Fire and Blood: Behavior and Thermoregulation in Small Nocturnal Mammals

Tal Kleinhouse-Goldman Gedalyahou

University of Maine

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FIRE AND BLOOD: BEHAVIOR AND THERMOREGULATION IN SMALL NOCTURNAL MAMMALS

by

Tal Kleinhause-Goldman Gedalyahou

A Thesis Submitted in Partial Fulfillment of the Requirements for a Degree with Honors (Wildlife Ecology)

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Advisory Committee:
Danielle Levesque, Assistant Professor of Mammalogy and Mammalian Health, Advisor
Stephen Coghlan, Associate Professor of Freshwater Fisheries Ecology
Michael Kinnison, Professor of Evolutionary Applications
Alessio Mortelliti, Assistant Professor of Wildlife Habitat Conservation
Jennie Woodard, Preceptor in the Honors College
ABSTRACT

As global temperatures continue to increase, so does the need to better assess the sensitivity of different species to climate change. Such assessments require accurate behavioral and physiological data, including on thermoregulation and the effects of high temperatures on the behavior of endotherms. Seeking to improve current knowledge and methodology, my research evaluated the connections between activity and thermoregulation, using deer mice (*Peromyscus maniculatus*) as a species model. By using a custom made maze, I was able to simulate an active foraging environment and examine both activity patterns and subcutaneous temperatures in mice during the active-phase of their activity cycle. Due to several circumstances, not all parts of the study were completed. Available data was analyzed for possible relationships between observed $T_{sub}$ frequencies and activity or consumed food. No significant relation between subcutaneous temperature waves and either activity or consumed food was found; as well as no relation between the sum of subcutaneous temperatures and consumed food. Nevertheless, my study emphasizes some of the benefits in using a behavioral maze. Protocols developed during this study could potentially assist in either the reconstruction of the full original study, or in future studies concerning variability in ecological and physiological data.
ACKNOWLEDGMENTS

There are not enough words to thank all who have been involved in this wild thesis journey. First and foremost, I wish to thank Dr. Danielle Levesque for all her help, teaching, support, encouragement and willingness to assist solving problems even if her location was all the way across the world. My interactions with Dr. Levesque were the most valuable learning experience I had the privilege to receive throughout this entire project. From sharing knowledge to sharing fun facts about tropical animals, I am honored for being able to spend the past three years learning in the Levesque Lab. Alongside, I wish to thank Vanessa Hensley, Jake Gutkes and Ana Breit for their help and guidance in developing the experiment protocol, the ongoing trapping attempts, analyzing the data and constant mental support. A special thank you to Dr. Joseph Zydlewski for his assistance with the antenna construction and patient while teaching me physics; to Dr. Naomi Jacobs for her time, encouragement and guidance; to the SBE administration for an irreplaceable backstage assistance; and to the Center of Undergraduate Research for funding my research and being the first (aside from my mom and my advisor) to believe in my abilities and in this project. I also wish to thank the members of this committee, Dr. Stephen Coghlan, Dr. Michael Kinnison Dr. Alessio Mortelliti and Dr. Jennie Woodard, for their incredible willingness to share knowledge and provide advice in times of need. Lastly, I wish to thank Kara Aiken, Patrick Hourihan and the rest of my friends and family for accepting me, encouraging me, having faith in my abilities, laughing at my maze jokes and most importantly, for buying me socks that have squirrels on them – I am so lucky to have you all in my life.
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CHAPTER 1

LITERATURE REVIEW AND GENERAL INTRODUCTION

Current Challenges and Assessing Species Vulnerability to Climate Change

As global climate continues to shift drastically, with potential severe implications for both humans and non-human animals, the need to better understand the potential for species to respond to change is becoming ever more urgent (Guisan & Thuiller, 2005; Huey et al., 2012; McKechnie et al., 2017). Although the study of temperature and precipitation fluctuations has been at the forefront of many recent studies, much is still unknown regarding the relationships between the expected increase in global temperature and the survival of different animal species (Guisan & Thuiller, 2005; Huey et al., 2012; Urban et al., 2016). Understanding and predicting species responses to the changing climate is essential for identifying conservation priorities as well as assessing species vulnerability and future trends in their health or distribution (Huey et al., 2012; Seebacher & Franklin, 2012). These kinds of assessments, called climate change vulnerability assessments (CCVA), are largely dependent on our ability to estimate the energetic costs for species to live under certain conditions (Ofori et al., 2017; Williams et al., 2008).

Understanding the potential for disparities between energy requirements in current and future environments is crucial for making science-based decisions concerning species conservation (Seebacher & Franklin, 2012; Urban et al., 2016). In recent years, different CCVA models were developed to represent the connection between changing climate and animal vulnerability, and have become an acceptable supporting tool (Carvalho et al.,
Most CCVA’s focus on assessing the degree of sensitivity to change, at the individual and the population level (Huey et al., 2012; Williams et al., 2008); while others emphasize the adaptation potential (Ofori et al., 2017) and the extent of exposure (Foden et al., 2013).

A common theme to all CCVA’s is the increasing need for accurate behavioral, physiological, and genetic data (Carvalho et al., 2010; Huey et al., 2012; Ofori et al., 2017; Williams et al., 2008). The lack of accurate information on essential traits for many species has created fundamental knowledge gaps, particularly in endotherms (Deutsch et al., 2008; Huey et al., 2012). Specifically, there is lack in a developed methodology for studying the effects of high temperatures on the thermoregulation and energy costs in endotherms, as well as some significant unknowns in endotherms thermoregulation as a whole (Hof et al., 2017; Levesque et al. 2016). These include a more accurate description of the basic thermoregulatory physiology of mammals. Such overarching questions of energy-saving strategies in relation to body temperature and behavior in the context of climate change are the basis for this research.

Endothermy in Mammals and Related Knowledge Gaps

Studying mammalian energetics requires an understanding of endotherm physiology. As endotherms, mammals are capable of producing, and regulating, body heat using physiological and biochemical processes (Mitchell et al., 2018; Rezende & Bacigalupe, 2015). Yet an endothermic regulatory strategy is highly demanding and can be combined with different physiological and behavioral mechanisms, expanding the range in which animals can survive (Humphries & Careau, 2011; St Juliana & Mitchell, 2016; Van Der Vinne et al., 2015; Van der Vinne et al., 2014). Mammals also show a
high degree of variability in activity patterns, from strictly diurnal, to strictly nocturnal, to a mix of both. Such variability is another strategy for maintaining heat and obtaining energy. Given the hot temperatures during the day and colder temperatures during the night, each timeframe presents its own specific challenges (Lovegrove, 2016; Mitchell et al., 2018). Nevertheless, our current understanding of the costs of endothermy and its relationship to temperature and performance is severally lacking (Levesque et al., 2016; Riek & Geiser, 2013; Van der Vinne et al., 2015).

Overall, night-active lifestyle is considered beneficial in terms of thermoregulation at high temperatures by avoiding the need to regulate body temperature during the hot hours of the day, saving both energy and water (Van der Vinne et al. 2015, Levesque et al. 2016). However, while average world temperatures increase as a whole, the average night temperatures are increasing more rapidly compared to the average day temperatures (NOAA, 2018). In the USA during the months of May-August 2018 alone, the average maximum daytime temperature was 29°C, 1.47°C higher than the 20th century average. The maximum nighttime average temperatures during these months was 15°C, the highest average to ever be recorded, and a 1.76°C higher than the 20th century average (NOAA, 2018).

Such increases in nighttime temperatures presents further challenges to nocturnal species and could also have potential harmful effects on diurnal and crepuscular animals, as some might shift to night-activity in response to increasing day temperatures (Levy et al., 2016; Van der Vinne et al., 2014). These challenges further emphasize the need to better understand the physiology and behavior of less studied nocturnal species and the ways a changing climate might affect them.
Specific Challenges in Studying Thermoregulation

Thermoregulation can be defined as the overall process by which animals regulate their body temperature (McNab & Morrison, 1963). By regulating body temperatures, animals are capable of surviving under different conditions and stresses. Yet the range of ambient temperatures at which an endotherm can maintain constant body temperature without increasing metabolic rate is generally considered as preferable, and can be defined as the thermoneutral zone – or TNZ (Buckley, Hurlbert, & Jetz, 2012; Huey, 1991; Mitchell et al., 2018). An important feature of the TNZ also encompass the study of the upper critical limits of the thermoneutral zone; that is the temperatures at which active metabolism exceeds the rate of water loss in an endothermic animal (McNab & Morrison, 1963; Mitchell et al., 2018).

The costs that the thermal boundaries impose on animals, as well as the different ways water loss should be incorporated in models are all under constant study. Recently, attention has also been given to the question of how to study, calculate, and define those boundaries (Levesque et al., 2016; McKechnie et al., 2017). The study of the upper critical limits of animals under lab conditions can easily be misrepresentative. More often than not, animals are not exposed to high enough environmental temperatures to stimulate an increase in the metabolic rate above minimum demands; while measurements are taken only from one individual out of each species (McKechnie et al., 2017). Such measurements do not necessarily represent the physiology of wild populations and are crucial in evaluating animal survivability in changing environments (Buckley et al., 2012; Huey et al., 2012; Humphries & Careau, 2011; Juliana & Mitchell, 2016).
However, to explore such questions, more physiology and behavior data in field conditions is needed (Deutsch et al., 2008; Huey et al., 2012). Unfortunately, this kind of information is mostly still missing for many species across different habitats and taxa. Moreover, most of the existing information on the critical limits of different species is focused on ectotherms or on cold temperatures and the lower critical limits of survival (Huey et al., 2012; McNab & Morrison, 1963). Given the current predictions of global warming, this lack of accurate data on the heat extremes that different species can experience is highly valuable for assessing sensitivity to climate change.

In order to better understand these relationships between thermoregulation and climate in endotherms and the way body temperatures is measured should also be considered. Given the close connection between temperature regulation and behavior (Humphries & Careau, 2011; Juliana & Mitchell, 2016), using measurements taken under high-stress lab conditions or during the rest phase might not provide the most accurate data for estimating natural conditions. As an attempt to provide more information on the upper critical limits of mammals (Huey et al., 2012), my thesis seeks to examine the current methodology for measuring body temperature during the active phase.

**The Behavioral Component**

Animal behavior studies emphasize the need to incorporate a behavioral approach in the overall study of animals (Bell, 2007; Réale et al., 2007). Specifically, behavior is one of the major factors affecting body temperature and the regulation of the internal environment in both endotherms and ectotherms. Environmental temperatures were found to be a source for variation in the behavior of different species in both cold and hot condition (Brodie & Russell, 1999; Rezende & Bacigalupe, 2015).
Any assessment of thermoregulation has to consider behavioral mechanisms that might be affecting thermoregulation. Within those behavioral mechanisms, the active phase is the time period in which an animal is foraging, breeding and operating; or in other words – the time during which animals spend most energy (Bieber et al., 2017; Du Plessis et al., 2012; Humphries & Careau, 2011). Successful foraging behavior has a crucial fitness role and is an important aspect in examining performance under different environmental conditions. Studying foraging behavior can be used to estimate different scenarios in species distribution in response to food availability or changes in habitat (Grácio et al., 2017; Mathias, Klunder, & Santos, 2003; Van der Vinne et al., 2014).

However, simulating foraging conditions in a manner sufficient enough to represent natural conditions is a difficult task. Such models will require both environmental variations such as micro-climate or available food sources, as well as behavioral triggers such as stress or activity time (Barnett & Smart, 1965; Bell, Hankison, & Laskowski, 2009). One tool designed to simulate foraging behavior is the use of mazes, especially in studying small mammals (Barnett & Smart, 1965; Jašarević et al., 2012). Maze-designs such as the Morris Water Maze, the Y-Maze, an Octopus Maze or other special learning tools are commonly used for evaluating small mammal learning (Gaulin & Wartell, 1990; Jašarević et al., 2012). However, those kinds of mazes are more focused on evaluating analytic components such as memory or problem solving, and less on measuring authentic physiological features such as body temperature and energetics costs.

For those reasons, my study seeks to explore the advantages of using a maze in evaluating the behavior of small mammals. By using a maze, both the behavior and environment of the animal can be monitored and evaluated (Jašarević et al., 2012). Using
a specialized maze will allow the adjustment of the lab conditions into a simulation of field and foraging conditions as much as possible. Such adjustment will have to include specific changes according to the study species. In this research, I used the deer mouse (*Peromyscus maniculatus*) – a common small mammal with many unknowns concerning its behavior, thermoregulation and responses to different environments.

**Species’ Description and Objectives**

The North American deer mice (*Peromyscus maniculatus*) are one of the most common rodents’ native to North America. Thanks to its large distribution in terrestrial environments and both wild and laboratory valid populations, deer mice are commonly used as a species model for small mammals (Chappell, 2004; Vrana et al., 2014). Adult deer mice from laboratory populations weigh 24.5g on average and have an average body temperature of 23°C (Hayes & Chappell, 1986; Rezende, 2004). Most of the research on deer mice focuses on responses to cold, different elevation and oxygen consumption (Chappell, 2004; Dane et al., 2018; Demas & Nelson, 1996; Demas & Nelson, 1998; Hayes, 1989; Hayes & Chappell, 1986; Rezende, 2004; Vrana et al., 2014). Nevertheless, current data on wild populations of *Peromyscus maniculatus gambeli* indicates deer mice can reach body temperatures of 40.3°C under heavy activity and to an average $T_b$ of 36.8°C under light activity (McNab & Morrison, 1963).

To improve knowledge of the relations between increasing ambient temperatures, foraging behavior and energetics in deer mice – my objectives are 1) to develop a more realistic behavioral and mechanistic model for ambient temperatures in endotherms using deer mice as a model species; and 2) review the effects of high temperatures on the body temperatures and activity level of a nocturnal mammal. By doing so, my study seeks to
provide more information on thermoregulation in endotherms as well as to assist in developing a methodology for estimating species vulnerability to climate change.

To address these objectives, my study offers an original design for a study-maze, which combines observations of foraging behavior with real-time measurements of $T_b$ while the animal is engaging with a new environment. I expect an increase in body temperature at higher environmental temperatures; as well as a decrease in activity as environmental temperatures increases. This hypothesis is based on the understanding that activity is affected, among other factors, by environmental conditions (Bieber et al., 2017; Brodie & Russell, 1999; Buckley et al., 2012; Levesque et al., 2016). As external temperatures increase, regulating body heat becomes more energetically demanding.

These demands are added to the risks of foraging behavior and could affect decisions such as niche preference (Van der Vinne et al., 2014) or potentially the choice between actively foraging or remaining in resting mode. Assuming such variations in energetic demands, a change in behavior can be expected as animals are exposed to higher temperatures and specifically, animals might show higher body temperatures with activity or when exposed to increasing external temperatures, followed by a decrease in activity levels in order to maintain and lower internal temperatures.
Maze Construction

The first step in this study was to design a maze. Since the objectives of this study all concern questions regarding performance in activity, the maze is intended to provide an active environment for the mice. The maze itself was made from standard hamster plastic tubes (57mm in diameter, Kaytee, Chilton, WI), and is 82cm in length, 56 cm in width and 31cm tall (Fig 1). The maze includes a combination of turns, climbs, loops and a spinning wheel. The simulation of foraging behavior was achieved by planting food items in different areas along the maze. The maze was put in a cardboard box that could later be transported between different areas and conditions.

The maze was also intended to provide a technique for accurately obtaining body temperatures from the mice as they are active, without disturbing their behavior. To allow those measurements, temperature-sensitive passive integrated transponder tags (12.5mm, BioThermo13, Biomark, Boise ID, USA) were injected subcutaneously to the inter-scapular region of the mice. Signals from these tags were received from a custom built antenna wrapped around the maze box connected to a reader base unit (HPR Plus, Biomark, Boise, ID, USA). The antenna was built with the help of Dr. Zydlewski by extending a 3m cable that was later welded to a connector (Biomark, Boise, ID, USA) and wrapped around the maze box. Under regular conditions, subcutaneous temperatures were recorded every 10 seconds.
Figure 1. Maze design used to simulate foraging behavior, made out of standard plastic tubes. Maze is measured as 82cm by 56cm by 31cm and includes turns, climbs, loops and a spinning wheel.

Figure 2. An external view of the activity maze setup, used for small mammals foraging behavior testing under different environmental conditions. Include customized antennae and recording equipment.
**Trapping Efforts**

Small mammal trapping was conducted during the months of September through October as part of Jake Gutkes’ Masters project. 20 live-catch Sherman traps (H. B. Sherman Traps Inc., Tallahassee, FL, USA) were placed in several locations in the University of Maine forest with peanut-better balls as bait. Trapping was only conducted on nights that were clear of rain and above 7°C. At capture each mouse was sexed, weighed, measured for length of hind foot, hind foot with toes, forearm, ear and urogenital in males (area too small to be measured in females for field conditions) and injected with a PIT tag. All trapping efforts were done under the University of Maine IACUC permit number A2018-04-03 and State Permit number 2017-516 (Appendix I and II).

**Experimental Procedure**

Upon capture, all mice were evaluated in the field in order to determine if they could participate in the study. I evaluated mice based on health conditions (no obvious signs for injuries, parasites, or low wait) and potential pregnancy for females (no visible signs of nipples). The first mouse to be found suitable was brought back to the Levesque Lab in 321 Murray Hall; except for the last night of trapping (06/10/2018), in which two mice were brought back and were kept separately throughout the entire process. In the lab, mice were kept in a plastic animal container (3G Critter Keeper, Lee’s, Nashville, TN) and were provided with apple and bedding. The apple was taken out of the animal container at least four hours before the experiment began, in order to promote the mouse’s motivation to search for food in the maze.
Each experiment included one mouse, lasted for one hour and was conducted after 20:00, in a dark environment. Before the mouse was transported to the maze, four apple pieces were placed in the maze; the total mass of the apples was recorded before and after the experiment. During the experiment, the mouse was let free in the maze, as temperatures are recorded every ten seconds. Manual recording of \( T_{\text{sub}} \) from the recorder were taken every five minutes. In addition, a video camera (IP5180VD, ELP, South Korea) was set above the maze in order to record the entire setup and obtain behavioral records. However, due to a malfunction in the recording protocol, all videos were lost.

According to the original study design, experiments were to occur in three stages. The first stage focused on controlled activity preformed in room conditions and was conducted in the 321 Murray Hall under an average room temperature of \( \sim 22.8^\circ \text{C} \). The second and third stages of the experimental procedure were to occur in temperature-controlled chambers, housed in the basement of Murray Hall, in order to manipulate external environments. Experiments were to be conducted in the chambers under 30°C for the second stage and 35°C for the third stage. However, setbacks in trapping efforts and in the construction of the maze prevented from implementing the second and third stages of the experimental protocol before field conditions prohibited further trapping.

In addition, for reasons unknown, during the last four experiments the antenna was unable to record and transmit the observed subcutaneous temperature from specific points in the maze. As a result, there are some large time gaps between measurements. Some of the time gaps were overcome by a manually recording of the temperatures using an GPR Plus hand reader (Biomark, Boise, ID, USA), yet all analysis and conclusion describe below are based on data obtained from the first stage alone.
Data Analysis

After completing data collection, all temperature measurements were exported to Excel and were analyzed using R-studio. Given the setbacks and malfunctions in the data collecting phase of the study, available data for analysis consisted the subcutaneous temperature measurements, some behavioral notes (active vs. resting), some apple consumption data, and initial field measurements. As such, during the analysis portion of the research I have tried to avoid over-interpretating the available data, while still seeking to find patterns that may be indicative for trends in a futuristic, more complete dataset. My first stage was to account for any time-gaps in the body temperature database, which I did by manually averaging subcutaneous temperatures for every 30-second intervals.

Using the 30-seconds averages, I focused on variability in observed $T_{\text{sub}}$ using a non-stationary waveform analysis in order to quantify variations in thermoregulation patterns (Levesque et al., 2017) as a connection link between $T_{\text{sub}}$ and activity (Bieber et al., 2017). The distribution of such patterns was then used for an intraspecific comparison and detecting patterns the timing of “temperature waves” and activity. Next, I performed a correlation analysis between the number of waves per individual and the mass each individual ate from the available apples during the experiment.

To test for the link between the sum of $T_{\text{sub}}$ and the mass of apple consumed, I followed the wave-form analysis with a correlation analysis. In order to perform this particular analysis, I used the manually recorded subcutaneous temperatures to account for missing measurements. Doing so, I insured each animal had the same temperature records– 2 data points each minute, 122 in total. I used the sum of temperatures instead of averages since I wanted to include the actual records of high temperatures in the analysis.
Lastly, I plotted a histogram based on the frequencies in which each subcutaneous temperature was recorded (McKechnie et al., 2007) in order to represent the relationship between activity and T_{sub}. To this histogram plot, as well as to the other regression plots, I added a normal curve distribution line or a best-fitted line, respectively.
RESULTS

Between the months of August to October of 2018, ten deer mice (six males, four females) were captured and tested using an activity maze in order to record behavior and body temperature (Fig 3). The average mass for all mice was 17.7g (14.7 – 22.8), while the average mass for males was 16.7g compared to the average female mass was 18.4g (Table 1). From the ten experiments preformed, the mass of the apple before and after the experiment was recorded during only five trails, since it was not included in the original protocol. The average mass of the apple before the experiment was 7.7g, while the average mass of the apple after the experiment was 6.1g. A control trial on apple evaporation without a mouse feeding on it was set on a loss of 0.1g per hour (Table 1).

Table 1. Summary of data obtained from 10 deer mice during an hour of behavioral maze experiments. Average mice mass is 17.71g and subcutaneous temperature is measured in ºC using pit tags. Average mass of consumed apple is 0.7g. Waves obtained from quantify thermoregulation variations patterns in a non-stationary analysis.

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Sex</th>
<th>Body Mass (g)</th>
<th>Sum of Subcutaneous Temperatures (ºC)</th>
<th># of Temperature Waves</th>
<th>Average Temperature</th>
<th>Apple Consumed (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>f</td>
<td>14.7</td>
<td>4331.0</td>
<td>6</td>
<td>35.5</td>
<td>x</td>
</tr>
<tr>
<td>2</td>
<td>f</td>
<td>22.8</td>
<td>4620.6</td>
<td>4</td>
<td>37.9</td>
<td>x</td>
</tr>
<tr>
<td>3</td>
<td>f</td>
<td>18.5</td>
<td>4395.5</td>
<td>4</td>
<td>36.1</td>
<td>x</td>
</tr>
<tr>
<td>4</td>
<td>f</td>
<td>18.7</td>
<td>4556.0</td>
<td>5</td>
<td>37.4</td>
<td>x</td>
</tr>
<tr>
<td>5</td>
<td>m</td>
<td>16.0</td>
<td>4480.1</td>
<td>2</td>
<td>36.8</td>
<td>x</td>
</tr>
<tr>
<td>6</td>
<td>m</td>
<td>17.3</td>
<td>4556.3</td>
<td>6</td>
<td>37.3</td>
<td>0.9</td>
</tr>
<tr>
<td>7</td>
<td>m</td>
<td>16.5</td>
<td>4589.4</td>
<td>3</td>
<td>37.5</td>
<td>0.4</td>
</tr>
<tr>
<td>8</td>
<td>f</td>
<td>17.7</td>
<td>4394.2</td>
<td>3</td>
<td>36.1</td>
<td>0.5</td>
</tr>
<tr>
<td>9</td>
<td>m</td>
<td>16.9</td>
<td>4224.0</td>
<td>2</td>
<td>35.5</td>
<td>0.6</td>
</tr>
<tr>
<td>10</td>
<td>f</td>
<td>18.0</td>
<td>4593.6</td>
<td>5</td>
<td>37.6</td>
<td>1.1</td>
</tr>
</tbody>
</table>
The overall comparison between the number of increases and decreases in $T_{\text{sub}}$ (used as indicative of a change in activity) and the actual activity patterns recorded for each animal showed no visual correlation. There was no correlation found between number of subcutaneous temperature waves per hour, obtained from the non-stationary waveform analysis, and the consumed apple mass (g) per individual per hour ($F_{1,3} = 4.75$, $p = 0.1175$, $n = 5$, Fig. 4. Significant correlation was also not found during the linear regression analysis of the relation between the sum of overall temperatures recorded for each animals and the consumed apple mass ($F_{1,3} = 0.50$, $p = 0.53$, $n = 5$, Fig. 5). Since both analyses involve the apple mass measurements, both only include data from the six trials in which mass of the apple was measured.

Figure 3. Individual variation of subcutaneous temperature ($^\circ\text{C}$) from 10 deer mice during activity maze experiment in room temperature ($23^\circ\text{C}$).
Figure 4. Relationship between subcutaneous temperature waves per hour, and the mass of consumed apple (g) per individual per hour for five deer mice. Reported p-value of the regression is 0.12.

Figure 5. Linear regression analysis between sum of subcutaneous temperatures and the mass of consumed apple (g) per individual per hour in five deer mice. The reported p-value of the regression is 0.53.
Meanwhile, the histogram showed the most frequently recorded $T_{sub}$ was at about 37.5 °C, yet another peak could be detected at about 36°C (Fig. 6) based on the distribution of the recorded frequencies. Initially, I was expecting to see the two peaks as clearly indicating the differences between the $T_{sub}$ of the resting phase vs. the active phase as a binomial distribution, like shown in other studies (Bieber et al., 2017; Cohen & Kronfeld-Schor, 2005). However the current data did not show such results and so no further analyses were performed based on the frequencies distribution.

**Figure 6.** A histogram for the frequency of subcutaneous temperatures recorded from 10 deer mice during an activity maze experiment.
Overall, my study found preliminary data on the possibility of using an activity maze to assess behavioral changes in response to increasing environmental temperature. Although my current work did not find any significant relationships between the tested variables, this study emphasized the difficulties in using subcutaneous temperatures as an indicator for activity and could assist in improving future models. Specifically, the results of this study have shown no significant correlation between the measured subcutaneous temperatures and activity in the maze. There was also no significant correlation between temperature waves or sum of temperatures and consumed apples during the experiment. In addressing original objectives, I could not prove a relation between high $T_a$ and $T_{sub}$ or activity level in deer mice. This is a result of the incompletion of all experimental protocols, including the manipulation of the experimental environment. Available data showed no relationship between the study’s different variables.

Such results are to be expected given low number of replicates for the experiment ($n=10$) and the initial variance in experimental procedure (apple mass was only recorded during the last five trails). In addition, the lack of behavioral data from the video recording severely limited the extent of potential interpretations to the $T_{sub}$ temperature measurements. A small sample size of 10 animals, especially given the trial-and-error nature of this study, could have affected result distribution. Meanwhile,
measurements of the subcutaneous temperature could have varied depending on tag location.

However, I have shown some success in addressing my first objective of developing a behavioral and mechanistic model for ambient temperature in endotherms such as deer mice. Despite some technical difficulties, the behavioral maze and custom antenna have been shown to be functional, providing an engaging environment to the mouse during its active phase. Although measurements from the study have proven to be insignificant, the methodology and study design could be improved and potentially shown to be of value. In addition, the obtained data emphasize to some degree the variability in different individuals and the potentials in pursuing such research (Cohen & Kronfeld-Schor, 2005). Overall, despite the lack of significant results, the current study emphasized the need in accurate activity data, rather than just measurements of increasing and decreasing subcutaneous body temperatures, as an indication of activity (Bieber et al., 2017)

Possible Improvements to Current Research

As part of the original objectives of this research, my goal was to examine the differences in behavior and body temperature of deer mice as they are exposed to different and increasing external temperatures. Nevertheless, several setbacks to the current study resulted in implementing only the first experimental protocol under room temperature conditions. In addition, malfunction in the recording protocols for the behavioral videos initially taken during each trail, resulted in lack of continuous behavioral data throughout the entire experiment. These factors have limited the available
data and possible statistical analysis that can be performed as part of this study. In addition, while in the original study plan I intended to obtain about 30 mice for each experimental phase, circumstances have limited the available time window for trapping to such that only 10 mice were part of the first room temperature experiment.

Given these differences between the original study design and practical outcome, I believe some improvement for the research outline can be made. Such improvements would be fairly easy to implement, given that the main obstacle to the previous setup – the construction and operation of the antennae and maze – have already been completed. If the study were to ever be repeated, I would recommend for trapping efforts to begin as early as possible in order to obtain the goal of 30 mice per experimental temperature. I would also recommend based on the current experimental design, to continue weighing combined apple pieces before and after the experiment as a way to calculate consumed food and potentially gained energy. Furthermore, I would recommend changing the current design such that the hour of day in which the experiments are performed is constant.

In addition to these changes, I would also recommend using a different platform to record the experiment itself, such as the Sony video camera that has already been purchased. If implementing the second and third parts of the experiment, which includes manipulating the outside environment of the experiment using the temperature chambers, some additional setbacks are to be expected. However, during the preparations for this study, the chambers have been tested as active and could be used for such a purpose.
Directions in Future Research

Overall, this research has aimed to provide information on the ways different components in endotherms ecology, such as physiology and behavior, can interact into a more comprehensive knowledge. In particular, evaluating animals’ sensitivity to climate remains relevant and with it the need for obtaining more accurate data on different life traits of each species (Huey et al., 2012; McKechnie et al., 2017). Particularly, energy-saving strategies such as a nocturnal lifestyle could potentially become more common among endotherms as a response to increasing temperatures (Levy, Dayan, Porter, & Kronfeld-Schor, 2018; Maor, Dayan, Ferguson-Gow, & Jones, 2017).

Assuming the original objectives of the study could be achieved in an adjusted and improved version of this study, more could potentially be done using the behavioral maze. Such follow up studies might include further examination of different energetic demands imposed by high environmental temperatures. In particular, the relation between the different activity patterns of diurnal and nocturnal could be examined by changing the experimental protocol to account for a day-active small mammal. Assuming the maze could also be reconstructed in a smaller, or with a potential increase to the antenna size and range, the current objectives could also be combined with respirometry experiments.

Using the maze could assist in demonstrating the relationships between all the different life-history traits and environmental pressures that effect animals in their natural environment. As current climate continues to impose severe threats to many species, the ability to understand such relations and pressures is crucial in developing both survival estimates and management plans. While still requiring improvements, the behavioral maze shows potential as a way to encourage animal activity and simulate field condition.


APPENDICES
From: Paula Portalatin  paula.portalatin@maine.edu
Subject: IACUC protocol A2018-04-03
Date: May 8, 2018 at 08:47
To: Danielle Levesque  danielle.i.levesque@maine.edu

Protocol #: A2018-04-03
Protocol Title: Modeling the thermoregulation of Maine small mammals
PI: Danielle Levesque
Species/approved:
White Footed Mouse/30
Deer Mouse/30
Red-backed Voles/30
Approval Period: 5/7/2018-5/9/2021

Dear Danielle,

The above referenced protocol has been approved by the University of Maine IACUC. As a courtesy the IACUC Office will generally send out reminders for annual and de novo reviews however, it is ultimately the responsibility of the PI to ensure that the protocol is renewed on time.

All of the proposed methods, procedures, and conditions have been approved AS STATED IN THE PROTOCOL APPLICATION. The IACUC must approve any changes or deviations from the approved protocol prior to being initiated.

University of Maine Animal Welfare Assurance #: A3754.01
The University of Maine is registered as a research facility in accordance with the U.S. Department of Agriculture Animal Welfare Act and the Public Health Service Policy on the Humane Care and Use of Laboratory Animals. The University of Maine holds the Office of Laboratory Animal Welfare (OLAW) of the National Institutes of Health assurance for vertebrate animals used in research, teaching and outreach.

The Animal Welfare Assurance: (1) confirms the commitment that the University of Maine will comply with the PHS Policy, with the Guide for the Care and Use of Laboratory Animals; and with the Animal Welfare Regulation; (2) describes the institution’s program for animal care and use; and (3) designates the institutional official responsible for compliance.

I have attached the final approved version to this email.

Once you have the lab set up in Presque Isle and before any animals are captured please send us pictures and a description in place of an official inspection.

In addition, since your lab will be moving to Murray Hall the IACUC will need to inspect the space once it is set up and before any animals are housed there and you will need to fill out a Satellite Plan for Murray (I have attached the form to this email)

Thank you,

Sincerely,
Paula

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Paula Portalatin, M. Ed., CPIA
Research Compliance Officer II
Office of Research Compliance
University of Maine
Room 402 Corbett Hall
Orono, Maine 04469-5717
(207) 581-2657
hereby applies for a permit to collect, possess, and transport wild animals and/or birds or their nests and eggs in Maine in accordance with the laws of the State for scientific or educational purposes only during the calendar year 2018-2019.

1. I understand that in the case of migratory birds, a permit from the U.S. Fish and Wildlife Service is also required. (Migratory Bird Permit Office, U.S. Fish and Wildlife Service, 300 Westgate Center Drive, PO Box 779, Hadley, MA 01035-0779 (413) 253-8643). However, the Maine Scientific Collector's permit must first be issued before a federal permit will be approved.

2. If an applicant plans to utilize firearms for any purpose while conducting activities covered by a scientific collecting permit, the application must be accompanied by proof that the applicant possesses a current valid Maine hunting license. This requirement also applies to any sub-permittee listed on the application. Also, if applicable, proof must be furnished of a valid federal scientific collector's or special purpose permit.

Do you plan to use firearms? Yes_______ No_______ X_______

If yes, my current State of Maine hunting license number is . (IMPORTANT - If you do not already have a valid Maine hunting license, do not purchase one just for scientific collection purposed until your application has been approved.)

3. Are you over 16? YES.
4. If, while you are collecting, your residence will be different from the one stated above, please list the address of that residence.

N/A

5. Did you have a Maine permit last year for the same study? If so, do not answer question 6.
   Yes (Permit #: 2017-516)

6. Describe the studies in which you are engaged that make it necessary for you to collect the specimens desired. Attach your study proposal.

The primary objective of this research is to broaden our knowledge of thermoregulation in the only two species of flying squirrel present in North America by examining the effects of high ambient temperatures on metabolic rate and body temperature. Focusing on two related species can provide insight on physiological adaptations across habitats and life history traits, as well as determine energy usage in relation to climate. Being nocturnal and tree nesting, flying squirrels may be particularly susceptible to climate change because they will experience the highest temperatures during their daily resting phase. Flying squirrels may need to devote more energy to thermoregulating during daytime rest instead of conserving it for use during their nighttime active phase. Dramatic changes in energy allocation will undoubtedly affect all aspects of flying squirrel biology and ecology.

The specific objectives are as follows:
1) Determine metabolic rate and evaporative water loss of northern flying squirrel (Glaucomys sabrinus) and southern flying squirrel (Glaucomys volans) under different ambient temperatures.
2) Measure internal body temperature of northern and southern flying squirrels in the wild and compare to ambient temperature fluctuations.
3) Repeat objectives for American red squirrel (Tamiasciurus hudsonicus) as comparative outgroup (larger, diurnal, arboreal, high likelihood of trap success).

7. List, by species, the number of specimens you require:
   Northern Flying Squirrel (Glaucomys sabrinus) - 40
   Southern Flying Squirrel (Glaucomys volans) - 40
   American Red Squirrel (Tamiasciurus hudsonicus) - 40
8. Where in Maine do you plan to make collections?

Dwight B. Demeritt Forest located in Old Town and Orono, Maine immediately adjacent to the University campus and the forests surrounding U Maine Presque Isle

9. When do you plan to make collections?

April 15, 2018 – April 30, 2019

10. What disposition will be made of the specimens you collect?

Collected specimens that do not undergo surgery (30/40) will be held no longer than 24 hours and will be released at their site of capture after completion of the respirometry experiments (see attached proposal). Collected specimens that undergo survival surgery (data logger implantation, n=10 per species) will be subject to the same, previously listed protocols. Specimens collected in order to retrieve the data-loggers will be euthanized via isoflurane overdose. Their pathology and parasites will be studied for an undergraduate capstone research project (student to be assigned), tissue samples and the skulls will be kept as voucher specimens in the Levesque Lab.

11. Please specify bird banding or endangered species requests.

N/A

12. List individuals authorized by you to assist in, or carry out, the work described in this Application.

(Name)                     (Address)
Danielle Levesque (PI)        347 Hitchner Hall, U Maine
Vanessa Hensley (Graduate student, co-PI)       (Same as above)
Jake Gutkes (Graduate student)       (Same as above)
undergraduate field assistant (will be hired April 2018)

I certify that the answers to the preceding questions are correct to the best of my knowledge and belief.

Jan 30, 2017 (Date) ____________________ (Signature)

RETURN TO: Maine Department of Inland Fisheries and Wildlife
Wildlife Resource Assessment Section, 650 State Street
Bangor, ME 04401-5654
Tal Kleinhouse-Goldman Gedalyahou is a sweet summer child, born in California to a Moroccan-Caucasian mother and a Native Chilean father. Due to a Moroccan tradition forbidding buying baby items before the child is born, Tal spent the first few weeks of her life in a dog bed, sleeping with three Boxer dogs. Full with ideology and hope that the child will stop naming animals “Matilda”, the family moved to Israel where Tal grew up as a desert creature. After graduating high-school as the top camel-rider of her class, Tal joined the Israeli Defense Force where she did secret things that should not be spoken of in case the enemy is listening. Tal left the military as a super cool Sergeant and decided to join a one-woman expedition to confirm the rumors about Maine as a state with a thing called ‘snow’, in which everyone is named “Bud”. In Maine, Tal went as an undercover college student majoring in Wildlife Ecology with a concentration in Wildlife Science and Management. Thanks to her love to long titles (such as her last name) Tal decided to add a minor in International Relationship with a concentration in International Security.

Trying to hide her mission, Tal participated during her college years on various activities her mother promised her are cool, such as being a Lead Community Assistant for three years; being part of the Xi Sigma Pi Honors Society; and being the first person who have their own assigned booth at the Bear’s Den Pub. Upon graduation, Tal is hoping to focus on being a better hippy by going to save some animals in places other people don’t want to go to, or any place where her accent sounds cool so she can pretend to be the real Wonder Woman. Her biggest wish is to be able to say the word “Wicked” without getting weird looks before leaving Maine.