 20°C saw a slight decrease in their fecundity as density increased.

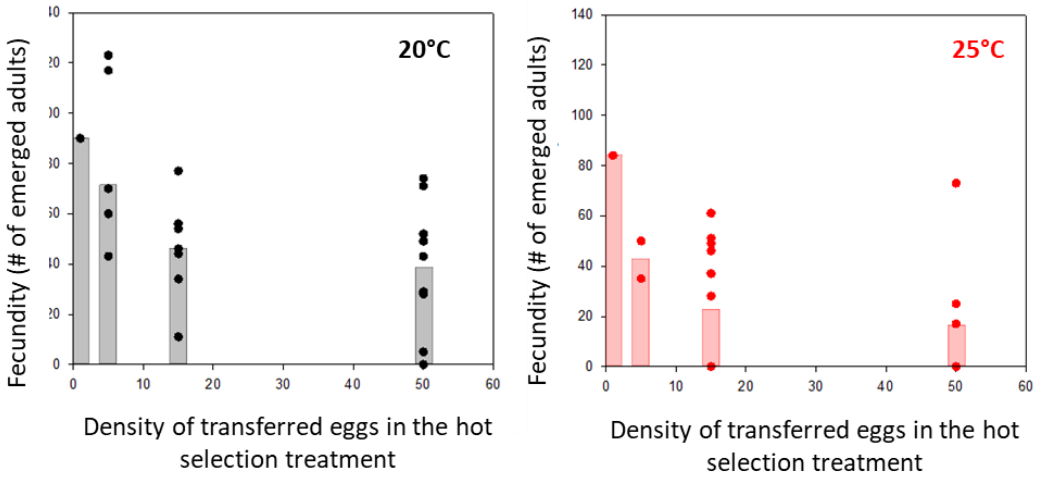

Figure 3.9. To parameterize my game theoretical model, I used fecundity data from 10 isofemale lines created from fly lines that have been experimentally selected and allowed to evolve at hot (25°C) for multiple years. Detailed descriptions of how the experimental populations were developed is available in Yeaman *et al.* (2010). I transferred either 1, 5, 15, or 50 eggs to a petri dish and maintained the flies in an incubator kept at either 20°C (black) or 25°C (red) with a 12:12 light cycle. I measured fecundity, by outbreeding adult female flies from my treatment trays with a male fly from a control line (Cantonese) for four days at a common temperature of 20°C and counting the total number of emerged flies from the vial. Each dot represents data from one female fly. Each bar represents the predicted model fit from the generalized linear model. Flies kept at 20°C had higher fecundity than flies kept at 25°C at all densities. Flies at both temperatures saw a decline in their fecundity at higher densities.

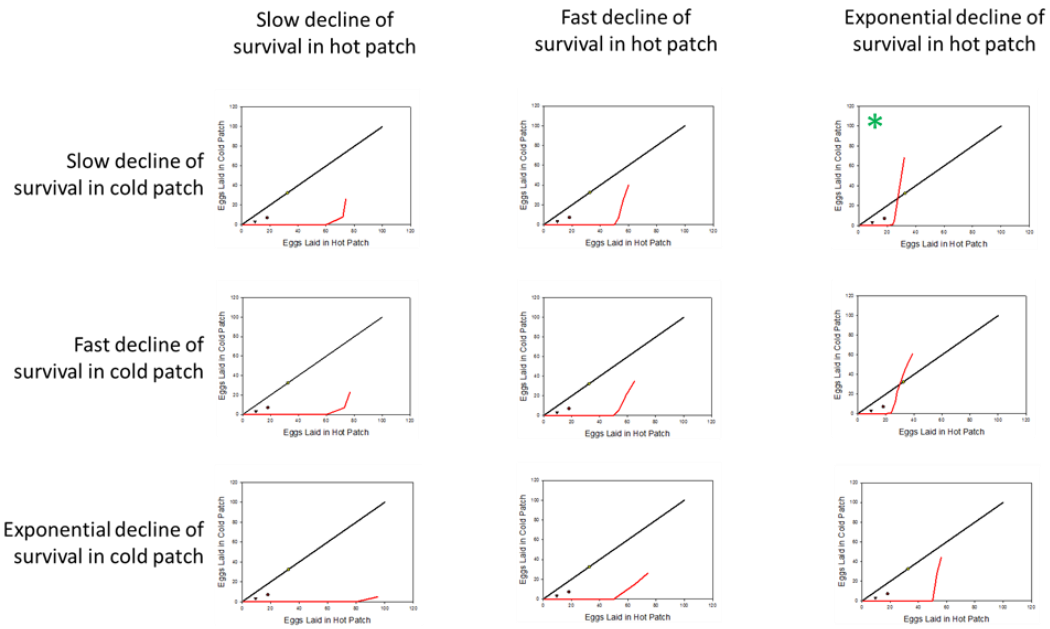

Figure 3.10. My models predict that flies from a low latitude environment should predominately lay their eggs in the warm patch as long as competition is low. As competition increases, flies should begin to lay more eggs in the cold patch especially if the rates or survival in the warm patch decline rapidly or if the flies in the cold patch experience only a slow decline in its survival rate under increased densities. The straight black line represents a trend line where the portion of eggs in each patch should be equal. The red line shows where I predict the flies to lay their eggs based on varying levels of survivorship if future fecundity and developmental time remain constant. The predicted model fit that most closely resembles the empirical data collected on fly fitness is denoted with a green apostrophe. The average egg density laid in the empirical, low-density fly treatment is represented with a burgundy circle and the average egg density laid in the empirical, high-density fly treatment is represented with an olive circle.

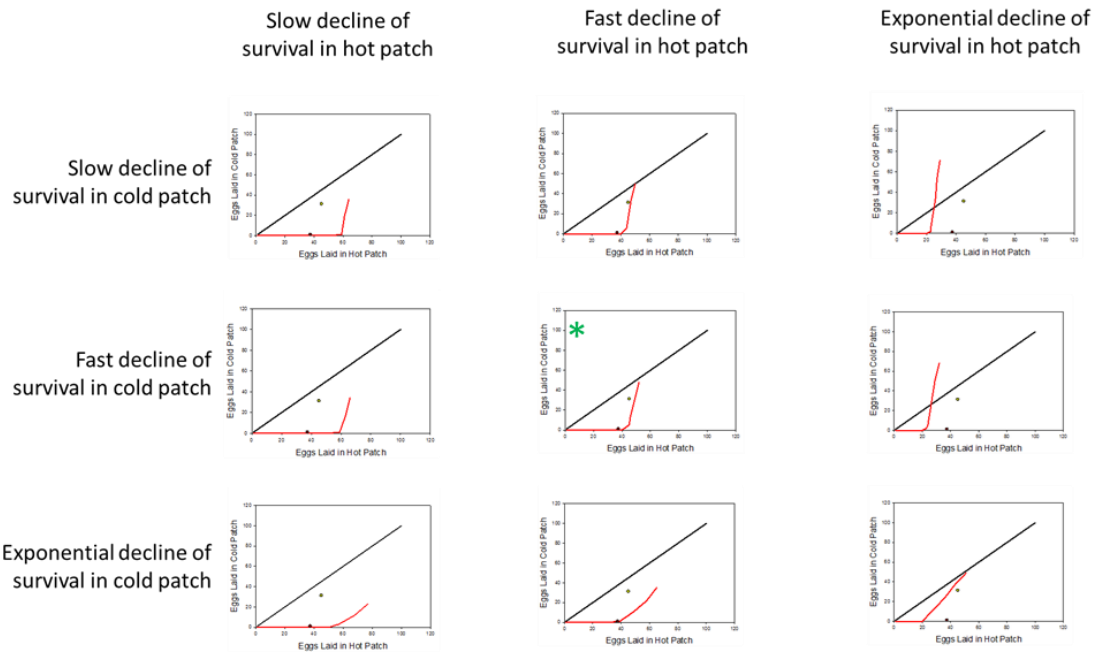

Figure 3.11. My models predict that flies from a high latitude environment should predominately lay their eggs in the warm patch as long as competition is low. As competition increases, flies should begin to lay more eggs in the cold patch especially if the rates or survival in the warm patch decline rapidly or if the flies in the cold patch experience only a slow decline in its survival rate under increased densities. Predictions for how flies from a high latitude should choose to lay their eggs based upon a game theoretical model that predicts flies' behavior as their fitness changes. The straight black line represents a trend line where the portion of eggs in each patch should be equal. The red line shows where we predict the flies to lay their eggs based on varying levels of survivorship if future fecundity and developmental time remain constant. The predicted model fit that most closely resembles the empirical data collected on fly fitness is denoted with a green apostrophe. The average egg density laid in the empirical, low-density fly treatment is represented with a burgundy circle and the average egg density laid in the empirical, high-density fly treatment is represented with an olive circle.

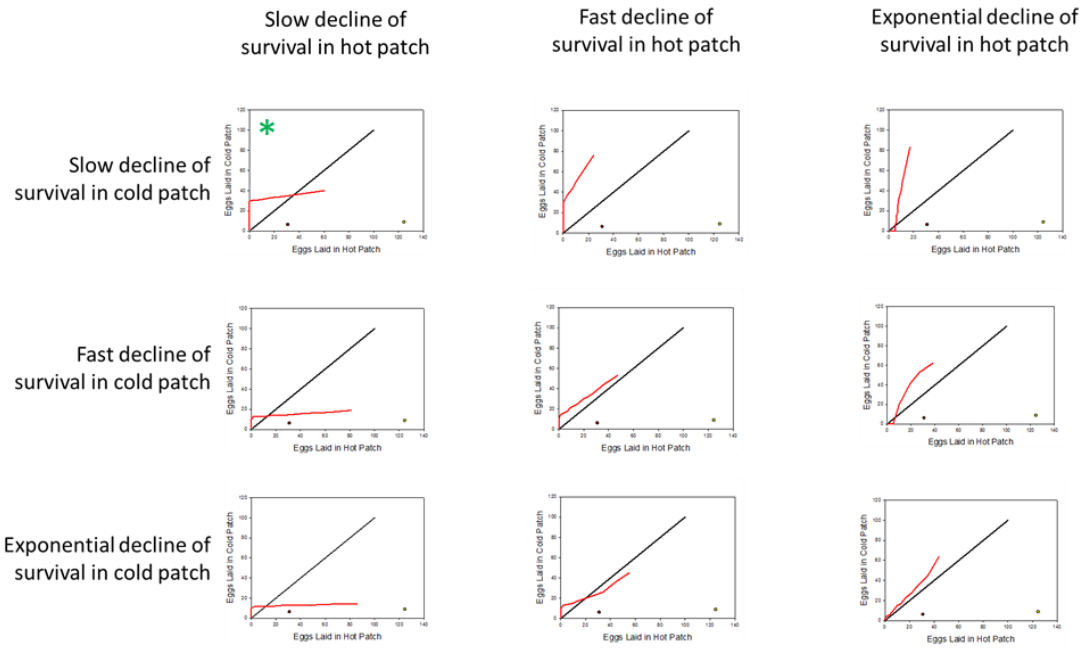

Figure 3.12. My models predict that cold selected flies should initially lay their eggs in the cold patch when competition is low. As competition increases, flies should continue to primarily lay their eggs in the cold patch if there is a fast or exponential decline of survival in the hot patch. However, if there is a slow decline of survival in the hot patch, flies should quickly switch to predominantly laying their eggs in the warm patch. The straight black line represents a trend line where the portion of eggs in each patch should be equal. The red line shows where we predict the flies to lay their eggs based on varying levels of survivorship if future fecundity and developmental time remain constant. The predicted model fit that most closely resembles the empirical data collected on fly fitness is denoted with a green apostrophe. The average egg density laid in the empirical, low-density fly treatment is represented with a burgundy circle and the average egg density laid in the empirical, high-density fly treatment is represented with an olive circle.

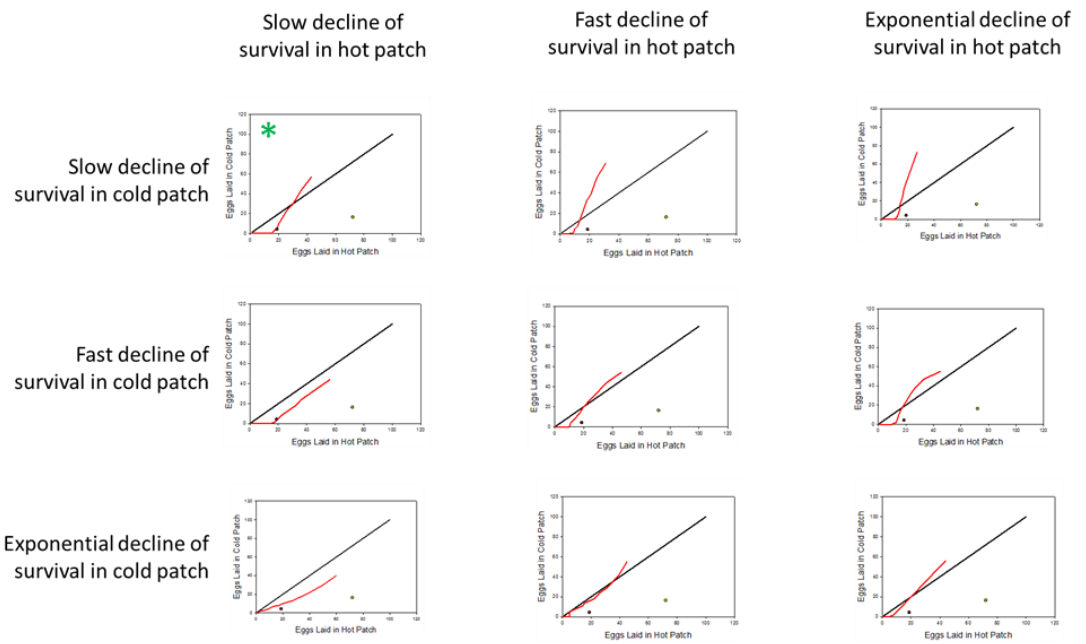

Figure 3.13. My models predict that hot selected flies should predominately lay their eggs in the warm patch as long as competition is low. As competition increases, flies should quickly switch to primarily laying eggs in the cold patch except in cases where there is a slow decline of survival in the hot patch and an exponential decline of survival in the cold patch. The straight black line represents a trend line where the portion of eggs in each patch should be equal. The red line shows where we predict the flies to lay their eggs based on varying levels of survivorship if future fecundity and developmental time remain constant. The predicted model fit that most closely resembles the empirical data collected on fly fitness is denoted with a green apostrophe. The average egg density laid in the empirical, low-density fly treatment is represented with a burgundy circle and the average egg density laid in the empirical, high-density fly treatment is represented with an olive circle.

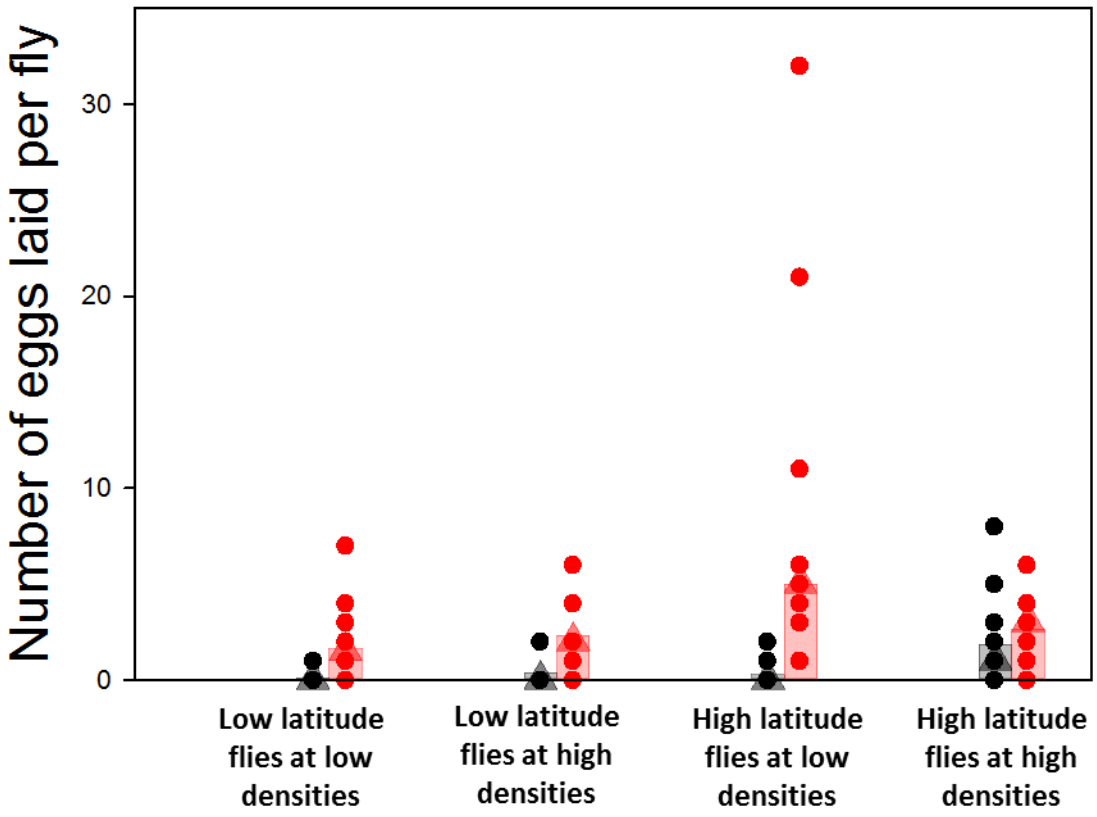

Figure 3.14. I added either 4 flies (low-density) or 15 flies (high-density) to each lane within a thermal arena. Flies then chose to oviposit their eggs in a 20°C patch (black) or a 25°C patch (red) within the thermal arena. Each dot in the graph represents the raw data for where flies chose to lay their eggs, while the triangles represents the median of where each fly laid their eggs, and each bar represents the predicted model fit for a linear mixed-effects model adjusted for zero-inflated data (Zuur 2009). I found significant differences from our low latitude, low-density treatment in relation to temperature (P = 0.004) and compared to flies from a high latitude and high-density (P = 0.003). I found significant differences from my high latitude, low-density treatment in relation to temperature ($P < 0.001$) and amount of competition (P = 0.004) from adult flies.

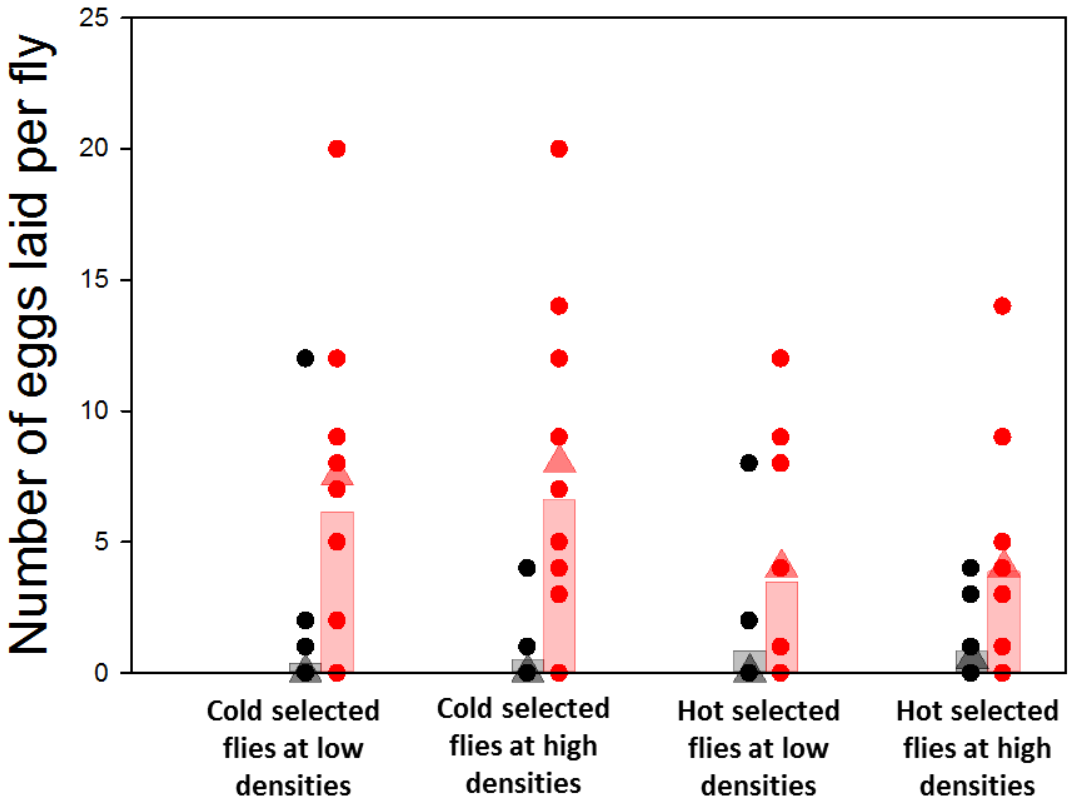

Figure 3.15. I added either 4 flies (low-density) or 15 flies (high-density) to each lane within a thermal arena. Flies then chose to oviposit their eggs in a 20°C patch (black) or a 25°C patch (red) within the thermal arena. Each dot in the graph represents the raw data for where flies chose to lay their eggs, while the triangles represents the median of where each fly laid their eggs, and each bar represents the predicted model fit for a linear mixed-effects model adjusted for zero-inflated data (Zuur 2009). I found significant differences from our cold selected, low-density treatment in relation to temperature ($P < 0.001$) and when compared to flies from the hot selection lines at 25°C for both low ($P = 0.024$) and high ($P = 0.041$) densities. I found significant differences from my hot selected, low-density treatment in relation to temperature ($P < 0.001$), but did not find any significant differences due to competition ($P = 0.79$).

Table 3.1. Results from a linear mixed-effects model adjusted for zero-inflated data for number of eggs laid by a fly in a patch that differed in temperature. Treatment groups differed in the number of female flies (4 or 15) and whether the isofemale line was created from flies found in a high latitude environment (Vermont) or low latitude (Southern Indiana). I found significant differences from our low latitude, low-density treatment in relation to temperature and high latitude, high-density, but did not find a significant difference with low latitude, high-density or high latitude, high-density.

	Estimate	SE	*P*
Intercept	-2.5267	1.0265	0.0138
High-Density	1.4465	1.1582	0.2117
High Latitude	1.1765	1.1961	0.3253
High Latitude High-Density	3.1326	1.0672	0.0033
25°C	2.9958	1.0247	0.0035
High-Density:25°C	-1.0863	1.1563	0.3475
High Latitude:25°C	-0.0469	1.1859	0.9684
High Latitude High-Density:25°C	-2.6391	1.0635	0.0131

Table 3.2. Results from a linear mixed-effects model adjusted for zero-inflated data for number of eggs laid by a fly in a patch that differed in temperature. All flies were from isofemale lines collected from the same natural, high latitude environment (Vermont) and only differed in the number of female flies (4 or 15). I found significant differences from my low-density treatment in relation to temperature and amount of competition from adult flies.

	Estimate	SE	*P*
Intercept	-1.341	0.620	0.0305
High-Density	1.952	0.684	0.0043
25°C	2.949	0.597	$P < 0.001$
High-Density:25°C	-2.593	0.661	$P < 0.001$

Table 3.3. Results from a linear mixed-effects model adjusted for zero-inflated data for number of eggs laid by a fly in a patch that differed in temperature. Treatment groups differed in the number of female flies (4 or 15) and whether the isofemale line was created from artificially selected flies that were allowed to evolve in either a constant cold environment (16°C) or a hot environment (25°C). I found significant differences from our cold selected, low-density treatment in relation to temperature but did not find a significant difference due to competition alone.

	Estimate	SE	*P*
Intercept	-1.046	0.571	0.067
High-Density	.301	0.742	0.685
Hot Selection	.845	0.704	0.230
Hot Selection High-Density	0.824	0.696	0.236
25°C	2.859	0.526	$P<0.001$
High-Density:25°C	-0.232	0.675	0.731
Hot Selection:25°C	-1.424	0.633	0.024
Hot Selection High-Density:25°C	-1.291	0.631	0.041

Table 3.4. Results from a linear mixed-effects model adjusted for zero-inflated data for number of eggs laid by a fly in a patch that differed in temperature. All flies were from isofemale lines evolved in a hot environment (25°C) and only differed in the number of female flies (4 or 15). I found significant differences from my hot selected, low-density treatment in relation to temperature but did not find any significant differences due to competition.

	Estimate	SE	*P*
Intercept	-0.2268	0.43700	0.60
High-Density	-0.00854	0.59319	0.99
25°C	1.43508	0.35187	$P < 0.001$
High-Density:25°C	0.13353	0.49461	0.79

CHAPTER 4

SPACE, FOOD, OR TEMPERATURE? EVALUATING IMPORTANCE OF ABIOTIC FACTORS WHEN FLIES CHOOSE OVIPOSITION SITES FOR THEIR YOUNG

Chapter 4: Space, Food, or Temperature? Evaluating importance of abiotic factors when flies choose oviposition sites for their young

Summary

How an organism thermoregulates depends on its own thermal physiology as well as the biotic and abiotic factors within its community. Therefore, we need models that integrate factors such as competition, food quality, and habitat size when predicting the body temperatures of animals. Such a model should consider how microhabitats limit rates of development, survivorship, and fecundity. I developed and tested a thermal game model of how flies should choose to oviposit their eggs. In this model, flies compete to lay their eggs in patches that vary in temperature, food quality, and space. I predicted that flies should initially lay their eggs in thermally optimal patches but switch to using both patches as competition increases. Additionally, flies in patches that are smaller or of lower food quality, should more readily switch to using patches that are thermally, suboptimal for development under competition. For a given density of competitors, flies laid more eggs in cooler patches when food quality was better. This behavior conflicted with a game theoretical model that predicted flies should continue to lay in warmer patches even at high densities of competitors. Competition did not alter how flies chose to lay their eggs in patches that varied in size. Further, flies preferred to lay their eggs in thermally poor patches when a large amount of space was available and preferred to lay their eggs in thermally optimal patches when the patches were smaller. Consequently, flies did not behave in accordance with my apriori predictions.

Introduction

Organisms, particularly ectotherms, engage in behavioral thermoregulation to maintain their preferred body temperature. The current theory of thermoregulation predicts how an organism should thermoregulate within its environment by calculating the costs and benefits an organism occurs due to the availability and time spent in patches that differ in temperature from the organisms optimal temperature (Huey and Slatkin 1976). However, organisms often thermoregulate much more accurately than predicted when preferred microclimates are rare (Blouin-Demers and Nadeau 2005). This mismatch might stem from competition for non-thermal resources that interact with body temperature to determine an animal's performance (Beitinger et al. 1975, Magnuson et al. 1979, Medvick et al. 1981, Seebacher and Grigg 2000, Stapley 2006, Rusch and Angilletta 2017). In addition to competition, one's ability to thermoregulate depends on environmental conditions, such as food availability (Riechert and Tracy 1975, Wildhaber 2001, Kessler and Lampert 2004, Sims et al. 2006), food quality (Underwood 1991, Pulgar et al. 2003) and size and frequency of different microclimates (Huey 1974, Withers and Campbell 1985, Tracy and Christian 1986, Huey 1991, Sears 2006, Sears and Angilletta 2015).

The size of a food patch can alter the amount of competition between organisms within the patch. With greater levels of density comes a greater level of competition for resources. As a population grows, resources such as food and space become limiting. Consequently, as density increases, individuals should shift their use of resources in ways that maximize their share of limiting resources (Maynard Smith 1976, Brown 1988, Brown et al. 1997, Brown 1998, Sih 1998). Consider an environment where food and

heat are disbursed across patches. If food becomes limited, the preferred temperature of the organism may change as organisms will begin to prefer cooler temperatures to reduce energetic costs (Magnuson et al. 1979, Crowder and Magnuson 1983). Given that organisms need to compete for both food and temperature resources, how they engage in balancing these needs will impact how they choose to spend time in patches that vary in temperature and food (Hughes and Grand 2000).

The idea that as competition increases, organisms should change how they exploit resources from their environment, offers some unique game theoretical predictions about how organisms should orient themselves within their environment. Fretwell and Lucas (1969b) developed a formula to determine the suitability of a patch by finding the ideal free distribution of a group of organisms in a patch as a product of the quality of the patch and competition for resources in the patch resulting from density of competitors. Additionally, Lancaster and Downes (2004) developed a model that analyzes how organisms exploit resources in spatially diverse patches. These models can further be modified to account for how organisms should utilize patches that have both finite and non-finite resources such as temperature. For example, Hughes and Grand's model (2000) considers how organisms should exploit patches given the resources in each patch and the competition for these resources.

A model by Hughes' and Grand (2000) predicts that organisms with access to more food should prefer warmer patches. This novel prediction comes from the interactive effect of food and temperature on growth rate. As organisms begin to compete more strongly for food, they should prefer cooler temperatures in order to decrease their metabolism and growth rate as they now have fewer resources to fuel their development

and maintenance that occurs at high temperatures (Hughes and Grand 2000). Although the model has rarely been tested directly, we know that temperature and food influence patch use. For example, zooplankton distribute themselves, in accordance with the ideal free distribution, within water columns to optimize their ability to utilize patches that differ in food and thermal quality (Lampert et al. 2003, Lampert 2005). Similar decision making trade-offs between patches that vary temperature and food availability have been found in beetles (Halliday and Blouin-Demers 2014) as well as predicted in bluegills (Wildhaber 2001), spiders (Riechert and Tracy 1975), and sharks (Sims 2003, Sims et al. 2006). Additionally, organisms are known to see various fitness benefits and costs associated with the type and timing of their diet (Raubenheimer and Simpson 1999, David et al. 2009, Raubenheimer et al. 2009, Meunier et al. 2017). Further, these different diets can affect their thermoregulation strategies (Underwood 1991, Pulgar et al. 2003). Consequently, without knowing accurate trends of how these abiotic and biotic factors influence each other we are unable to adequately model how organisms thermoregulate across habitat types.

In this chapter, I aim to determine how the size of food patches interacts with temperature and density to affect the oviposition preference of flies, *Drosophila melanogaster*. When developing evolutionary game models, or predicting how organisms will orient themselves within their environment, we need to be able to use thermoregulation models in conjunction with niche dynamics to model habitat benefits. By bringing in space use and food quality to my earlier work (Chapters 2 and 3), we will have a much better conceptual understanding of how organisms thermoregulate in nature. Fruit flies are a model system to test how thermoregulation differs across habitat types.

since in nature, larval fly communities can consist of a single piece of rotting fruit filled with various densities of fruit fly larvae. Consequently, at least relative to other species, we can recreate a close approximation of a community that a fruit fly offspring might experience in nature inside of a petri dish in the lab.

I created a game theoretical model of oviposition behavior (Chapter 2) that can be used to predict how organisms should lay their eggs in habitats that vary in food quality and size. I then empirically tested this model by allowing flies to lay eggs in patches that varied in either food quality or size. I predict that habitats that are of lower thermal quality for development, but higher nutritional quality will be more appealing to flies as competition increases than patches with high thermal quality and lower nutritional quality. Additionally, I predict that flies experiencing high levels of competition will more readily switch to less thermally beneficial patches when the patch is smaller in size.

Methods

Maintenance of *Drosophila melanogaster*

I used flies descended from females collected in Beasley Orchid, Indiana, during the fall of 2011. Twelve isofemale lines were created by mating a single adult virgin female fly with a male sibling for two generations (Cooper et al. 2014). By using isofemale lines, I controlled for genetic effects on behavior. I maintained the isofemale lines on a standard diet (recipe of the Bloomington Stock Center, Bloomington, IN) in 25 x 90 mm vials (Genesee Scientific, San Diego, CA) inside of an incubator (Percival Scientific, Perry, IA) at a temperature of 21°C and a light cycle of 12:12 L:D. Flies were transferred to new vials with fresh food approximately every three weeks.

Females in our experiments were raised at controlled density for three generations. Two males and two females from the same isofemale line were added to a vial for 48 hours. When the next generation of flies emerged in these vials, I transferred two males and two females to a new vial for 48 hours. I repeated this protocol for three generations. Adult females from the third generation were used in the experiments described below.

Parameterize the model

I used data collected from flies in the lab to parameterize of my model. To estimate the fitness of flies at each combination of density and temperature, I measured the survivorship, fecundity, and developmental time. To obtain flies for my experiment, I allowed females from 12 isofemale lines to lay eggs in petri dishes with a grape agar and yeast solution for eight hours at 23°C. I then transferred either 1, 5, 15, or 50 eggs to new petri dishes containing the grape agar and yeast. In the food quality experiment, the grape agar in each petri dish was mixed with either 0.033g of yeast (low-food quality environment) or 0.33g of yeast (high-food quality environment) while holding the circumference of the petri dish constant at 35mm. For the space availability experiment, I used either a 35mm (low-space) or 50mm (high-space) circumference petri dish while holding the amount of yeast present in each dish constant at 0.033g. These petri dish were kept an incubator at either 16°, 20°, 25°, or 30°C with a 12:12 light cycle. I measured survivorship by counting the number of adults that survived to adulthood.

To measure fecundity, I outbred adult female flies from my treatments with male flies from a control line (Cantonese, Bloomington Stock Center, Bloomington, IN) to capture only variation in fecundity because egg production, not sperm production, is typically the limiting factor for population growth. Additionally, since I was concerned about the effect of larval developmental temperature and adult flies would be able to freely move to new patches that varied in temperature, I moved all vials to a common temperature of 20°C for my fecundity experiments. I placed each pair of flies into a vial (25 x 95 cm) containing a standard cornmeal-yeast medium for 4 days in an incubator at 20°C with a 12:12 light cycle. I then removed the adult breeding pair and allowed any eggs laid by these fly pairs to develop to adulthood. From the adult flies that emerged within each of these vials, I again recorded developmental time (egg to adult) to the nearest day and counted the total number of flies emerged (fecundity).

I estimated how density and temperature related to fecundity using a generalized linear model while I estimated survivorship using a logistic regression curve. I then used Akaike information criterion (AIC) to pick the best polynomial fit for each curve in R (Version 3.3.1 R-Core-Team 2016) (Zuur 2009). I then developed additional models based on predictions of how one might expect the fitness of flies to vary under different circumstances. I used the best fit curve developed from the raw parameters to find an initial curve which could explain how survivorship and fecundity change with increasing density. Furthermore, I hypothesized additional parameter data that was in line with findings on fitness data to create additional models regarding how you would expect flies to behave if their survival parameters were slightly altered. To create this additional parameter data, I also increased or decreased how quickly survivorship changed under

increased competition since in nature you might have conditions changing within these patches causing survivorship to differ from what we measured. For example, resources might be replenished within these patches, which would cause survivorship to decline more slowly. Alternatively, predators might be attracted to higher densities of prey, which would cause survivorship to decline more quickly and possibly at an exponential rate.

Experimental tests of the model

I used the same Plexiglas thermal arena for the experimental tests of the model as in Chapter 2 (Figure 2.3). I used the thermal arenas to test where flies chose to lay their eggs under different intensities of competition. In each arena, a fly could choose between a petri dish at a preferred temperature of 25°C and a petri dish at 20°C. For each isofemale line, I ran trials in which either 4 or 15 adult females were in an arena at a time. Additionally, I simultaneously ran the same isofemale line for both cross-comparison treatment groups when testing the flies in the arenas. For the food quality experiments, I used petri dishes with a 35mm circumference, but varied the amount of yeast in the agar so that the low-food quality experiment used 0.033g of yeast and the high-food quality experiment used 0.33g of yeast (Figure 4.1). For the experiments looking at space availability, I used 0.033g of yeast in each petri dish, but varied the size of the petri dish so that the low-space experiments used a petri dish with a circumference of 35mm and the high-space used a petri dish with a circumference of 50mm (Figure 4.2). After four hours, I removed the flies and froze the petri dishes from each side of the arena. Later,

these dishes were thawed, and the number of eggs was counted under a dissecting scope (Zeiss Stemi 2000-C) at each temperature.

I performed a linear mixed-effects model adjusted for zero-inflated data (Zuur 2009) using the glmmADMB package (Fournier et al. 2012) in R (Version 3.2.3, R-Core-Team 2016) to determine whether the number of eggs laid in each patch in each treatment type differed. The fixed factors in my model were temperature and density/treatment. I used trial as a random factor to compare eggs laid in the two sides of the arena while accounting for variation in the number of eggs among trials. The experimental date and isofemale line was dropped from my model due to poor fit using AIC values.

Results

I modeled the fitness of flies in different environments using data on survivorship and fecundity. Survival of flies at different densities and temperatures depended on food quality and space availability (see Figures 4.3-4.5). Survivorship at different temperatures was similar for flies in low-food and low-space environments. In high-food quality environments, survivorship was lower in 25°C patches than 20°C patches. In large patches, survivorship was highest at low densities in 20°C patches. Fecundity of flies also changed with both density and developmental temperature depending on food quality and space availability (see Figures 4.6-4.8). In low-space and low-food environments, fecundity was highest in 25°C patches at low-density. While in high-food environments, flies had a fairly constant fecundity across densities in patches at 20°C, while the highest fecundity in 25°C patches was at the lowest and highest densities. In large patches, flies

had higher overall fecundity in 25°C patches and saw a decrease in fecundity with an increase in density.

My game theoretical models illustrate how competition affects the fitness of fruit flies. The model predicts that under scenarios where survivorship in hot patches falls quickly, fruit flies should lay eggs in the colder patch at lower density levels. In scenarios where survivorship in cold patches falls quickly, fruit flies should lay fewer total eggs in the cold patch. Flies in a low-food quality environment are also expected to initially lay their eggs in the warm patch and then spread their effort to the cold patch as the density of eggs in the hot patch continues to increase (Figure 4.9). However, flies in the high-food quality environment are expected to only lay their eggs in the hot patch under most survivorship scenarios (Figure 4.10). The only time that flies in the high-food environment are expected to use the cold patch at all is when there is an exponential decline of survivorship in the hot patch. The models for the flies in both high (Figure 4.11) and low-space (Figure 4.9) environments, predicts that flies should initially lay eggs in the hot patch than shift to the cold patch as the density of eggs increases. Additionally, the model predicts that flies in the high-space environment should shift their effort to the cold patch when there are fewer total eggs than the flies in the low-space environment.

I found that temperature and competition both affected patch choice in high-food environments, but only temperature affected patch choice in low-food quality environments. Low densities of flies laid eggs in high-food quality patches almost exclusively at 25°C ($P = 0.02$) while high densities of flies laid more eggs at 20°C than flies at low-density ($P = 0.03$). I did not find any significant differences in the amount of

eggs laid by low-density flies between low and high-food quality patches (Table 4.1). Flies at low-density laid eggs in low-food quality patches almost exclusively at 25°C (P = 0.005) and did not differ in the amount of eggs laid at high densities (P = 0.49) (Figure 4.12, Table 4.2). Flies in low-food quality environments did not behave differently when laying eggs at low vs high densities. At low-density, flies in low-food quality environments laid a median of 1.5 eggs at 25°C and 0 eggs at 20°C. At high-density, low-food quality flies laid a median of 2.6 eggs at 25°C and still 0 eggs at 20°C. Flies in high-food quality environments behaved differently when laying eggs at low vs high densities. At low-density, high-food quality flies laid a median of 0.5 eggs at 25°C but 0 eggs at 20°C. At high-density, high-food quality flies laid a median of 0.5 eggs at 20°C and 3 eggs at 25°C.

In patches that vary in size, I found that competition did not alter flies' preference for where they chose to lay their eggs, however, flies in small patches preferred to lay their eggs in warm environments while flies in large patches preferred to lay their eggs in cold environments. Low densities of flies in 35mm patches laid eggs almost exclusively at 25°C (P < 0.001) and did not significantly change their egg laying behavior under increased competition (P =0.34). I found that flies in 50mm patches laid significantly more eggs in the 20°C patches than flies in 35mm patches at both low-density (P=0.006) and high-density (P=0.004) (Table 4.3). Low densities of flies in 50mm patches laid eggs almost exclusively at 20°C (P = 0.003), but did not change their behavior at high-density (P =0.94) (Figure 4.13, Table 4.4). Flies in low-space environments did not behave differently when laying eggs at low vs high densities. At low-density, flies in low-space environments laid a median of 2 eggs at 25°C and 0 eggs at 20°C. At high-density, low-

space flies laid a median of 1 egg at 25°C and still 1 egg at 20°C. Flies in high-space availability environments did not behave differently when laying eggs at low vs high densities. At low-density, flies in high-space environments laid a median of 1 egg at 25°C and 2 eggs at 20°C. At high-density, high-space flies laid a median of 1 egg at 25°C and still 3 eggs at 20°C.

Neither the flies in the food quality or space availability treatments followed my models' predictions. Flies in high-food quality environments had a density effect while the flies in the low-food quality environment did not. These findings are completely opposite from the models' predictions that flies in low-food quality environments should see a density effect, but not those flies in high-food quality environments. While my findings for space use were also not in line with my predictions. Neither the flies in the low-space nor high-space availability environments saw a density effect. In fact, flies in the high-space environment showed a preference for the patch that was the sub-optimal temperature for their young.

Discussion

I found the opposite of what I expected in patches that varied in food quality. I found that organisms were likely to disperse their effort between both patches in the high-food environment as opposed to the low-food quality environment. However, the density of the eggs in my low-food quality patches also never got high enough to warrant a change in behavior according to my model. Consequently, flies laying their eggs predominantly in the cold patch was the optimal behavior in the low-food quality patches. While in the high-food quality patches, the flies behaved sub-optimally by spreading their effort out to

both the warm and cold patch as opposed to laying only in the warm patch as predicted by the model. In nature, food resources typically vary throughout the environment. This variation in food resources affects how organisms should utilize their environment. In Hughes' and Grand's model (2000), which makes predictions based on which patch offers the highest potential growth rate, they found that in high-food quality patches organisms should prefer warmer temperatures as opposed to organisms in low-food quality patches where organisms should show a preference for colder temperatures. This prediction contrasts with my findings, where flies switched to colder patches faster in high-food quality environments than in low-food quality environments.

These results might be due to flies viewing a high-food environment as very rewarding and consequently immediately lay their eggs despite potential other negatives such as temperature. If food is the primary driver of choice within a habitat, a habitat that is of very high-food quality may be too rewarding for the flies to bypass and flies may choose to lay their eggs in the high-food quality patch regardless of temperature or any other factor. Previous work has shown that flies do actively probe and strongly choose sites that are bitter smelling or plain over sites that are high in sucrose (Yang et al. 2008b). Additionally, flies have been shown to have a strong preference for ovipositing on acetic acid rich media despite a strong behavioral avoidance of the same media when not engaging in oviposition behavior (Joseph et al. 2009). This strong preference for certain odors could explain why flies in the high yeast treatment did not change their strategy as competition increased.

Flies develop much slower at cold temperatures (Bennett 1987, Frazier et al. 2006). Consequently, flies may need a larger food resource available to them for them to

choose colder patches for their young. Since in cold patches, that food supply is going to have to last for a longer period of time than a food patch in a hot environment. In a patch with a small quantity of food available, flies need a hotter patch so that they develop faster so they can reach a stage where they can more readily move before food runs out in their patch. This conclusion is in line with the temperature size rule that states organisms grow at a much quicker, albeit less efficient rate, in hotter environments (Atkinson 1994, 1995, Noach et al. 1996, Bochdanovits et al. 2003). Because faster growth requires more food, flies may be more sensitive to increasing density at high temperature. Although organisms need more food per day to grow fast at higher temperatures, they do not need the food to last as long since they will also develop quicker and be able to move to a new patch. Consequently, in patches of low-food quality, flies might have maintained a strong preference for the warm patch so that offspring could develop quickly and move to a patch of higher quality. While in a patch of high-food quality, flies might not have been as concerned with temperature as in a low-food quality patch, despite developmental effects of temperature, since flies will have a food supply that should last much longer.

Flies may have switched to cooler patches in high-food environments more readily than low-food environments due to the way different foods and food qualities covary with temperature in their natural habitat. If certain temperatures are more likely to contain food with high nutritional content in their habitat, flies may assume that the food quality of a patch may vary in the future. At warmer temperatures, other organisms, such as yeast, also have a faster growth and reproduction rate (White and Munns 1951, Merritt 1966). Therefore, a warm environment that contains some yeast, may actually have more yeast available later, than a colder patch that initially starts with more yeast. Further my

findings that flies are more willing to use a cold patch when it is also a of high-food quality is consistent with results found in red flour beetles that showed they had a higher preference for food than temperature (Halliday and Blouin-Demers 2014). My flies' willingness to switch to cold patches in high-food quality treatments may have simply been the result of flies simply basing their choice on seeing a patch that had high-food quality. This finding is also consistent with a previous finding where ants only switched to cold temperature patches if the patches were high enough in food quality (Lachlan and Latty 2016).

Adult flies may also be attracted to the high-food quality patch purely for their own selfish reasons. The additional food quality is not only beneficial to the fly larvae, but also to the adult flies as the additional yeast has a strong odor and can be consumed by the adult flies (Becher et al. 2012, Marshall 2015) (Figure 4.1). Consequently, fruit flies are very attracted to yeast and so the fruit flies might just be spending disproportionate amounts of time in the high-food quality patch for themselves and not for their young. Consequently, fruit flies may lay lots of eggs in the thermally poor, but high-food quality patch simply because they are spending time in the patch to consume food for themselves.

Larger areas likely contain more heterogeneous microclimates. Consequently, fly larvae might have greater motility and access to more temperatures in a large patch than in a small patch. Organisms that live in patchier, more thermally heterogenetic patches are more capable of thermoregulating than organisms in more uniform environments as organisms are able to utilize different microclimates (Tracy and Christian 1986, Huey 1991, Sears and Angilletta 2015). If flies assume that there more microhabitats and

greater mobility of their young, fruit flies may assume that once their larvae will be able to move to a more thermally beneficial portion of the habitat regardless of where they are initially laid. In fact, fly larvae have shown a strong ability to thermoregulate within a small habitat (Wang et al. 2008). Therefore, flies may be expecting their larvae to better utilize the habitats within the larger patches. Further since these patches are cooler, the flies may be more confident that temperatures will not become lethal for their larvae (Feder et al. 2000). Further, it has been found that interference competitive for food between larvae has a large influence on population size (Gilpin 1974). If selection is primarily taking place in the larvae, then it would make sense that the adult flies are simply choosing sites that enable their larvae to have the most options available to them. Consequently, my fruit flies may have seen the larger patches of food as potentially having more microhabitats and thus being a better habitat for their young regardless of temperature.

While my results for the low-food quality and low-space availability, line up with each other and one of my previous studies (Chapter 3) using that flies from that same population, the results do not match up with the results from my first study (Chapter 2). In the first experiment, the flies changed their behavior as competition increased while the flies in the second and third experiment did not change their behavior under increased competition the second time. These differences in results could be due to a couple of reasons. The timing of the experiments and isofemale lines from both the Chapter 3 and 4 experiments were the same. However, the experiments from Chapter 2 were carried out almost two years prior and with different isofemale lines. Consequently, there are different fly lines used in the two experiments. These isofemale lines may have differed

from the earlier isofemale lines that I previously used in how they respond to temperature or competition differences. Further, these isofemale lines may have just differed in their food or humidity preferences which may have caused a conflating factor that was unaccounted for. As a result, I might not have been able to pick up some of the same differences that I could with the earlier experiment.

A potential logistical issue is also that there may just not have been adequate space between patches in high-space treatment. I used the same size arena for both the large and small patches. Consequently, there was less distance between the optimal and suboptimal thermal patches in the arena with large patch size (Figure 4.2). Therefore, it is possible that our patches within our thermal arena were too close together for the adults to recognize them as distinct patches that maggots could not move between. Temperatures vary on a very fine scale in nature (Sears et al. 2011, Potter et al. 2013, Ficetola et al. 2018), however, these small patches may not remain at a stable temperature for an extended period of time especially if they are that close to a patch of another temperature. If the temperature of these patches varies too much, flies should just lay randomly since the current temperature of the patch may not be a good predictor of future temperatures (Huey 1991, Feder et al. 1997b, Kingsolver and Huey 1998). Further, maggots may also be able to move between the patches themselves when they are close enough together. If the patches were close enough together, it is possible that they were simply thought of as patches that larvae could alternate between to obtain benefits from both patches similar to how zooplankton will shuffle between patches that vary in resources (Lampert et al. 2003, Lampert 2005). While there is no food or beneficial substrate between the two patches, the high-space patches are closer together than the

low-space patches which may allow for the maggots to move between the patches. Therefore, it could be beneficial for flies to lay their eggs in cold patches, since that patch may be less likely to get as lethally hot temperatures in the next 24 hours - despite the developmental cost of being at a lower temperature as an egg. Then once the fly enters the larval stage, it can move between the patches to maximize its thermal benefits. Consequently, this brings us back to the idea that selection maybe taking place entirely in the larvae.

There is still much we do not understand about how factors such as temperature, competition, space, and food influences one and another. However, it is clear that we need to be more capable of predicting how organisms respond to competition for resources in patchy environments. Empirically examining how biotic and abiotic factors influence niche dynamics can offer surprising results that incorporate numerous different factors that may not even be initially considered when developing complex game theoretical models. However, it is important that we continue to make quantitative predictions and empirically check these same predictions so that we can better understand how these animals behave and produce better evolutionary game models for them in the future.

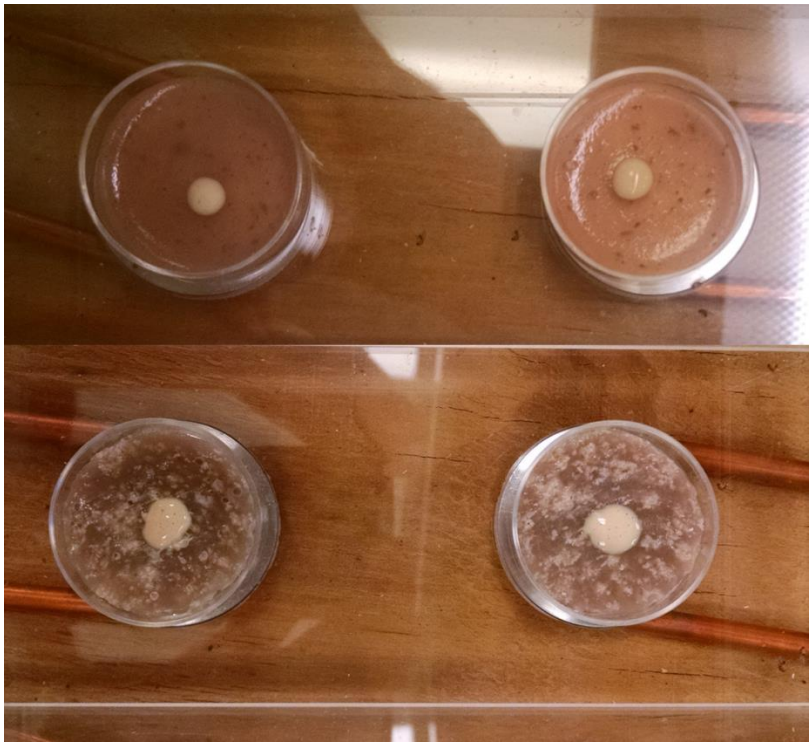

Figure 4.1. My thermal arena consisted of a Plexiglas container that consisted of 6 independent lanes by running copper tubing below small aluminum plates. Each lane within the arena had two aluminum plates on opposite ends of the lane where I could place an agar-filled petri dish with a drop of yeast. Flies could fly and lay their eggs in either the 16°C petri dish or the 25°C petri dish. Food quality differed between treatments as a function of the quantity of yeast available in each patch between the low-food quality treatment (top) and high-food quality treatment (bottom).

Figure 4.2. My thermal arena consisted of a Plexiglas container that consisted of 6 independent lanes by running copper tubing below small aluminum plates. Each lane within the arena had two aluminum plates on opposite ends of the lane where I could place an agar-filled petri dish with a drop of yeast. Flies could fly and lay their eggs in either the 16°C petri dish or the 25°C petri dish. Flies in the low-space treatment chose between 35mm diameter patches (top) while flies in the large space treatment chose between 50mm diameter patches (bottom). The total amount of yeast present in patches was constant between treatments.

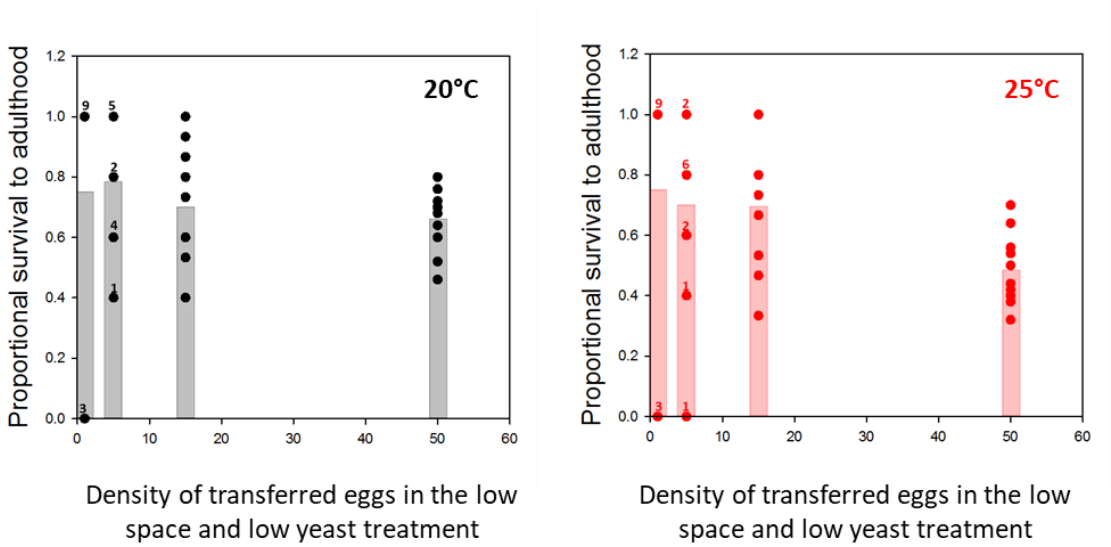

Figure 4.3. To parameterize my game theoretical model, I used survivorship data from 12 isofemale lines created from wild caught flies collected in Indiana. I transferred either 1, 5, 15, or 50 eggs to a 35mm petri dish (low-space) with a low yeast to grape agar ratio and maintained the flies in an incubator kept at either 20°C (black) or 25°C (red) with a 12:12 light cycle. I measured survivorship by counting the proportion of adults that survived to adulthood. Each dot represents data from one isofemale line. For densities kept at either one or five individuals, the number of overlaid dots at each proportion is denoted with a number above the dot. Each bar represents the predicted model fit from the logistic regression curve. While survivorship was similar for both temperatures at low-density, survivorship dropped off much quicker as density increased for flies that were kept at 25°C.

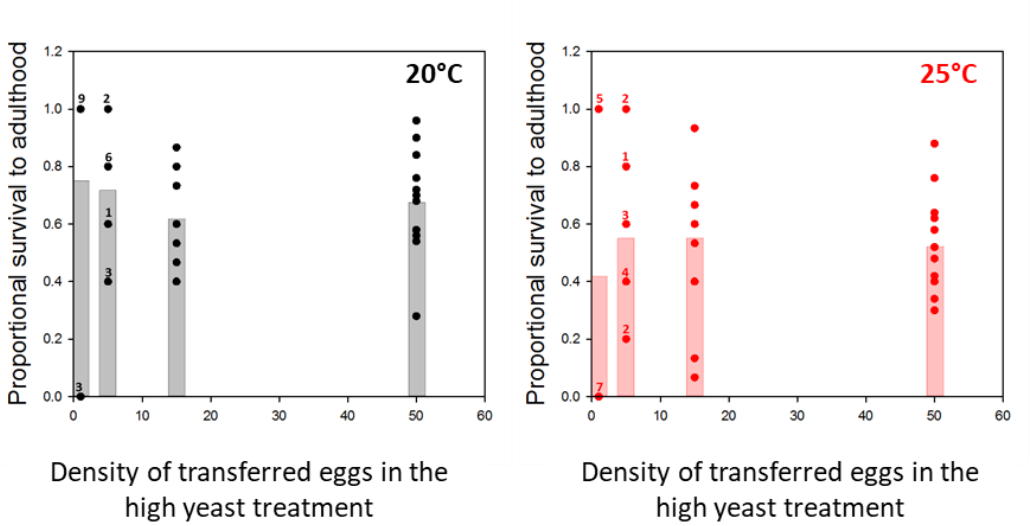

Figure 4.4. To parameterize my game theoretical model, I used survivorship data from 12 isofemale lines created from wild caught flies collected in Indiana. I transferred either 1, 5, 15, or 50 eggs to a 35 mm petri dish with a high yeast to grape agar ratio and maintained the flies in an incubator kept at either 20°C (black) or 25°C (red) with a 12:12 light cycle. I then measured survivorship by counting the proportion of adults that survived to adulthood. Each dot represents data from one isofemale line. For densities kept at either one or five individuals, the number of overlaid dots at each proportion is denoted with a number above the dot. Each bar represents the predicted model fit from the logistic regression curve. Flies kept at 20°C had higher survivorship than flies kept at 25°C at all densities. Flies kept at 20°C also had a slight increase in their survivorship as density increased while flies kept at 25°C saw a decline in their survivorship as density increased.

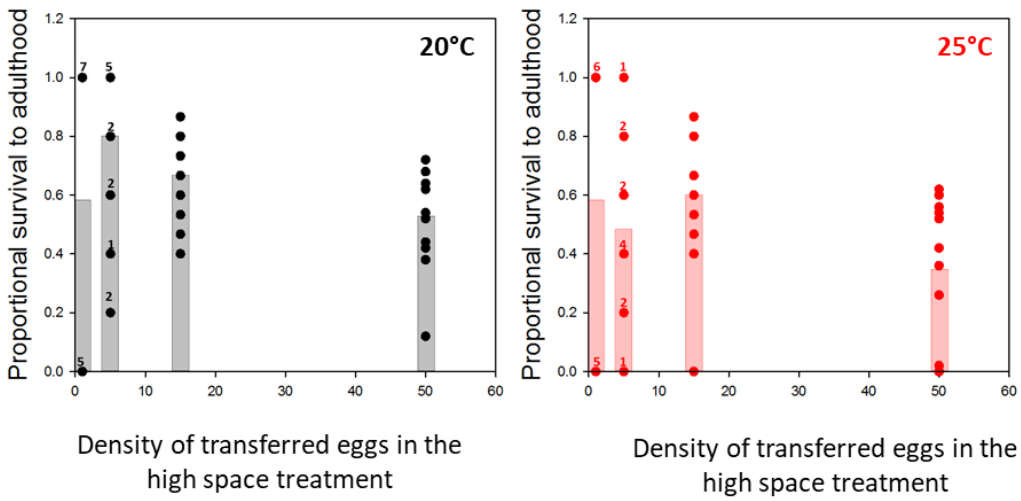

Figure 4.5. To parameterize my game theoretical model, I used survivorship data from 12 isofemale lines created from wild caught flies collected in Indiana. I transferred either 1, 5, 15, or 50 eggs to a 50mm petri dish with 0.033g of yeast and maintained the flies in an incubator kept at either 20°C (black) or 25°C (red) with a 12:12 light cycle. I measured survivorship by counting the proportion of adults that survived to adulthood. Each dot represents data from one isofemale line. For densities kept at either one or five individuals, the number of overlaid dots at each proportion is denoted with a number above the dot. Each bar represents the predicted model fit from the logistic regression curve. Flies kept at 20°C had higher survivorship than flies kept at 25°C at all densities. Flies at both temperatures saw a decline in their survivorship as density increased.

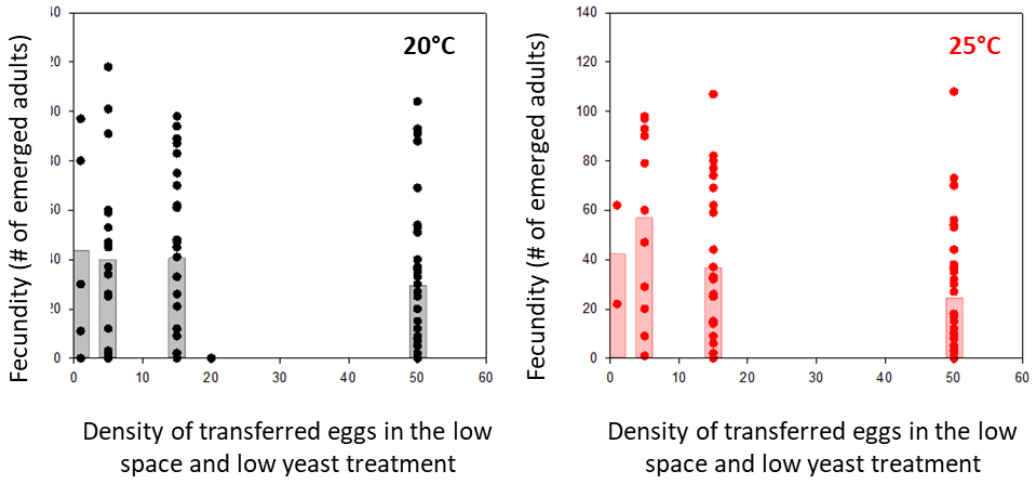

Figure 4.6. parameterize my game theoretical model, I used fecundity data from 12 isofemale lines created from wild caught flies collected in Indiana. I transferred either 1, 5, 15, or 50 eggs to a 35mm petri dish (low-space) with a low yeast to grape agar ratio and maintained the flies in an incubator kept at either 20°C (black) or 25°C (red) with a 12:12 light cycle. I measured fecundity, by outbreeding adult female flies from my treatment trays with a male fly from a control line (Cantonese) for four days at a common temperature of 20°C and counting the total number of emerged flies from the vial. Each dot represents data from one female fly. Each bar represents the predicted model fit from the generalized linear model. The fecundity of flies at 25°C was higher at low densities, but decreased faster than the fecundity of flies at 20°C as densities increased, causing flies at 20°C to have a higher fecundity at high densities.

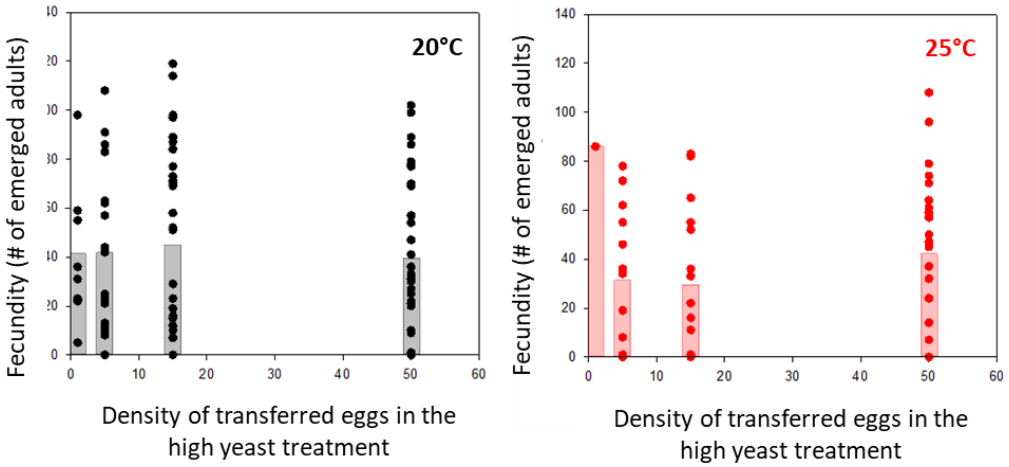

Figure 4.7. To parameterize my game theoretical model, I used fecundity data from 12 isofemale lines created from wild caught flies collected in Indiana. I transferred either 1, 5, 15, or 50 eggs to a 35 mm petri dish with a high yeast to grape agar ratio and maintained the flies in an incubator kept at either 20°C (black) or 25°C (red) with a 12:12 light cycle. I measured fecundity, by outbreeding adult female flies from my treatment trays with a male fly from a control line (Cantonese) for four days at a common temperature of 20°C and counting the total number of emerged flies from the vial. Each dot represents data from one female fly. Each bar represents the predicted model fit from the generalized linear model. While fecundity was similar for both temperatures at high densities, fecundity of flies at 20°C was higher at lower densities.

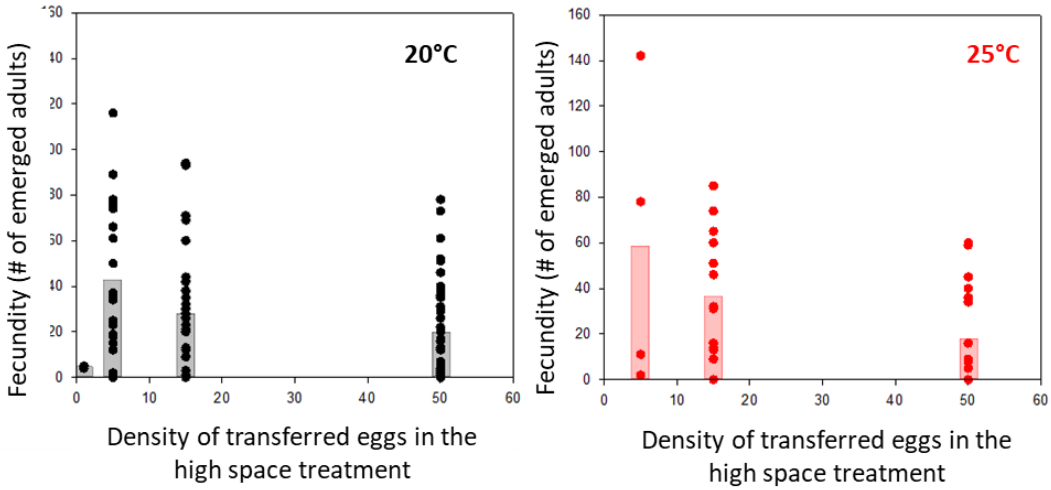

Figure 4.8. To parameterize my game theoretical model, I used fecundity data from 12 isofemale lines created from wild caught flies collected in Indiana. I transferred either 1, 5, 15, or 50 eggs to a 50mm petri dish with 0.033g of yeast and maintained the flies in an incubator kept at either 20°C (black) or 25°C (red) with a 12:12 light cycle. I measured fecundity, by outbreeding adult female flies from my treatment trays with a male fly from a control line (Cantonese) for four days at a common temperature of 20°C and counting the total number of emerged flies from the vial. Each dot represents data from one female fly. Each bar represents the predicted model fit from the generalized linear model. While fecundity was similar for both temperatures at high densities, fecundity of flies at 25°C was higher at lower densities.

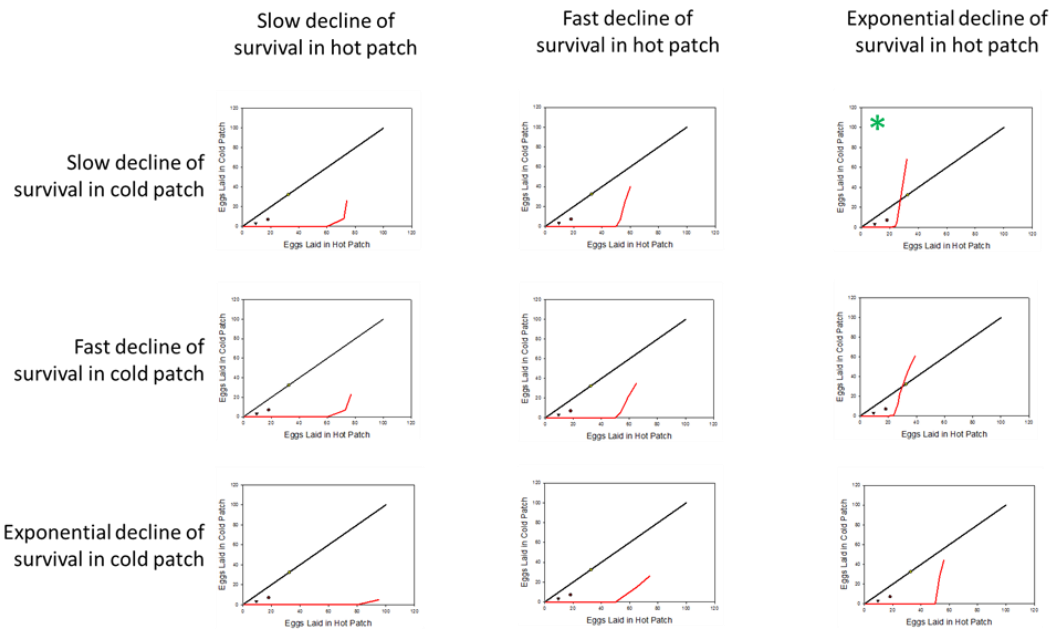

Figure 4.9. My models predict that flies in a low-space and/or low-food quality environment should predominately lay their eggs in the warm patch as long as competition is low. As competition increases, flies should begin to lay more eggs in the cold patch especially if the rates or survival in the warm patch decline rapidly or if the flies in the cold patch experience only a slow decline in its survival rate under increased densities. The straight black line represents a trend line where the portion of eggs in each patch should be equal. The red line shows where I predict the flies to lay their eggs based on varying levels of survivorship if future fecundity and developmental time remain constant. The predicted model fit that most closely resembles the empirical data collected on fly fitness is denoted with a green apostrophe. The average egg density laid in the empirical, low-density fly treatment is represented with a burgundy circle and the average egg density laid in the empirical, high-density fly treatment is represented with an olive circle.

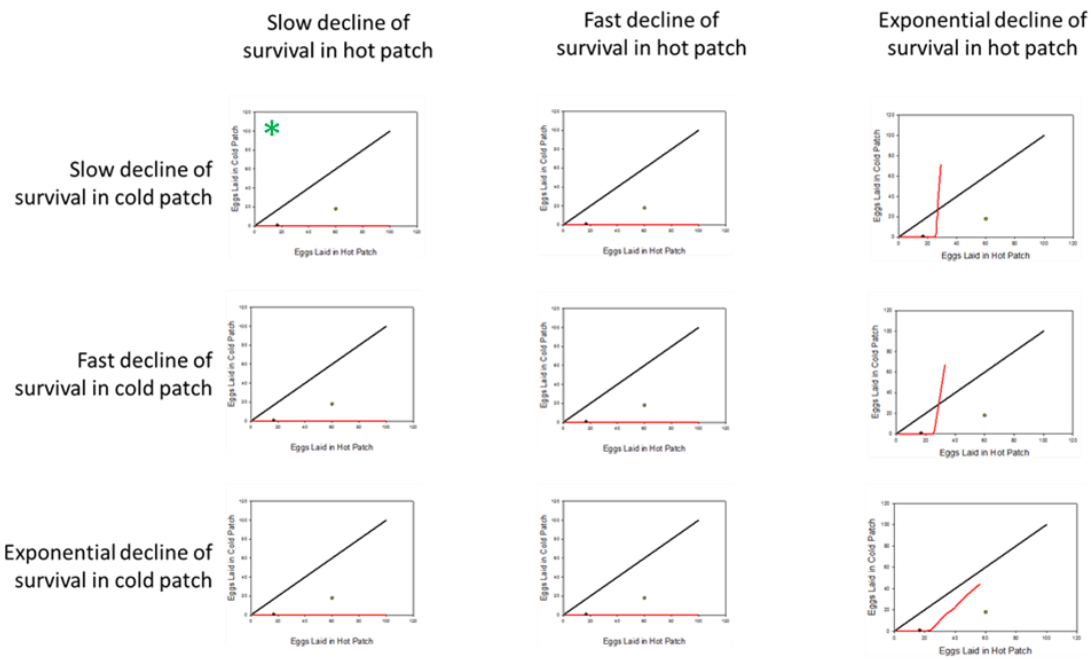

Figure 4.10. My models predict that flies in high-food quality patches should predominately lay their eggs in the warm patch as long as competition is low or the rate of decline of survivorship in the warm patch is linear. However, if there is an exponential rate of decline of survivorship in the warm patch, flies should begin to lay more eggs in the cold patch or if the flies in the cold patch experience only a linear decline in its survival rate under increased densities. The straight black line represents a trend line where the portion of eggs in each patch should be equal. The red line shows where we predict the flies to lay their eggs based on varying levels of survivorship if future fecundity and developmental time remain constant. The predicted model fit that most closely resembles the empirical data collected on fly fitness is denoted with a green apostrophe. The average egg density laid in the empirical, low-density fly treatment is represented with a burgundy circle and the average egg density laid in the empirical, high-density fly treatment is represented with an olive circle.

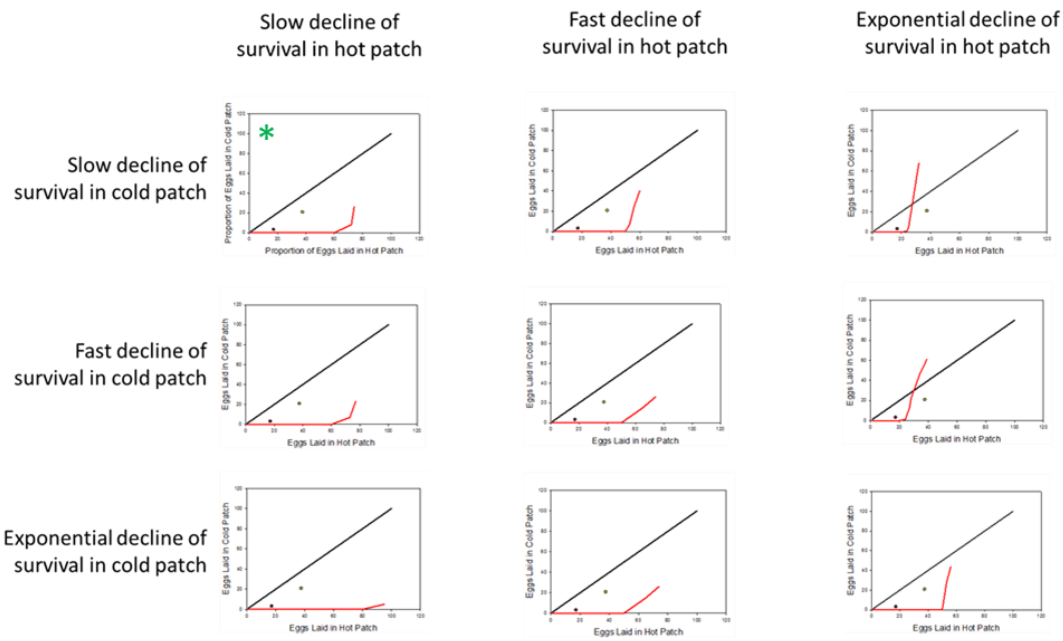

Figure 4.11. My models predict that flies in high-space environments should predominately lay their eggs in the warm patch as long as competition is low. As competition increases, flies should begin to lay more eggs in the cold patch especially if the rates or survival in the warm patch decline rapidly or if the flies in the cold patch experience only a slow decline in its survival rate under increased densities. The straight black line represents a trend line where the portion of eggs in each patch should be equal. The red line shows where we predict the flies to lay their eggs based on varying levels of survivorship if future fecundity and developmental time remain constant. The predicted model fit that most closely resembles the empirical data collected on fly fitness is denoted with a green apostrophe. The average egg density laid in the empirical, low-density fly treatment is represented with a burgundy circle and the average egg density laid in the empirical, high-density fly treatment is represented with an olive circle.

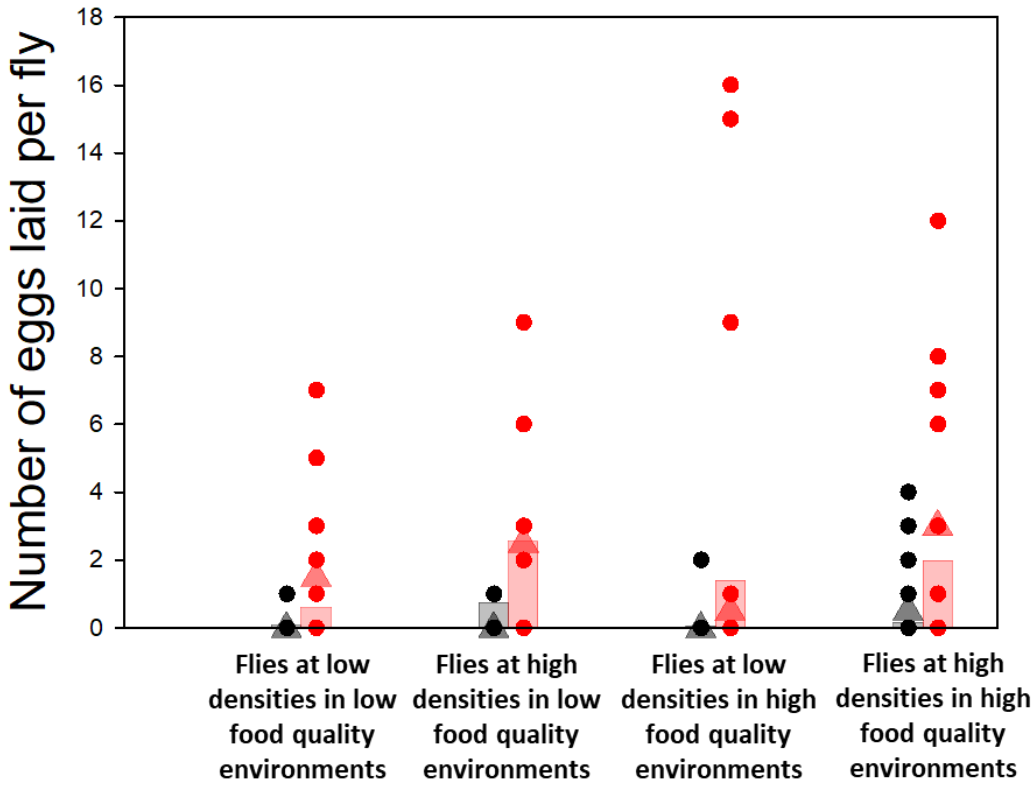

Figure 4.12. I added either 4 flies (low-density) or 15 flies (high-density) to each lane within a thermal arena. Flies then chose to oviposit their eggs in a 20°C patch (black) or a 25°C patch (red) within the thermal arena. Each dot in the graph represents the raw data for where flies chose to lay their eggs, while the triangles represents the median of where each fly laid their eggs, and each bar represents the predicted model fit for a linear mixed-effects model adjusted for zero-inflated data (Zuur 2009). I found significant differences from our high-food quality, low-density treatment in relation to temperature (P = 0.018) and due to increased competition from adult flies in the high-food patch (P = 0.029). I found significant differences from my low-food quality, low-density treatment in relation to temperature (P = 0.005), but did not find a significant difference due to competition (P = 0.49).

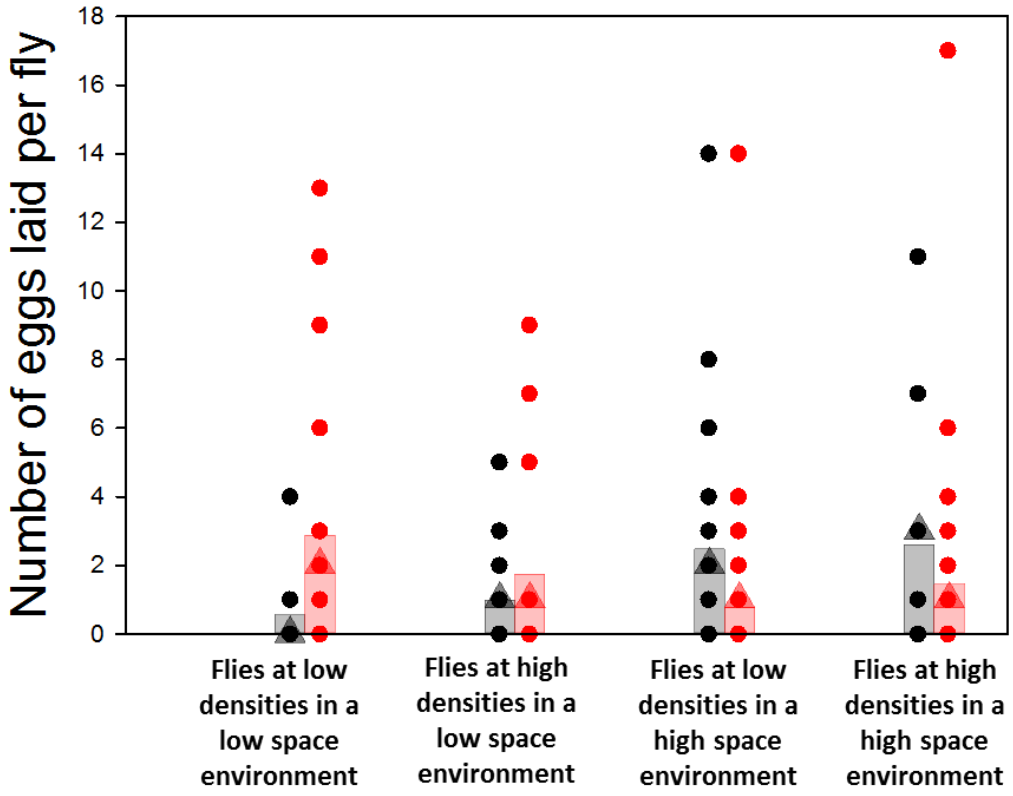

Figure 4.13. I added either 4 flies (low-density) or 15 flies (high-density) to each lane within a thermal arena. Flies then chose to oviposit their eggs in a 20°C patch (black) or a 25°C patch (red) within the thermal arena. Each dot in the graph represents the raw data for where flies chose to lay their eggs, while the triangles represents the median of where each fly laid their eggs, and each bar represents the predicted model fit for a linear mixed-effects model adjusted for zero-inflated data (Zuur 2009). I found significant differences from our 35mm, low-density treatment in relation to temperature (P <0.0001) and plate size at low densities (P = 0.006) and high densities (P = 0.004). I found significant differences from my 50mm, low-density treatment in relation to temperature (P = 0.003), but did not find a significant difference due to competition (P = 0.942) in the 50mm patches.

Table 4.1. Results from a linear mixed-effects model adjusted for zero-inflated data for number of eggs laid by an isofemale fly line in patches that differed in temperature. Treatment groups differed in the number of female flies (4 or 15) and whether the patch was rich in food quality (high yeast) or poor in food quality (low yeast). I found significant differences from our high-food quality, low-density treatment in relation to temperature and amount of competition from adult flies in the high-food patch, but did not find a significant difference due to lowering the food quality of the patch.

	Estimate	SE	*P*
Intercept	-2.335	0.845	0.0058
High-Density	2.076	0.952	0.0292
Low-Food Quality	-0.212	1.364	0.8763
Low-Food Quality High-Density	0.659	1.075	0.5399
25°C	1.844	0.777	0.0177
High-Density:25°C	-0.640	0.844	0.4483
Low-Food Quality:25°C	1.046	1.288	0.4166
Low-Food Quality High-Density:25°C	0.523	0.984	0.5950

Table 4.2. Results from a linear mixed-effects model adjusted for zero-inflated data for number of eggs laid by an isofemale fly line in patches that differed in temperature. All patch sizes were poor in food quality (low yeast) and only differed in the number of female flies (4 or 15). I found significant differences from my low-food quality, low-density treatment in relation to temperature, but did not find a significant difference due to competition.

	Estimate	SE	*P*
Intercept	-2.487	1.081	0.0215
High-Density	0,867	1.259	0.4914
25°C	2.890	1.027	0.0049
High-Density:25°C	-0.523	1.192	0.6606

Table 4.3. Results from a linear mixed-effects model adjusted for zero-inflated data for number of eggs laid by an isofemale fly line in patches that differed in temperature. Treatment groups differed in the number of female flies (4 or 15) and whether the size of the patch was either 50mm or 35mm in diameter. I found significant differences from our 35mm, low-density treatment in relation to temperature and plate size, but did not find a significant difference due to increased competition in the 35mm patches.

	Estimate	SE	*P*
Intercept	-0.599	0.442	0.1750
High-Density	0.565	0.589	0.3376
50mm	1.500	0.541	0.0056
50mm High-Density	1.550	0.540	0.0041
25°C	1.653	0.364	$P < 0.001$
High-Density:25°C	-1.073	0.494	0.0298
50mm:25°C	-2.822	0.514	$P < 0.001$
50mm High-Density:25°C	-2.242	0.462	$P < 0.001$

Table 4.4. Results from a linear mixed-effects model adjusted for zero-inflated data for number of eggs laid by an isofemale fly line in patches that differed in temperature. All patch sizes were 50mm in diameter and treatment groups only differed in the number of female flies (4 or 15). I found significant differences from my 50mm, low-density treatment in relation to temperature, but did not find a significant difference due to competition in the 50mm patches.

	Estimate	SE	*P*
Intercept	0.974	0.287	0.0007
High-Density	0.028	0.388	0.9424
25°C	-1.156	0.392	0.0032
High-Density:25°C	0.573	0.485	0.2369

CHAPTER 5

DIMINISHING RETURNS LIMIT ENERGETIC COSTS OF CLIMATE CHANGE

ABSTRACT

Changes in the time available for organisms to maintain physiologically preferred temperatures (thermal opportunity) is a primary mechanism by which climate change impacts the fitness and population dynamics of organisms. Yet, it is unclear whether losses or gains in thermal opportunity result in proportional changes in rates of energy procurement and use. We experimentally quantified lizard food consumption and energy assimilation at different durations of thermal opportunity. We incorporated these data in an individual-based model of foraging and digestion in lizards to explore the implications of nonlinear responses to shifts in thermal opportunity across a wide geographic range. Our model predicts that shifts in thermal opportunities resulting from climate change alter energy intake primarily through digestion rather than feeding, because simulated lizards were able to fill their gut faster than they can digest their food. Moreover, since rates of energy assimilation decelerate with increasing thermal opportunity, shifts in daily energetic assimilation would depend on the previous opportunity for thermoregulation. In particular, the same changes in thermal opportunity will have little impact on lizards from warm locations, while having a large impact on lizards from cold locations where thermoregulation is possible for only a few hours each day. Energy expenditure followed spatial patterns in thermal opportunity, with greater annual energy expenditure occurring at warmer locations. Our model predicts that lizards will spend more energy under climate change by maintaining higher body temperatures and remaining active longer. However, the predicted changes in energy assimilation following climate change greatly exceeded the predicted increases in energy expenditure. Simple models, which assume

constant rates of energy gain during activity, will potentially mislead efforts to understand and predict the biological impacts of climate change.

INTRODUCTION

Because climate change has shifted the distributions (Parmesan 2007) and phenologies (Root et al. 2003) of species, biologists have become increasingly concerned with predicting future responses (Kearney and Porter 2009, Buckley et al. 2010). By quantifying the times when animals can thermoregulate accurately, one can predict the potential to forage, digest, grow, and reproduce (Buckley et al. 2010, Kearney 2011, Gunderson and Leal 2016). Under climate change, a warmer environment may limit the amount of time at optimal temperatures (thermal opportunity) for growth and reproduction (Sinervo et al. 2010, Kearney 2013). Sinervo et al. (2010), for example, suggested that global warming decreases thermal opportunities for lizards around the globe, leading to reduced food intake, reproduction, and eventually to local extinctions. On the other hand, warming may offer more opportunities for thermoregulation in colder environments, increasing the fitness of species at high latitudes (Buckley 2008, Kearney 2013, Gunderson and Leal 2016, Levy et al. 2016b) or altitudes (Huang et al. 2013, Huang et al. 2014).

To understand and predict shifts in energetics and phenology, we must consider how climate constrains the time and energy available for reproduction (Levy et al. 2016b). This task is easier said than done, because many physiological and ecological processes scale nonlinearly with the time or energy available to organisms. Consequently, the benefits or costs of shifts in thermal opportunity differ among populations that currently experience different climates. Energy intake, through foraging and digestion, is an excellent example of a process that depends nonlinearly on temperature and time (Angilletta 2001a). At low body temperatures, a small degree of warming would confer a

substantially greater rate of energy gain. By contrast, at high body temperature, the same degree of warming would confer little increase or even decrease the rate of energy gain. Even if an animal were to remain at its optimal temperature indefinitely, the rate of energy gain would decrease over time. For example, an animal that forages for twice as long, does not necessarily gain twice the energy, since the animal might spend more time searching for food as its density decreases (Stephens and Krebs 1986). In such cases, a decrease in the time available for foraging might impose only a marginal cost. Similarly, the rate of energy assimilation also decreases with the time. Most of the ingested food is assimilated in the first few hours of digestion because the sequential processes of digestion, absorption, and excretion create a physiological bottleneck (Grant and Porter 1992). This phenomenon causes diminishing energetic returns on the time invested in thermoregulation and activity. For example, lizards grew faster when allowed to thermoregulate for 10 h d^{-1} instead of 6 h d^{-1}, but no faster when allowed to thermoregulate for 14 h d^{-1} (Adolph and Porter 1993, Sinervo and Adolph 1994). Hence, an animal with a moderate period of thermoregulation will do nearly as well as one with a longer period.

Because foraging and digestion occur only at certain body temperatures, energy balance requires opportunities to thermoregulate, without paying costs that exceed the benefit (e.g., energy loss or predation risk). As climates warm, thermal opportunity for temperate species should expand (Deutsch et al. 2008, Levy et al. 2016b), enabling ectotherms to spend more time at preferred body temperatures. Whether extended thermal opportunity will enhance the energy budget of an organism depends on its current thermal opportunities. If an animal currently spends only a few hours per day at its

preferred temperature, a little warming would confer a large energetic benefit. However, if a species currently spends many hours at its preferred temperature each day, a little warming would either confer a small energetic benefit or impose a small energetic loss (Dillon et al. 2010). For widespread species, these impacts should vary systematically along a latitudinal or altitudinal cline; animals at higher latitudes or altitudes should be more likely to benefit energetically from extended hours at preferred temperatures in a warming climate. Impacts of climate change will depend on other factors that vary regionallu, such as densities of vegetation and prey.

Using an individual-based model, we explore the impacts of projected changes in climate on the energy budgets of lizards throughout a wide geographic range. First, we experimentally quantified food consumption and energy assimilation at different durations at preferred body temperatures. Then, we used the data to parameterize our model and simulate foraging and assimilation in past and future climates. We show that shifts in thermal opportunities may alter their energy intake primarily through energy assimilation, and less by shifts in food consumption, since lizards need only a few hours of foraging to fill their gut, but may benefit from long periods of energy assimilation. Climate change will limit opportunities for foraging in warm places while expanding opportunities in cold places, but diminishing energetic returns from digestion will weaken effects on energy assimilation.

METHODS

Modeling energy gain

We modeled the relationships between time budgets and energetics, and how shifts in time budgets due to climate change may affect feeding and assimilation rates in North American lizards. We developed an individual-based model of an adult lizard (snout-vent length = 63 mm, mass = 8.9 g) based on a *Sceloporus* model, developed by Buckley (2008) and expanded by Levy and colleagues (2015, 2016b). We used a published set of hourly microclimates (Levy et al. 2016a) to calculate the operative temperatures of lizards (i.e., the steady state temperature in a particular microclimate, Bakken 1992) on surfaces ranging from 0 to 100% shade. The microclimates represent 11,407 locations across the United States and Mexico with a spatial resolution of 36 x 36 km for the past (1980-2000) and the future (2080-2100, assuming a radiative forcing of +8.5 W m^{-2} at year 2100, RCP 8.5 scenario). At each location, the dataset includes thermal conditions at various heights above and below the ground, and under different levels of shade. In natural habitats, distances between these microhabitats are often a few meters, enabling animals to shuttle between sun and shade. Every hour in the dataset, we tracked the feeding and digestion of the lizard based on potential body temperatures (T_b). Hourly air temperatures, radiative loads, and wind speeds were used to calculate the lizard's operative temperature in each microhabitat and whether this temperature enabled foraging and digestion. We calculated these body temperatures as

$$T_{b,t} = T_{b,t-1} + \Delta T_b, \qquad [1]$$

by solving heat-exchange equations in Fei et al. (2012). The parameters and equations used are described in Table S1. We selected a small value for Δt (120 s) to yield small values of ΔT_b, which enhanced the stability of the model. In the model, the lizard is able to forage and assimilate energy whenever it can attain a body temperature between 29.4°

and 36.3°C (central 80% of field body temperature; Angilletta 2001a). For simplicity, we use the term *thermal opportunity* to refer to the number of hours that a lizard could attain a body temperature in this range. During the period of thermal opportunity, we assumed that a lizard maintains its preferred temperature (33.1°C; Angilletta 2001a) by shuttling between exposed and shaded microclimates. Outside of the period of thermal opportunity, we assigned the lizard the closest available temperature to its preferred temperature. During the night, we assumed that lizards rest on the ground surface, under full cover, which has been observed during field studies (M. J. Angilletta, unpublished). During winter, if activity was not possible for more than two weeks, we assumed that lizards retreated to a 12-cm burrow.

For each location in our domain, we calculated (1) time budgets as the number of foraging and assimilation hours, and (2) the amount of energy a lizard could ingest and assimilate (kJ h^{-1}). In each location, we also compared between current and future climates, by calculating the difference in the mean near-surface (3-cm above ground, 50% shade cover) temperature during 1980-2000 and 2080-2100.

Lizards were assumed to forage when their body temperature allowed activity. To determine the feeding rates for each hour of foraging, we first calculated the maximal velocity (v, m s^{-1}) of the lizard as

$$\log_{10}(v) = 0.044 + 0.2 \cdot \log_{10}(M_b), \qquad [2]$$

based on published observations where M_b equaled the mass of a lizard (Van Damme and Vanhooydonck 2001). Then, assuming lizards forage at 70% of their maximal velocity (Irschick and Losos 1998), we calculated the distance traveled (d, m) in one second as $0.7 \times v \times 1\ sec$. As in Buckley's analysis (2008), we assumed that the energy content of an

insect equals 30.12 J, the rate of insect encounter assuming foraging along a line equals 0.005 insects m^{-1} s^{-1} (Jones et al. 1987b, Niewiarowski and Roosenburg 1993), 50% of insects encountered are captured by a foraging lizard, and lizards assimilate 76% of ingested energy (Angilletta 2001a). Hence, at each hour, the energy intake ($e_{i,h}$) was

$$e_{i,h}\ (\text{J h}^{-1}) = 30.12\ (\text{J insect}^{-1}) \cdot 0.005\ (\text{insect m}^{-1}\ \text{s}^{-1}) \cdot 0.5 \cdot 0.76 \cdot d\ (\text{m}) \cdot 3600\ (\text{s h}^{-1}). \quad [3]$$

As the lizards feed, we modeled how feeding filled the gut, reducing the available space ($J_{available}$, kJ):

$$J_{available} = C_{max} - J_{daily\ max}, \quad [4]$$

where C_{max} (kJ) is the maximal gut space (2.55 kJ/d, based on our laboratory measurements) and $J_{daily\ max}$ (kJ) is the amount of energy consumed that day.

Lizards assimilated energy whenever they had food in their gut and body temperature was between 29.4° and 36.3°C. This range corresponds to the central 80% of field body temperatures, because digestion proceeds slowly at higher or lower temperatures (Angilletta 2001a). Each day in the simulation, the rate of energy assimilation was derived from our statistical analysis (see results for more details), suggesting that the rate of energy assimilation (E) depended on the interaction of maximal consumption and time budget:

$$E\ (\text{kJ}) = a \cdot C_{max} \cdot \log(t_d + 1), \quad [5]$$

where a is a constant fitted to our empirical data, t_d is the duration of assimilation since the first feeding event of that day. Assimilation rates did not exceed the energetic content of the gut:

$$E_{assim}(\text{kJ}) = \begin{cases} E & \text{when} \quad E \le J_{gut} \\ J_{gut} & \text{when} \quad E > J_{gut} \end{cases}, \qquad [6]$$

where J_{gut} is the amount of energy (kJ) in the gut.

We estimated energy expenditure from experimental studies of metabolic rate. Resting metabolic rate (RMR, J s^{-1}) was modeled according to Angilletta (2001b):

$$\ln(\text{RMR}) = -10.0 + 0.51 \cdot \log(M_b) + 0.12 \cdot T_b, \qquad [7]$$

We multiplied RMR by 1.5 to yield the resting metabolic rate of a digesting lizard (Roe et al. 2005) and then multiplied this rate by 2 to yield the metabolic rate of a foraging lizard (Bennett 1982). To calculate the energy balance of lizards, we subtracted the estimates of energy expenditure from the energy assimilated.

Parameterizing the assimilation model

To parameterize the function relating thermal opportunity to energy assimilation, we conducted experiments with lizards from three population of the *Sceloporus undulatus* complex (Leache 2009): *Sceloporus tristichus* from Pinal County, Arizona (33.308117, -111.049417) and Grand County, Utah (38.26044, 109.6962); and *Sceloporus consobrinus* from Ogallala and *Keith Counties,* Nebraska (41.336767, -102.008993). Lizards were collected in the spring of 2011 and transferred to an animal care facility at Arizona State University. Each lizard was housed in a plastic terrarium partially heated by Flexwatt™ heat tape (Calorique, West Wareham, MA, USA), allowing lizards to freely thermoregulate. Prior to our experiment, lizards had unlimited access to water and were fed crickets (*Acheta domestica*) three times per week.

Our experiment controlled the duration at which lizards experienced their preferred body temperature. We placed lizards in incubators with diel cycles of temperature and light that simulated three levels of thermal opportunity (6L:18D [n = 25], 10L:14D [n = 21], and 14L:10D [n = 19] light cycles). The temperature during the light phase (33.1°C) was chosen to match the body temperature of lizards during thermoregulation in natural environments and thermal gradients (Buckley et al. 2015). This temperature also maximized the rate of energy assimilation by *S. undulatus* when food is plentiful (Angilletta 2001a). The temperature during dark phase (20°C) was chosen to severely limit the rate of energy assimilation. In a fourth treatment, lizards (n = 17) were exposed to the preferred temperature for 24 h d^{-1} and a 14L:10D light cycle. We used a stratified design to randomly assign each lizard to a thermal treatment. In all treatments, lizards were kept in plastic terraria (32 cm x 38 cm x 63 cm) at 70% humidity. Feeding occurred about 2 hours after the start of each light phase.

We measured rates of feeding and assimilation during the experiment. First, lizards were fasted for 48 h. Then, each lizard was offered a cricket that was injected with a non-digestible, fluorescent dye (Scientific Marking Materials, Seattle, WA). We used this dye to mark the initial passing of fecal matter from the cricket consumed at the beginning of the trial. We inspected feces daily until this dye was observed. At that point, we began collecting all feces and urates. The trial lasted for 7 days, during which we fed lizards as many crickets as they would consume within 2 hours of each morning. More frequent feeding would likely have resulted in a similar energy intake, because these lizards required about 48 hours to digest a single cricket and consume multiple crickets when feeding (Angilletta 2001a). In fact, a previous experiment reported a similar rate of

consumption by the same species in less than an hour per day for feeding (Angilletta 2001a). Water was provided daily by misting the sides of the terraria.

After 7 days, lizards were fed a second cricket marked with fluorescent dye (a different color than the first dye). Because all crickets were weighed to the nearest 0.1 mg, we could calculate the total mass of food consumed between the two marked crickets. Feces were checked daily until the second marker appeared. Feces and urates collected between the two markers resulted from the known mass of food ingested during the trial. Lizards that refused to eat for several days or failed to eat one of the marked crickets were removed from the study.

We used bomb calorimetry to estimate the energy consumed and excreted by each lizard during the trial. A sample of 29 crickets was dried and combusted in a Parr 1425 semimicro bomb calorimeter to determine their caloric density. We then used the mean water content (25%) and the mean energetic density (22.187 kJ g^{-1}) to convert the wet mass consumed to the equivalent number of Joules. We also determined energetic content of the feces and urates produced by each lizard. For each lizard, we calculated feeding rates (kJ/d) as the energy consumed as crickets, and assimilation rates (kJ/d) as the difference between the feeding rates and the energy excreted as feces and urates.

Analysis of assimilation data

We used our experimental data to estimate two functions in the individual-based model. The first function related the body length (snout-vent length) of a lizard to its maximal daily consumption of food. Food consumption was the dependent variable and body length was a continuous independent variable. This model was fitted to estimates of food

consumption by lizards exposed to their preferred temperature for 24 h d^{-1}, because this treatment enabled the fastest digestion and hence the most consumption (but see Whelan and Brown 2005 for a discussion of factors that affect gut constraints). We used a log link function and a gamma distribution of residual variation.

The second function related the hours of thermal opportunity to the rate of energy assimilation. Energy assimilation was the dependent variable, population of lizards was a categorical predictor, and the log[(h d^{-1}) + 1] was a continuous predictor (see Eqn. 5). We forced the intercept of the model to equal zero, because lizards should assimilate little or no energy without access to preferred temperatures. We used an identity link function and a gamma distribution of residual variation. Based on Akaike Information Criterion (Burnham and Anderson 2002), we removed the population factor since it didn't contribute to the fit of the model (ΔAIC = 3.74). All data analysis was done in R version 3.2.3 (R Development Core Team 2011) using the *glm* function of the *nlme* library (Pinheiro et al. 2011). Descriptive statistics are means and standard deviation estimated from the final model.

Sensitivity analysis

To explore how predictions of our model depend on our assumptions, we altered the values of three parameters and quantified the effect on dependent variables. Specifically, we quantified how time budgets and rates of energy intake may differ when (1) decreasing the density of food by 50%, and when (2) assimilation rates (E in eq. 5) are assumed to either be constant over time (estimated as $E(t_d = 24)/24 \cdot t_d$) or decelerating faster than in our observations (estimated as $E(t_d = 24)\cdot(1 - e^{-0.3\cdot t_d})$). Moreover, we

quantified how energy expenditure and energy balance may differ when increasing the costs of activity by 50% to account for possible costs of foraging and thermoregulation (three times the RMR). Although all of these assumptions potentially vary among locations, such sensitivity analyses can help understand the effects of such assumptions at different conditions. Moreover, although Bennett and Dawson (1976) reported a maximal five-fold increase between standard metabolic rates and active metabolic rates, these rates were measured during induced activity in the laboratory and spontaneous activity in the field should be significantly lower. Unless otherwise noted, we report each prediction of the model as the mean of values among locations, plus or minus the standard deviation.

RESULTS

Our experiment confirmed the expected diminishing relationship between thermal opportunity and energy assimilation. Lizards that spent more hours per day at their preferred temperature assimilated more energy, but this effect diminished as access to the preferred temperature approached 24 hours per day (Fig. 1). The most likely statistical model resulted in the following relationship among thermal opportunity (t_d), maximal gut size (C_{max}, kJ/d), and energy assimilation (E, kJ/d): $E = 0.115 \cdot C_{max} \cdot \log(t_d + 1)$. Variation in body size within and among populations contributed indirectly to energy assimilation, because maximal gut size increased exponentially with body length (snout-vent length, *SVL*, mm): $\ln(C_{max}) = -2.23 + 0.05 * SVL$ (Fig. 1). These functions were used to model the impacts of climate on energy gain in past and future climates.

Our simulations characterized a latitudinal gradient in thermal opportunity, where lizards from lower latitudes could spend more time at their preferred temperature and

assimilate more energy each year (Fig. S1). However, simulated lizards spent much less time foraging than digesting (Fig. S2). Hence, time spent at the preferred temperature did not directly translate to animals eating more each day. In particular, a lizard needed only 2.3 hours each day (± 0.1 h d^{-1}) to fill its gut, regardless of the climate at its location (Fig. 2). When we halved the density of food, a lizard in any location needed to forage only one more hour per day (0.93 ± 0.05 h) to fill its gut (Figs. S3, S4). Thus, foraging time was nearly independent of climate, at least in the range of conditions that we explored with our model.

In contrast to foraging, energy assimilation through digestion and absorption proceeded slowly, such that every additional hour of thermal opportunity contributed to energy assimilation when animals had ingested food. In the past climate (1980-2000), lizards from warm locations could attain preferred temperatures up to 348 days per year for as many as 6.2 hours per day (Figs. S2, 3). When switching to the climate projected for 2080-2100 (RCP 8.5), a lizard either gained or lost thermal opportunity (Fig. 3a,b), depending on its current climate. The number of days with at least one hour of thermal opportunity increased by 21.1 days per year (± 9.0 d y^{-1}) at 99% of locations. At the remaining locations, the number of days decreased by 0.7 days per year (± 1.1 d y^{-1}). The daily duration of thermal opportunity increased in 86% of the locations, by 0.8 hours per day (± 0.4 h d^{-1}); these locations were relatively cool in the past climate, having a mean annual temperature of 10.0°C (± 9.0°C). At the remaining locations, with a mean annual temperature of 14.6°C (± 4.5°C), thermal opportunity decreased by 0.3 hours per day (± 0.2 h d^{-1}). Thus, simulated climate change enabled phenological shifts in activity, with

lizards gaining energy on more days during the summer at cold locations or more days during the winter at warm locations (Fig. 3a, b).

Given the decelerating relationship between thermal opportunity and energy assimilation, additional time at the preferred temperature would benefit lizards in cold locations more than lizards in warm locations, which had more time for digestion each day in the past climate (Fig. 4). Although lizards in the hottest locations spent less time at their preferred temperatures throughout the year, they still had time to digest most of the food in their gut each day. For example, in warm locations (mean temperature above 20°C), a decrease in thermal opportunity of 5 hours per day reduces energy assimilation by 0.23 kJ per day (± 0.01 kJ y^{-1}). By contrast, 5 additional hours of thermal opportunity for digestion increased energy assimilation in cold locations (mean temperature below 15°C) by 0.39 kJ per day (± 0.03 kJ y^{-1}) (Figs. 3c, 4). This asymmetry between the impacts of warming depended on the rate at which energy assimilation decelerated with thermal opportunity (Figs. S6, S7) and disappeared when energy assimilation increased linearly with thermal opportunity (Figs. S8, S9).

Energy expenditure followed spatial patterns in thermal opportunity, with greater annual energy expenditure occurring at warmer locations. Annual energy expenditure increased under the scenarios of climate change, because lizards maintained higher body temperatures and engaged in more activity (Fig. S10); overall, energy expenditure increased by 3.56 kJ per year (± 0.74 kJ y^{-1}) when switching from the past climate to the future climate (Fig. S10). Greater energetic demands occurred mostly as a response to the phenological changes in thermoregulatory behavior based on shifts in thermal

opportunity (Fig. 3d). Energy expenditure increased mostly in colder regions during summer and in warmer regions during winter.

The predicted increase in energy assimilation following climate change greatly exceeded the predicted increase in energy expenditure (Figs. 3, S10). With our initial parametrization, annual energy expenditure was only 21% of annual energy assimilation (± 5 %) (Fig. S11a,b). When we imposed a greater cost of activity (+50%), energy expenditure was still only 23% of energy assimilation (± 5 %) (Fig. S11c,d). Thus, energy balance in past or future climates was dominated by thermal effects on energy assimilation (Fig. 3e, S10). Consequently, daily shifts in energy balance of lizards reflected the decelerating relationship between thermal opportunity and energy assimilation (Fig. 4). The high correlation between energy balance and energy assimilation persisted when we simulated lizards with a greater cost of activity (Figs. S6, S7).

DISCUSSION

Time is an ecological resource that enables animals to feed, grow, and reproduce. Based on our model, climate change will limit opportunities for such activities in warm places while expanding opportunities in cold places. The model sheds light on the mechanisms by which phenological shifts might affect energy gain by animals, and hence influence the dynamics of populations and communities. In particular, diminishing returns during digestion affect changes in energy assimilation as the climate warms. Animals require more time at preferred temperatures to digest and absorb food than to consume it, regardless of body temperature (Angilletta 2001a). Thus, energy assimilation strongly

depends on the opportunity to thermoregulate after feeding (reviewed by Huey 1982, Waldschmidt et al. 1987).

Our model considers a scenario in which foraging depends only on body temperature and food density. However, foraging costs and benefits depend on other factors (e.g, water balance, interspecific competition, and predation risk) that reduce feeding (Dunham 1980, Lima and Dill 1990, Brown et al. 1999, Levy et al. 2016c), as well as prey density that may differ across locations and seasons with natural variations in temperature, rainfall, and vegetation. Incorporating such factors in future models should enhance our ability to predict impacts of climate change. For example, the marginal value of water may increase during a drought, causing lizards to forage during cooler hours of the day. Such responses were modeled in lizards (Kearney et al. 2013) and observed in other animals (Levy et al. 2016c), (ibex, Hochman and Kotler 2006), *Corvus coronoides* (Australian raven, Kotler et al. 1998), and *Capra hircus* (goat, Shrader et al. 2008). Moreover, although *Sceloporus* lizards are sit-and-wait predators, different modes of foraging (e.g., active-searching) may incur different exposures to competition and predation as well as different energetic and hydric costs. Thus, factors that affect foraging may vary across ecological communities and may shift under global change (Mack et al. 2000, Tylianakis et al. 2008, Hobbs et al. 2009), bringing further complexity to an energy balance model. Alternatively, the low energy demands of ectotherms may enable them to survive with only short bursts of foraging (e.g., Lagarde et al. 2003). Therefore, the time required for reptiles to digest food far exceeds the time required to forage, such that a digestive bottleneck limits feeding more than opportunities to forage (Congdon 1989). In our simulations, lizards needed only a few hours of

foraging to fill their gut, and could do so even when warming restricted foraging time or halved prey density. For these reasons, we think the major patterns described by our model would hold up under a wider range of conditions that we have considered.

Significant ecological patterns could emerge when the rate of energy assimilation decelerates with increasing thermal opportunity. Although lizards assimilated substantial energy when warmed for just a few hours per day, the rate of energy assimilation decelerated when lizards warmed for longer periods. Previous experiments have shown that lizards require more than 20 hours of continuous exposure to their preferred temperature to pass a single item of food (Beaupre et al. 1993, Angilletta 2001a). The longest period of exposure would be 10 and 14 h per day in past and future climates, respectively. Therefore, feeding could occur multiple times per day but food remains in the gut for multiple days. Consequently, the annual energy budget in our simulations depended more on the number of days that lizards could feed and digest than on the number of hours per day. Thermoregulation depends on access to preferred microclimates (Porter et al. 1973, Grant and Dunham 1988, Bashey and Dunham 1997), and just a few hours of effective thermoregulation during the day enables an individual to acquire enough energy to meet its energetic demands for maintenance. After a few hours, when the marginal value of thermoregulating decreases, lizards can either choose to abandon thermoregulation and seek shelter, perhaps to save energy or avoid predators, or continue to thermoregulate and gain more energy. Although we assumed that lizards only thermoregulate on the ground, lizards can climb or burrow to access microclimates above or below the ground (Norris and Kavanau 1966, Jacob and Painter 1980). The tradeoffs among energy gain, predation risk, and metabolic costs have been captured by foraging

models, in which rates of energy gain decelerate as foraging depletes patches. Foraging theory also predicts that animals will quit foraging earlier when the cost of foraging or the risk of predation increases (Brown 1988, Mitchell et al. 1990).

The diminishing return on thermal opportunity also determines how populations respond to a changing climate. As an environment warms, the energetic benefit of additional time to thermoregulate depends on the previous opportunity for thermoregulation. In particular, the marginal benefit of thermal opportunity was great for lizards that currently have only a few hours of thermal opportunity each day and miniscule for lizards that currently have many hours of thermal opportunity throughout the day. Previous models, in which the rate of energy assimilation was assumed to increase linearly with increasing thermal opportunity (Buckley 2008, Sinervo et al. 2010, Kearney 2013), either under- or over-estimated the energetic consequences of climate change by failing to consider diminishing energetic returns on activity. In particular, a linear function would underestimate the energetic benefit of climate change at cold locations (by 7.1 ± 4.6 kJ at 26% of locations; Fig. S9) and overestimate the energetic loss due to climate change at warm locations (by 17 ± 9 kJ y^{-1} at 74% of locations; Fig. S9). Global warming has already caused species of lizards to go extinct in tropical and subtropical regions (Sinervo et al. 2010), and tropical ectotherms in general seem especially vulnerable to further warming (Huey et al. 2012). Importantly, our model suggests that losing opportunities to forage on warm days might not drive such extinctions if lizards can feed during cooler times of the day and assimilate most of their food in 6 to 10 hours of digestion. On the other hand, warmer summers may decrease feeding and digestion while increasing energetic demands (Fig. 3), reducing the

probability of survival (Bestion et al. 2015, Levy et al. 2016b). Researchers should account for diminishing returns on thermoregulation when predicting energetics, growth, and reproduction of animals in future climates.

A disadvantage of mechanistic models, relative to climate-envelope models, is that one must define relationships between environmental variables and organismal performance, such as the function relating body temperature to energy assimilation. Any mathematical model is just a series of such functions, and many functions are linearized to make a model easier to analyze and interpret. For example, linear approximations were chosen to relate the duration of thermoregulation to the energetics (e.g., Porter et al. 1973, Kearney et al. 2009a, Kearney et al. 2009b), survival (Adolph and Porter 1993), phenology (Kearney et al. 2010), or life history (Adolph and Porter 1993, 1996). More complex models explicitly calculate an energy balance to predict food and water requirements (Kearney and Porter 2004), optimal behavior (Grant and Porter 1992), life history (Kearney 2011), reproduction (Grant and Porter 1992, Adolph and Porter 1993, Kearney 2011), or population growth (Buckley et al. 2010, Kearney 2011). Even in these models, thermoregulatory activity is translated to energy gain by assuming that assimilation rates are linear (but see Adolph and Porter 1993). In contrast to this simplifying assumption, we have shown that rates of energy assimilation diminish with increasing thermal opportunity in two species of *Sceloporus* lizards. Our observations agree with hypothetical arguments (Adolph and Porter 1993), as well as empirical rates of somatic growth in other *Sceloporus* lizards, which also decelerate with increasing thermal opportunity (Sinervo and Adolph 1989, Sinervo 1990, Avery 1994). Thus, our study provides a potential mechanism for the deceleration of somatic growth during previous

experiments. But more importantly, we show that accounting for such nonlinear relationships in mechanistic models can be crucial for understanding potential impacts of climate change on energy and water budgets, life histories, and population dynamics.

The ecological significance of future shifts in assimilation rates may differ between currently cold and warm locations. In previous studies, we found that climate change will enable lizards to remain active for wider spans of days (Levy et al. 2015, Levy et al. 2016b). At cold locations, where daily rates of assimilation increase substantially (as in Fig. 3c), females could reproduce more or store fat for use in winter. At warm locations, however, high mortality of offspring produced during the summer would favor females that avoid reproducing at this time, leading to a bimodal distribution of reproduction throughout the year (Levy et al. 2016b). The decrease in energy gain during summer would reinforce bimodal reproduction, since females that lay eggs during summer would not only put embryos at risk of overheating but also have less energy for reproduction later in the year. By contrast, lizards in colder locations might reproduce continuously throughout the year, because climate change would enhance energy assimilation (Fig. 3c) and offspring survival (Levy et al. 2016b).

Shifts in the availability of time for activity may also incur ecological consequences on populations and communities. With fewer hours of activity, for example, the trade-offs among feeding, mating, and defending a territory may become severe (Dunbar et al. 2009). For territorial animals, more or less time available for defending a territory may in turn increase or decrease territory sizes, respectively (Stiles 1971, Pyke 1979, Davies 1980). During days with little thermal opportunity, organisms would have more difficulty partitioning their activities throughout the day, intensifying

competition for space and potentially raising predation risk (Kronfeld-Schor and Dayan 2003). If temporal shifts in activity increase competition or predation, climate change can indirectly reduce survival rates as well as energy gains. At colder locations, on the other hand, an increase in thermal opportunities will not only enable more time for foraging and digestion, but could also promote temporal partitioning to avoid competition and predation (Kronfeld-Schor and Dayan 2003).

The relationship between thermal opportunity and energy gain will vary among species because of body mass, diet quality, handling time, and gut bacteria (Munn and Dawson 2006, Rall et al. 2012). For example, herbivorous species may be more sensitive to decreased foraging and digestion times than carnivorous species are. Herbivores consume food with high concentrations of indigestible fiber and secondary metabolites and low concentrations of protein (Clauss et al. 2013). To increase assimilation rate, herbivores consume large volumes and carry a microbiome that digests cellulose, hemicellulose, and pectin (Clauss et al. 2013). Rates of assimilation for herbivores might be constant or even accelerate with time, because they can absorb glucose faster after breaking down cellulose. Hence, herbivory may impose selection for longer periods of thermoregulation to speed energy assimilation. If climate change reduces opportunities for thermoregulation in herbivorous species, a shift in physiology, microbiome, or diet might be necessary to offset the loss of thermal opportunity (Hirakawa 1997). By contrast, herbivores at cold locations may experience a significant increase in energy assimilation if thermal opportunity will enable a substantial increase in cellulose breakdown. The diversity of physiological responses among species requires careful analysis of each species to determine the relationship between thermal opportunity and

energy gain. Simple models, which assume constant rates of energy gain during activity, will potentially mislead efforts to understand and predict the biological impacts of climate change.

ACKNOWLEDGEMENTS

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FIGURES

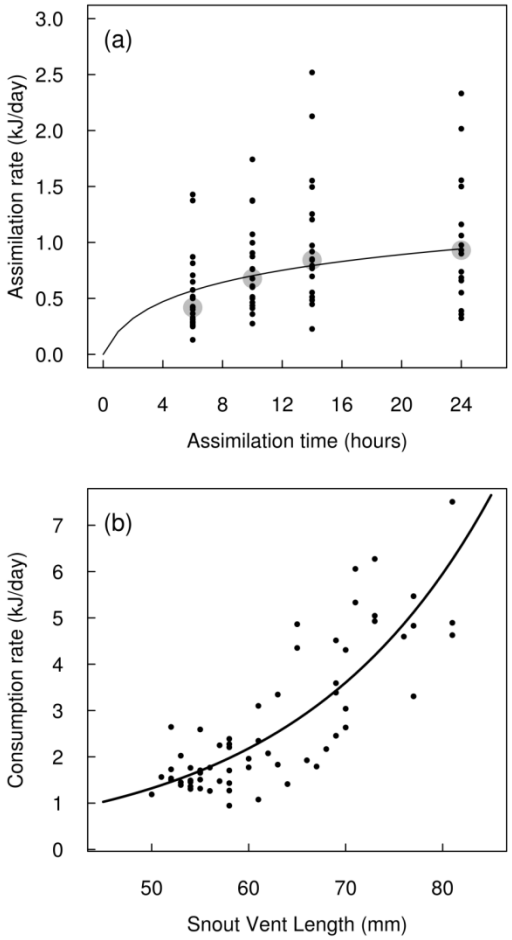

Figure 5.1. Our empirical observations (black points) support the hypothesis of diminishing assimilation rates as the daily duration of time suitable for assimilation increases (panel a, n=82). Bigger lizards assimilated more energy per day. We show the relationship between Snout-Vent length and daily assimilation rates for lizards exposed to 24-h of their preferred temperature (panel b, n=65). In panel a, grey circles represent the median of the observations for each assimilation time. In both panels, the line is the fitted curve used in the bio-energetic model.

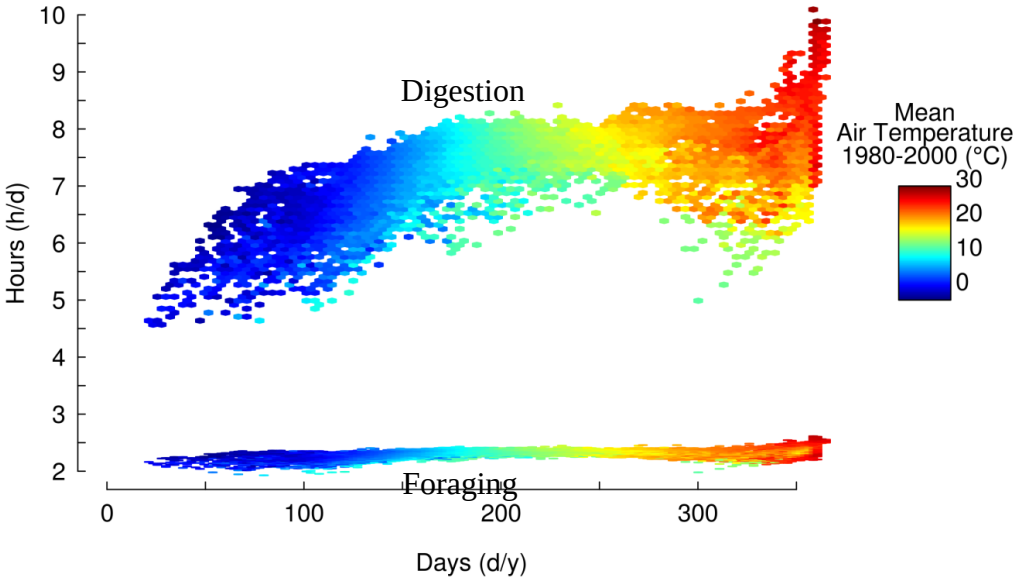

Figure 5.2. Thermal opportunity is strongly affected by climate, especially across days. Within each day, thermal opportunity for foraging is not affected by climate since lizards can fill their gut within 2-2.5 hours of feeding. Climate significantly affects the daily thermal opportunities for digestion, however, since digestion is a relatively long process. The color of the each point indicates the mean air temperature at one or more locations. See Fig. S4 for predictions when the abundance of insects is smaller than in our initial parameterization.

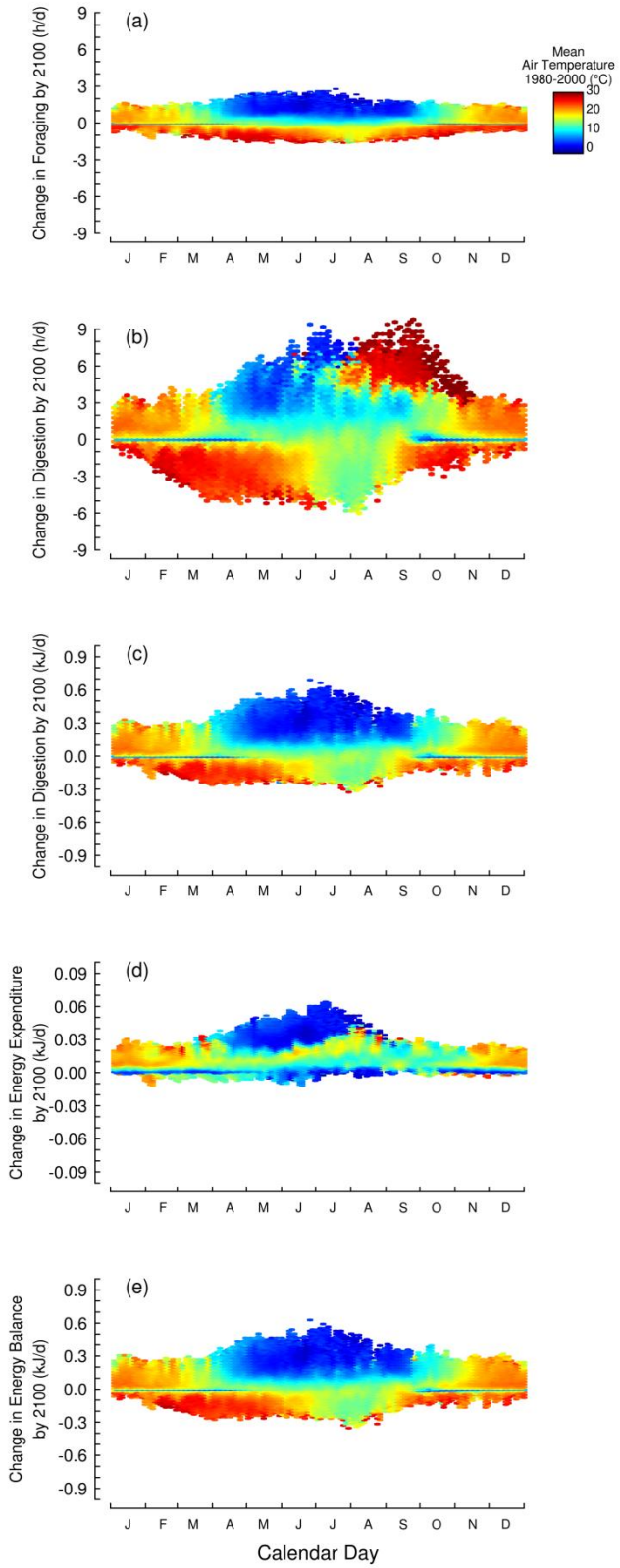

Figure 5.3. Phenological impacts of climate change on opportunities for energy intake. The effect of warming on energy intake depends on the current temperature and the time

of year. At cool locations, lizards will have more time for foraging (a) and digestion (b) during summer in future climate than in the past climate. At warm locations, however, lizards will have more opportunities for foraging (a) and digestion (b) in winter, but less in the spring and summer and fall. Climate change may offer more opportunity for digestion in the fall at few warm locations, where night temperatures may enable digestion. The effect of warming on energy assimilation matches the effect on the thermal opportunities for digestion (c). Given the deceleration of assimilation rates with thermal opportunity, the daily decreases in assimilation rates at warm locations are relatively small compared to the daily increases in assimilation rates that result from the phenological shifts. Daily energy expenditure of lizards will increase throughout the year, acccording to phenological increases in activity. Although metabolism proceeds more rapidly in a warmer climate (d), phenological shifts in energy balance will mostly resemble shifts in energy assimilation (e). The color of the each point indicates the mean air temperature at one or more locations. See Fig. S5 for changes in foraging time budgets when the abundance of insects is smaller than in our initial parameterization. See Figs. S7 and S9 for shifts in daily assimilation rates when lizards have faster rates of decelerating returns than in our initial parameterization, or have constant rate of assimilation, respectively.

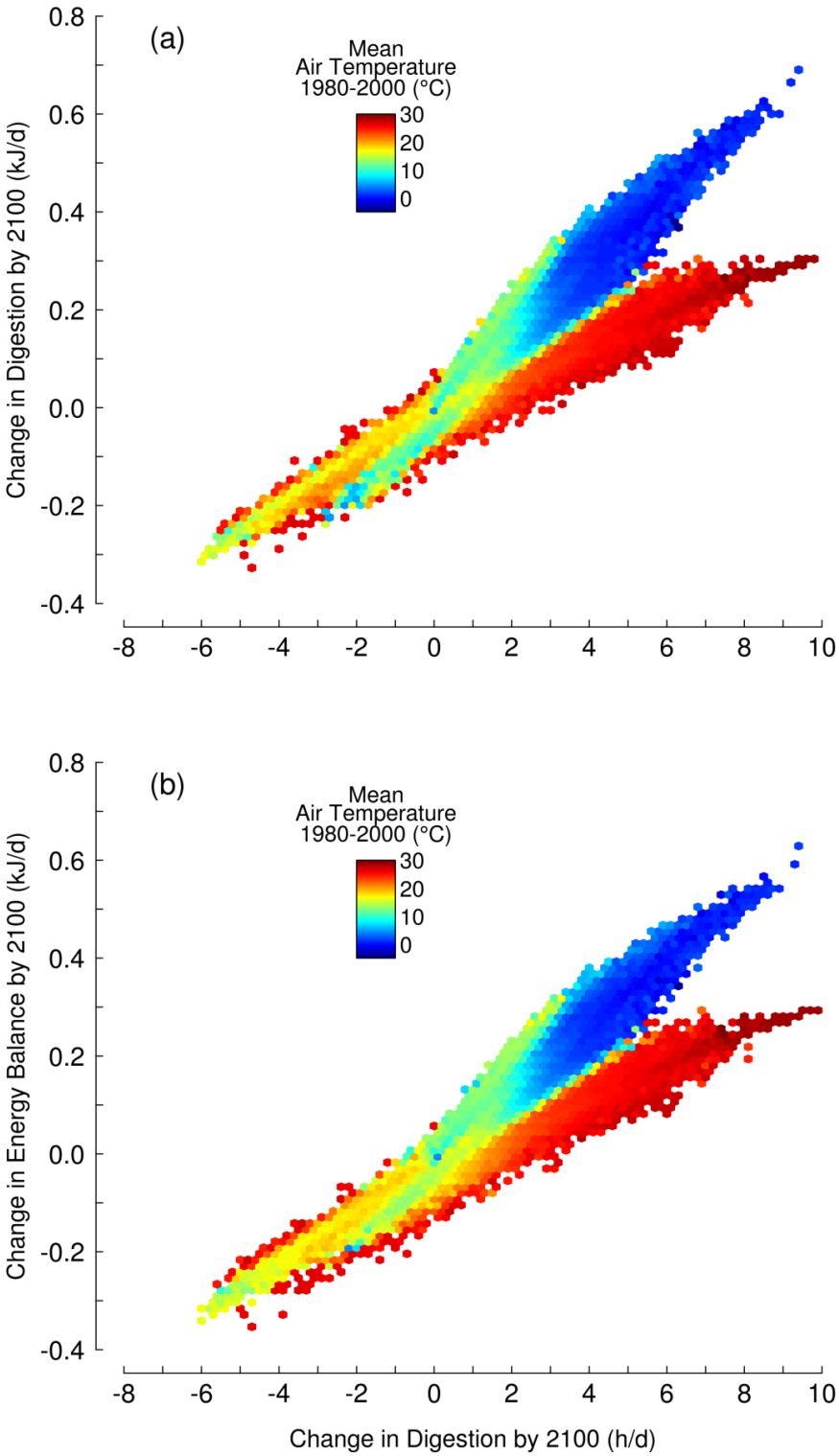

Figure 5.4. Impacts of climate change on daily assimilation rates (a) and energy balances (b) will depend on the current temperature. Given the deceleration of

assimilation rates with thermal opportunity, the impact of shifts in thermal opportunity for digestion will pose a lesser effect on daily assimilation rates at warm locations compared to cold locations. The color of the each point indicates the mean air temperature at one or more locations. See Figs. S6 and S8 for shifts in daily assimilation rates when lizards have faster rates of decelerating returns than in our initial parameterization, or have constant rate of assimilation, respectively.

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CHAPTER 6

REFLECTIONS ON THE ATTACHED CHAPTER

Engaging in this project (Chapter 5), taught me many things and helped shape my view of research. This project examined the assumptions that mechanistic models make regarding energetics and assimilation rate. If organisms spend a large amount of time at their optimal temperatures, they see reduced energetic benefits from continuing to spend time at this temperature. Most mechanistic models though assume that this relationship between energetics and temperature is linear. Therefore, we parameterized our mechanistic model using both a linear function and our asymptotic function for assimilation rate to see how this changes the predictions of the model. By incorporating functions with diminishing returns, we can significantly alter predictions being made by mechanistic models.

This manuscript originally started as a project to collect data for a mechanistic model. As we started analyzing the data I collected, we began having discussions about what the data meant within the context of a mechanistic model. As a result of these discussions, the scope of the project changed. As the data began to show that it violated some of the basic assumptions of mechanistic models, we decided to change the type of question we were examining. Rather than just using the data to parameterize a mechanistic model, we decided to see how predictions being made by the model were altered by calculating the energetics of these lizards in a more realistic manner.

From working on this project, I learned a lot about collecting data. This project was my first large scale project. Additionally, this project was the first time that I had to collaborate with more than just one or two other people. As a result, I became better at

organizing large groups of people – where each member of the group was working on different components of the project and had different goals. I learned how to meticulously collect numerous types of physiological data. In addition, I had to make sure that I devised very clear and detailed methodologies for collecting data so that everyone followed the same protocol. Further, I learned how to keep trying new methods even when the first attempts do not work. I also mentored my first undergraduates during the course of this project. I also learned about lizard husbandry and working with animal care. Lastly, by collecting all of this data, I greatly improved my understandings of metabolism and energetics.

From doing statistical analyses and writing on this project, I learned that science is a long and hard process filled with many bumps. I learned that you have to make numerous drafts and edits. Additionally, I learned that each of these drafts and analyses always take 2 or 3 times longer than you expect. I also learned to be open to changes that may occur throughout the course of the project. After presenting and discussing my talk at SICB, we decided to alter the scope of the project. Rather than just collect data to parameterize a mechanistic model, we decided to look at the fundamentals of the model itself. We then brought Dr. Ofir Levy on board and decided to test how using more realistic physiological data affects the predictions that mechanistic models make.

From the findings on this project, I concluded that it is always important to verify one's assumptions. This verification is especially true if you are trying to make predictions based on these assumptions. I now look at and probe the assumptions being made in a model rather than take them at face value. I also now have a much better realization about how much work is involved in creating a mechanistic model.

This project also helped shape my view towards the other chapters within my dissertation. Since all of the chapters in my dissertation, were focused on making quantitative predictions about how organisms thermoregulate and respond to temperature variation, this project helped forge my view that would be present in my work with flies as well. By helping to collect data to parameterize and develop a quantitative model for how organisms respond to changes in temperature, I developed skills that I continued to use during the other chapters of my dissertation as I created quantitative models predicting how flies should thermoregulate at different temperatures and densities. Consequently, this chapter not only taught me a lot about how science is completed, it helped set me up for the future by giving me insight into designing and implementing projects that I would continue to use in my future work.

This manuscript was my first large scale project. Consequently, it has changed my thought process by teaching me about the basics of running a large research project. After working on this project, I changed how I thought about interacting and organizing people within a large collaborative group. Afterwards, I realized just how much energy and effort goes into performing an experiment and seeing it through all the way to publication. As a result, in future research, I already have and will continue to build upon these lessons and skills that I learned during this project.

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APPENDIX A

CHAPTER 4 SUPPLEMENTAL INFORMATION

Table S1: Lizard parameters used to calculate changes in body temperature (ΔT_b) of a *S. undulatus* lizard.

Description	Value (units)	Source
Size of adults (Snout vent length)	0.063 (m)	our measurements
Body mass	$SVL^3*3.55*10^{-8}$ (kg)	(Tinkle and Ballinger 1972)
Surface area	$0.0314\pi M_b^{2/3}$ (m^2)	(Fei et al. 2012)
Projected area for direct and scattered solar radiation	0.4 A_L (m^2)	(Porter et al. 1973)
Projected area facing toward the ground	0 (m^2) – laying, 0.4 A_L (m^2) – standing	(Porter et al. 1973)
Projected area that touched the ground	0.35 A_L (m^2) – laying, 0.05 A_L (m^2) – standing	(Bartlett and Gates 1967)
Area facing toward the sky	0.6 A_L (m^2)	(Bartlett and Gates 1967)
Area that is exposed to air	0.9A_L (m^2)	(Fei et al. 2012)
Thermal absorptivity	0.965 (dec. %)	(Bartlett and Gates 1967)
Convective heat transfer coefficient	10.45 (W m^{-2} K^{-1}) †	(Porter et al. 1973)
Emissivity of skin	0.965	(Bartlett and Gates 1967)
Thermal conductivity	0.5 (W K^{-1} m^{-1})	(Porter et al. 1973)
Body thickness (diameter)	0.02 (m)	our approximation
Heat capacity	3762 (J kg^{-1})	(Porter et al. 1973)

†assuming that the wind speed at the height of a lizard is minimal (less than 0.5 m/s)

Figure S1

Latitudinal gradient in energy assimilation estimated by our model, in which lizards from lower latitudes could spend more time at their preferred temperature and assimilate more energy each year. Climate warming between 1980-2010 (left column) and 2080-2100 (right column) will increase the annual duration available for foraging and digestion (h/y) in most locations (a, b), which will increase annual energy intake (kJ/y; c, d). On each map, the black line represents current distribution of *S. undulatus*.

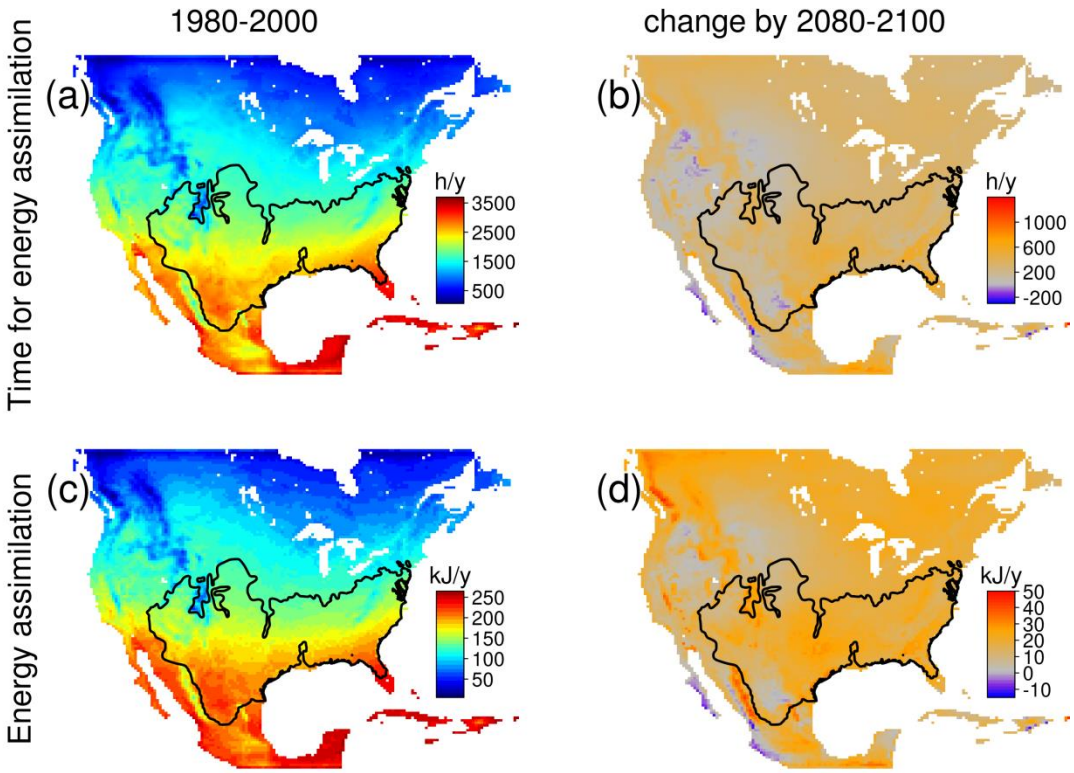


Figure S2

Impacts of climate on opportunities for foraging and digestion. Warmer locations enable lizards to forage and digest for more hours. The effect of climate on opportunities for foraging is less pronounced than on opportunities for digestion, since lizards are able to fill their gut faster than to digest the consumed food. The color of the each point indicates the number of locations. See Fig. S3 for predictions when the abundance of insects is smaller than in our initial parameterization.

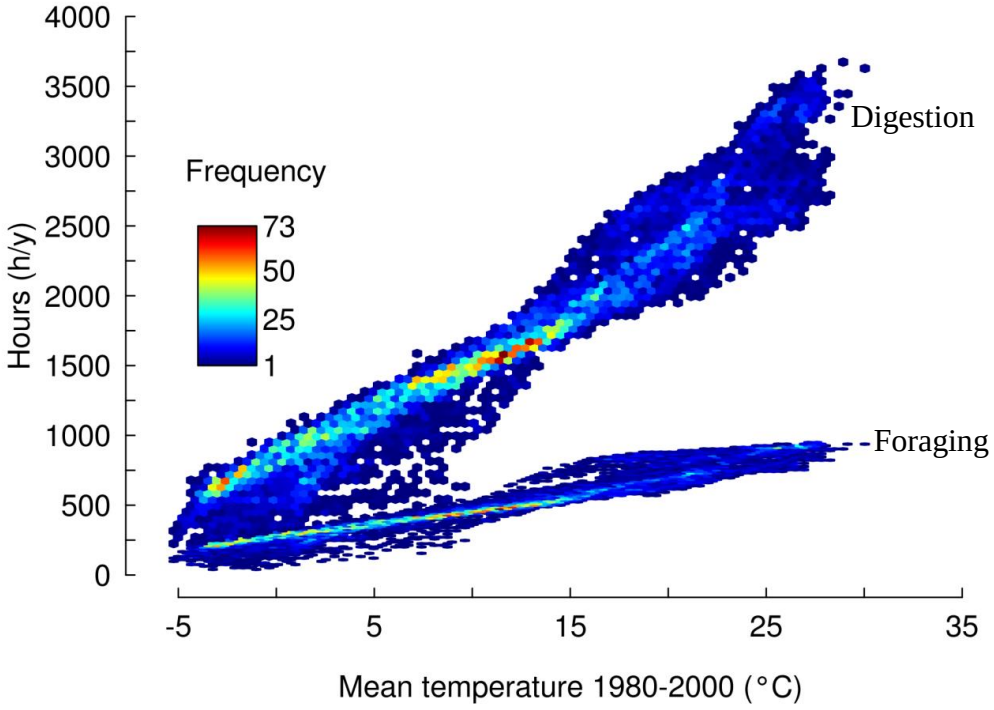


Figure S3

Impacts of climate on opportunities for foraging and digestion when the abundance of insects is smaller than in our initial parameterization (insect abundance = 0.0025 insect m^{-1} s^{-1}). Warmer locations enable lizards to forage and digest for more hours. The effect of climate on opportunities for foraging is less pronounced than on opportunities for digestion, since lizards are able to fill their gut faster than to digest the consumed food. The color of the each point indicates the number of locations.

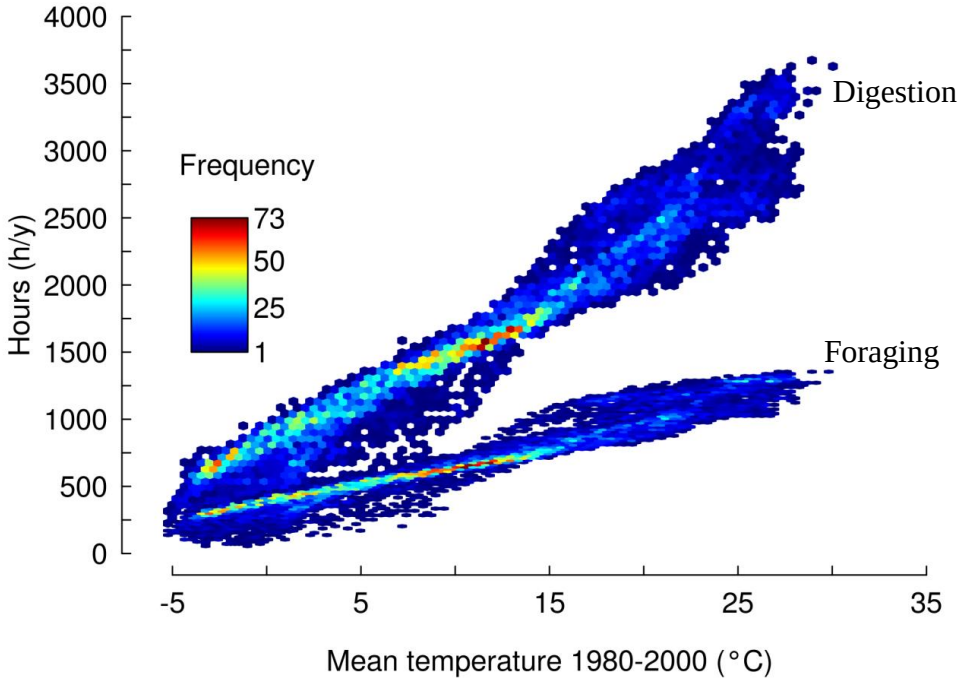


Figure S4

Impacts of climate on opportunities for foraging and digestion when the abundance of insects is smaller than in our initial parameterization (insect abundance = 0.0025 insect m^{-1} s^{-1}). Thermal opportunity is strongly affected by climate, especially across days. Here, even though the abundance of prey items is lower, thermal opportunity for foraging is not affected by climate since lizards can fill their gut within 2.5-3.5 hours of feeding. Climate significantly affects the daily thermal opportunities for digestion, however, since digestion is a relatively long process. The color of the each point indicates the mean air temperature at one or more locations.

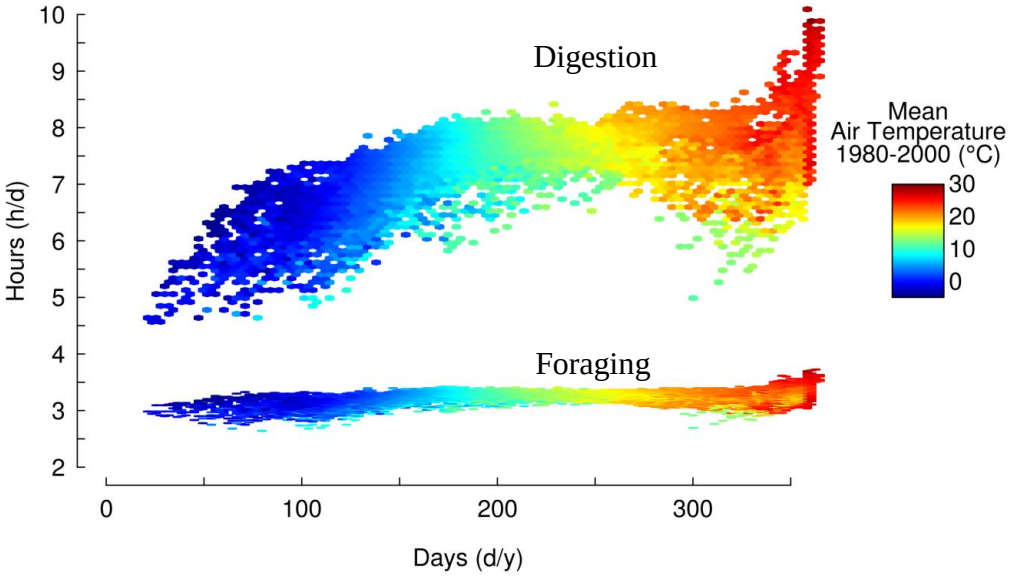


Figure S5

Phenological impacts of climate change on foraging time when the abundance of insects is smaller than in our initial parameterization (insect abundance = 0.0025 insect m^{-1} s^{-1}). The effect of warming on foraging time budgets depends on the current temperature and the time of year. At cool locations, lizards will have more time for foraging during summer in future climate than in the past climate. At warm locations, however, lizards will have more opportunities for foraging in winter, but less in the spring, summer and fall. The color of the each point indicates the mean air temperature at one or more locations.

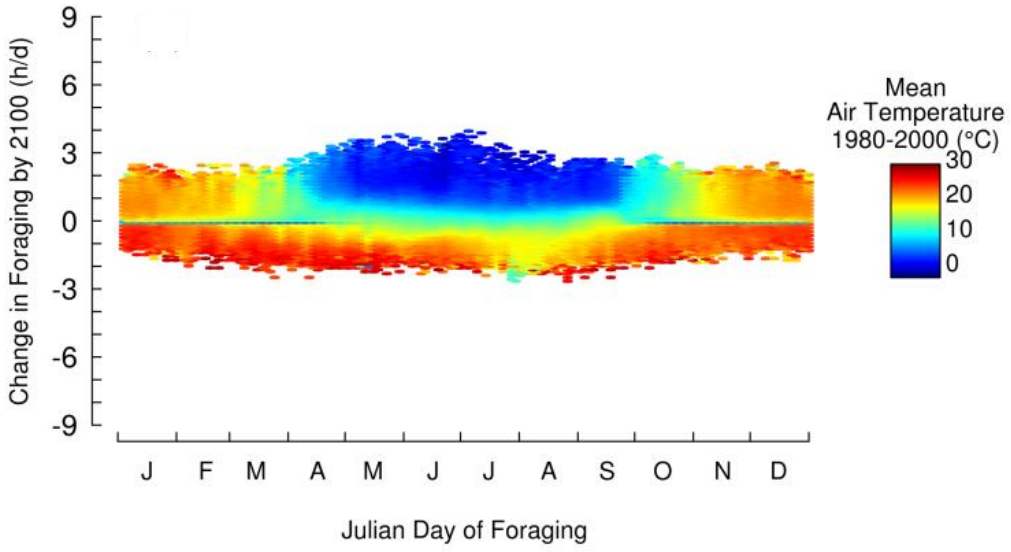


Figure S6

Impacts of climate change on daily assimilation rates (a) and daily energy balances (b) when energy assimilation decelerates with increasing thermal opportunity faster than in our initial parameterization (assimilated energy = $0.98 \cdot (1 - e^{-0.3 \cdot td})$, t_d - thermal opportunity). Here, given the faster deceleration of assimilation rates with thermal opportunity, the impact of shifts in thermal opportunity for digestion will pose even a lesser effect on daily assimilation rates at warm locations compared to cold locations. The color of the each point indicates the mean air temperature at one or more locations.

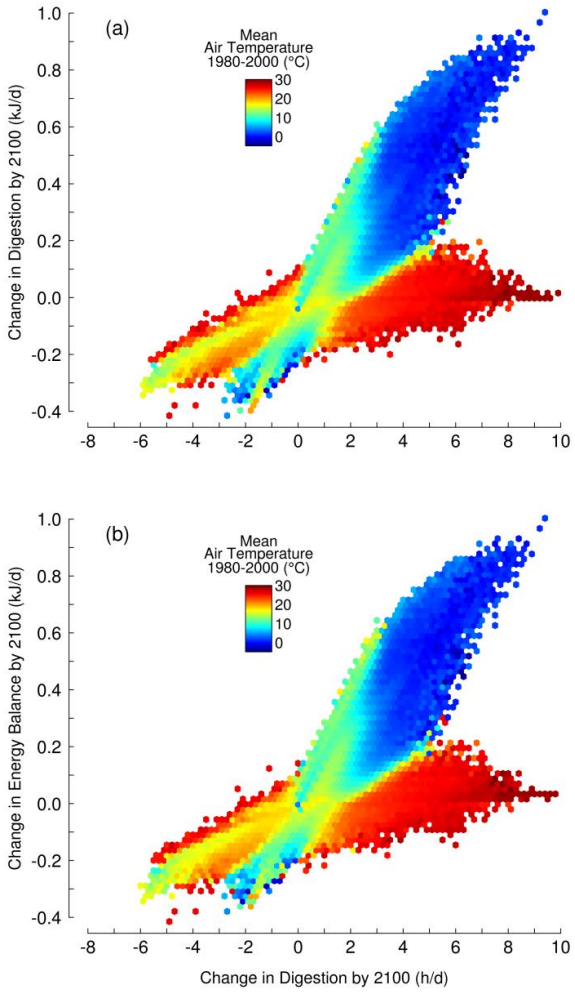


Figure S7

Phenological impacts of climate change on daily rates of energy assimilation when energy assimilation decelerates with increasing thermal opportunity faster than in our initial parameterization (assimilated energy = $0.98 \cdot (1 - e^{-0.3 \cdot t_d})$, t_d - thermal opportunity). The effect of warming on energy assimilation matches the effect on the thermal opportunities for digestion. At cool locations, lizards will assimilate more energy during summer in future climate than in the past climate. At warm locations, however, lizards will assimilate more energy in winter, but less in the spring, summer and fall. Here, given the faster deceleration of assimilation rates with thermal opportunity, the daily decreases in assimilation rates at warm locations are even smaller compared to the daily increases in assimilation rates that result from the phenological shifts. The color of the each point indicates the mean air temperature at one or more locations.

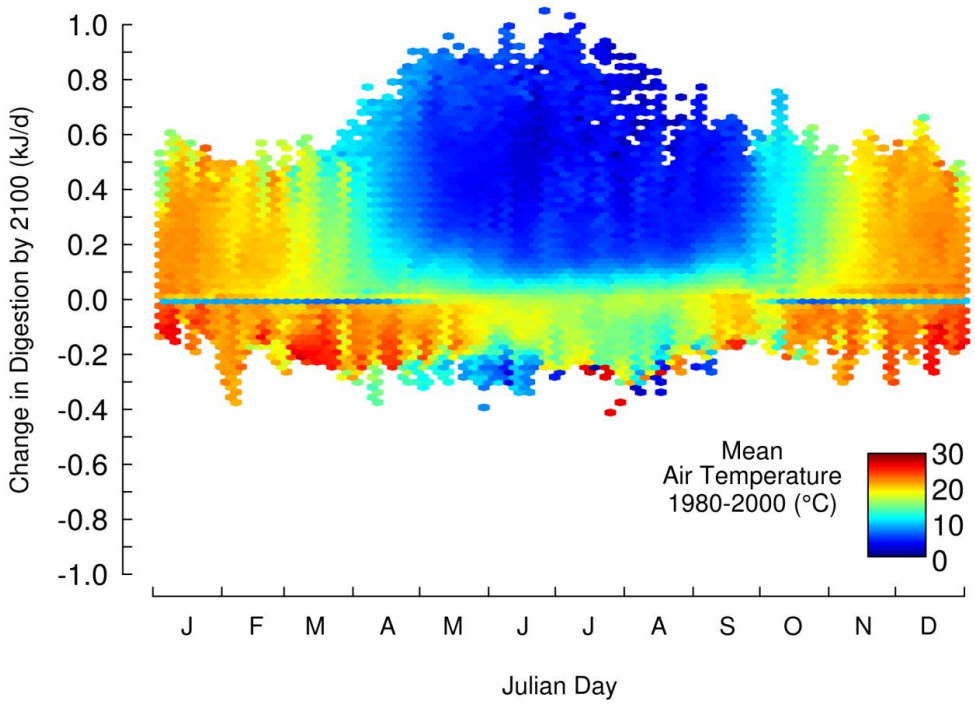
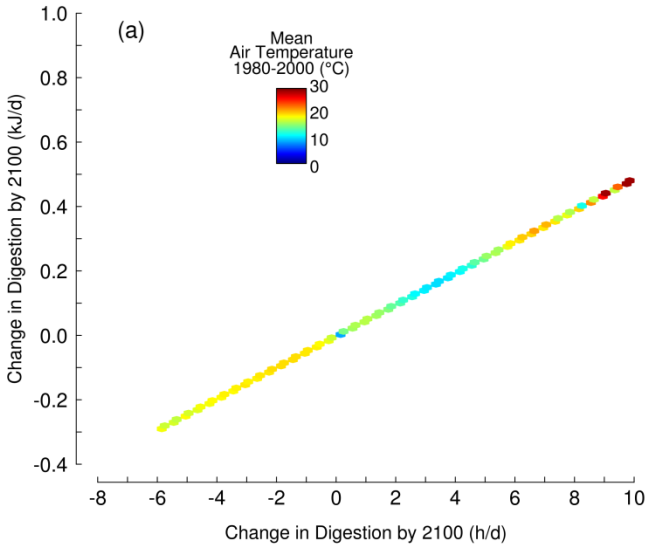


Figure S8

Impacts of climate change on daily assimilation rates (a) and daily energy balances (b) when lizards have constant rates of energy assimilation (assimilated energy = 0.98/24· t_d, t_d - thermal opportunity). Given the constant rates of assimilation with thermal opportunity, the impact of shifts in thermal opportunity for digestion will pose an equal effect on daily assimilation rates among warm and cold locations. The color of each point indicates the mean air temperature at one or more locations.



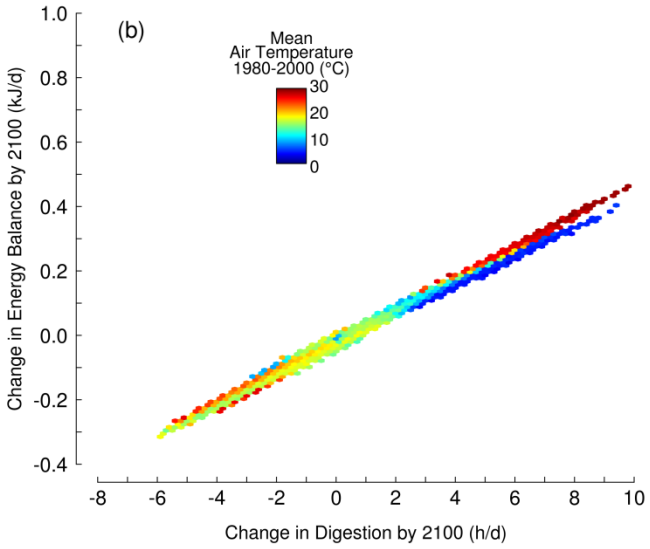


Figure S9

Phenological impacts of climate change on daily rates of energy assimilation when lizards have constant rates of energetic assimilation (assimilated energy = 0.98/24· t_d, t_d - thermal opportunity). The effect of warming on energy assimilation matches the effect on the thermal opportunities for digestion. At cool locations, lizards will assimilate more energy during summer in future climate than in the past climate. At warm locations, however, lizards will assimilate more energy in winter, but less in the spring, summer and fall. Given the constant rates of energy assimilation with thermal opportunity, the daily shifts in assimilation rates will be independent of the current climatic conditions. The color of each point indicates the mean air temperature at one or more locations.

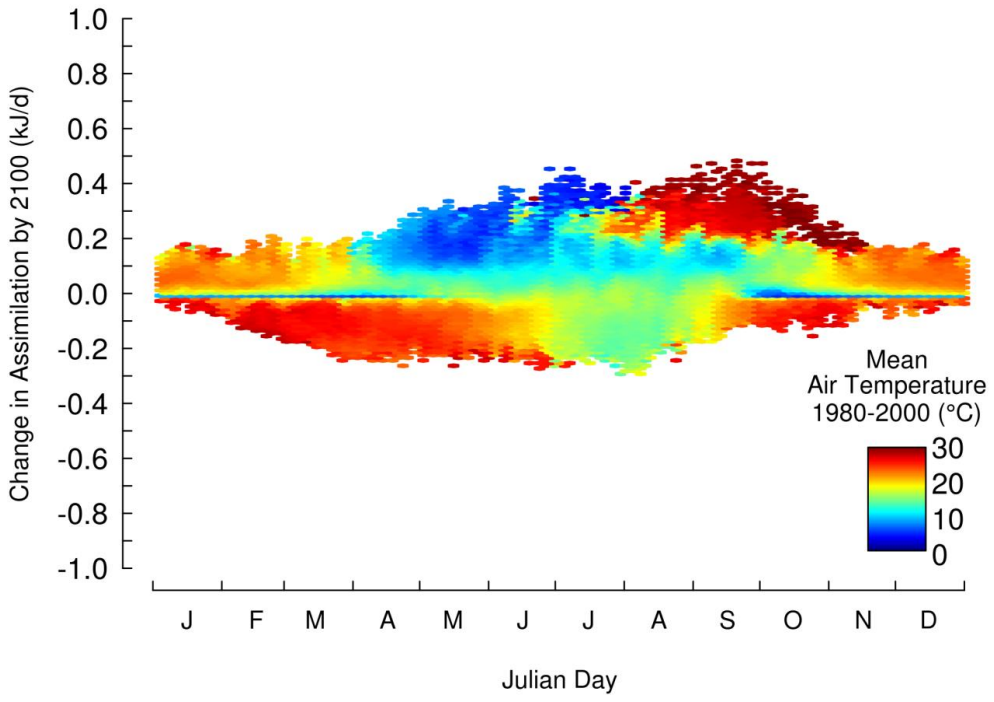


Figure S10

Latitudinal gradients in energy expenditure and energy balance estimated by our model. Lizards from lower latitudes could spend more time foraging and thermoregulating and spend more energy each year. Climate warming between 1980-2010 (left column) and 2080-2100 (right column) will increase the annual energy expenditure (kJ/y) in all locations (a, b). However, energy balance will increase at most locations because of greater energy assimilation (kJ/y; c, d). On each map, the black line represents current distribution of *S. undulatus*.

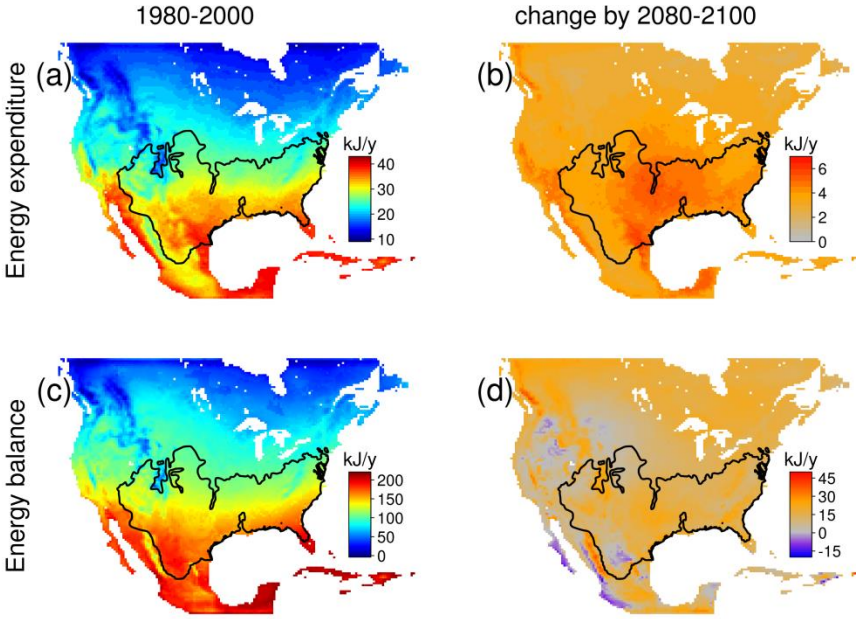
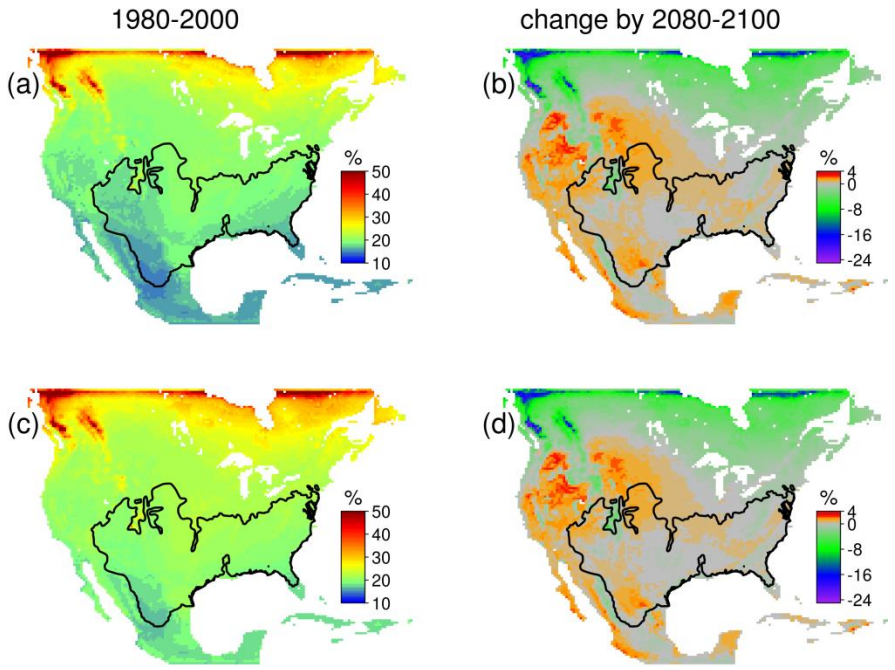


Figure S11

The ratio between annual energy expenditures and energy assimilation across North America, as estimated by our model. The ratios could be much lower in lizards from lower latitudes. Climate warming between 1980-2010 (left column) and 2080-2100 (right column) will increase the ratios at lower and middle latitudes, where current times for energy intake are high (See Fig. S1). For these calculations, energy expenditure during activity was either 300% (a, b) or 450% (c, d) more than the resting metabolic rate. On each map, the black line represents current distribution of *S. undulatus*.



APPENDIX B

STATEMENT REGARDING CO-AUTHORS OF CHAPTER 5

All co-authors of Chapter 5 (Ecology, 98: 1217-1228) have given their permission for it to be included in this dissertation. Permissions were made through email to Jason Borchert.