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**THE EFFECTS OF RISING AMBIENT TEMPERATURES ON  
THERMOREGULATION AND RANGE SHIFTS OF  
NORTHERN FLYING SQUIRRELS**

By Elise Gudde

B.S. University of Maine at Presque Isle, 2019

A Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

(in Ecology and Environmental Sciences)

The Graduate School

The University of Maine

August 2022

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Biology

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An Abstract of the Thesis Presented  
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August 2022

Climate change may potentially alter a species' range distribution and thus the relationship between environmental temperatures and animal performance as a response to climate warming has become an important area of research. Two species of flying squirrel in North America, the southern flying squirrel (*Glaucomys volans*) and the northern flying squirrel (*Glaucomys sabrinus*) have undergone northward range shifts, with climate warming being the suspected driver. Because they are nocturnal and tree nesting, flying squirrels may be particularly susceptible to warmer temperatures, as they will experience the highest daily ambient temperatures during their resting phase. I used *G. sabrinus* as a study species to determine the effect of high temperatures on thermoregulation and energy use to test if thermal limits will contribute to range shifts. I measured metabolic rate and evaporative water loss under different ambient temperatures using flow-through respirometry and estimated the responses to high ambient temperatures in the field by measuring body temperature. I used climate data from Arrowsic, Bangor, and Presque Isle, Maine over recent decades to determine if temperature increases would correspond to the northward movement of flying squirrels in Maine. *G. sabrinus* did not show visible or physiological signs of heat stress during respirometry

experiments, and an upper critical temperature was not reached at temperatures tested. Consistent daily rhythms in body temperature were observed in free-ranging flying squirrels, as predicted, and increased as ambient temperatures decreased. Additionally, high ambient temperatures were not likely to be a direct factor in northern flying squirrel range shifts, but that climate warming caused *G. volans* to move north and outcompete *G. sabrinus*.

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## CHAPTER 1

### GENERAL INTRODUCTION AND THESIS OVERVIEW

#### 1.1 General Introduction

Climate change has the potential to change ecological systems, and changes to phenology, migration patterns, ranges, and life history traits have been observed in many species over the past 20-140 years (Parmesan, 2006; Parmesan and Yohe, 2003). Climate warming is likely to cause loss of boreal conifer forests, reduce the extent of hardwood deciduous forests, and result in increases of mixed oak-hickory forests in New England (Tang and Beckage, 2010). Climate warming will increase the rate of insect development and expand ranges of pests and diseases (Iverson et al., 2008). Maine's present-day climate can be quantified by looking at year-to-year variations of monthly average temperature and precipitation in each climate zone, and this information can be used to assess long-term climatic changes (Jacobson et al., 2009). Statewide, the warmest month is July, but viewed seasonally, monthly average high temperatures vary from north to south (Jacobson et al., 2009). The Southern Interior climate zone is warmer than both the Northern and Coastal divisions. Maine is expected to have higher average temperatures due to the lengthening of summer and the increased incidence of high heat days (Fernandez et al., 2020), and these rising temperatures may bring changes to ecological communities in the state, especially boreal habitats (Tang and Beckage, 2010). Because climate warming has the potential to impact ecological systems, including species' range shifts, the relationship between environmental temperatures, energetics, and animal performance as a response to climate warming has become an important area of research (Huey et al., 2012). One approach for predicting the effects of climate warming on animal species is a bioclimatic envelope model. Bioclimatic envelope models use climate and species' occurrences to identify

conditions that are needed to maintain sufficient populations (Araujo and Peterson, 2012). Bioclimatic envelope models are simplistic as they do not consider biotic factors, and only use abiotic factors as predictor variables, (Araujo and Peterson, 2012; Kearney et al., 2009). Bioclimatic envelope models also do not consider physiological factors even though physiology can provide mechanistic explanations for species' responses to climate warming (Huey et al., 2012; McCain and King, 2014; Mitchell et al., 2018). Researching the physiological ramifications of high temperatures on animal species can improve our use of predictive mechanistic models, as these parameters can provide explanations for ecological changes (Helmuth et al., 2005). Although data necessary for physiologically informed models are available for many ectotherms, little research has focused on the physiological effects of high temperatures on endotherms. Endotherms have a more complex relationship between their environmental temperature and body temperature (Levesque and Marshall, 2021; Scholander, 1955), and therefore research into their physiology is important to improve our understanding of endothermy and to predict their responses to a changing climate (Levesque and Marshall, 2021).

## **1.2 Study System- Species Description**

Two species of flying squirrel occur in eastern North America, the northern flying squirrel (*Glaucomys sabrinus*) and the southern flying squirrel (*Glaucomys volans*). Flying squirrels differ from diurnal, ground-dwelling animals, as they rest in trees during the daytime. They also are able to glide from tree to tree, and their glide differs from powered flight used by birds or bats, allowing flying squirrels to cover large distances quickly and efficiently (Vernes, 2001). Flying squirrels do not hibernate, although they can use shallow torpor (Olson et al., 2017). Flying squirrels use food caches, well-insulated nests, and social thermoregulation to survive cold temperatures (Olson, 2016; Muul, 1968). Although at first glance the two species of

flying squirrel in eastern North America are similar in appearance, they differ in size, habitat type, diet, nesting habits, and range.

The southern flying squirrel is active year-round and is widely distributed in northern North America, ranging from the central Appalachians to the Sierra Nevada and north into Alaska (Smith, 2007). The southern species occupies hardwood forests and primarily feeds on hard mast (Weigl, 1978). Mating takes place in the spring and summer and an average litter size is 3-4 young (Dolan and Carter, 1977). The northern species is found in eastern North America, from Minnesota to Nova Scotia (Weigl, 1978) and is identifiable by its size, as it is larger than the southern flying squirrel (average of 103.7g vs 70.1g) (Olson et al., 2017). Northern flying squirrels' diet mainly consists of hypogeous fungi (Blois, 2000) which are usually patchily distributed throughout forests (Vernes, 2001). Tree cavities are used as shelter and breeding nests for many squirrel species; as in cold environments, cavities provide protection from precipitation, wind, cold temperatures, and predators (Trudeau et al., 2011). The northern flying squirrel was thought to be a coniferous forest specialist based on their diet of hypogeous fungi and their reliance on tree cavities (Blois, 2000). Recently, however, the northern flying squirrel has been described as a habitat generalist, abundant in mixed or deciduous forests and uses a variety of nest types including tree cavities and external nests built on branches in tree canopies such as witches' brooms (Trudeau et al., 2011; Smith, 2007). When tree cavities are used, heartwood decay and tree size provide important habitat for northern flying squirrels (Trudeau et al., 2011; Holloway and Malcolm, 2007). Breeding season for northern flying squirrels begins with male testes developing in winter and spring, followed by female pregnancy and lactation in late spring and summer, and it has also been found that northern flying squirrels may have an

additional fall breeding season (Vernes, 2004). Females produce a litter of 2-4 young (Wells-Gosling and Heaney, 1984; Smith, 2007), and young are weaned at 2 months of age.

The flying squirrel (*Glaucomys sp.*) is a good model species to study the relationship between physiology and environment as it lives in various habitat types over a large geographic range and a large range of climate conditions (Muul, 1968). Additionally, in the Northeast of North America, flying squirrels have recently undergone northward range shifts, with climate warming being the suspected driver (Wood et al., 2016), making them a useful species to test the efficacy of physiologically informed models in predicting species distributions and responses to climate warming. There is no single consensus held by researchers on the explanation for flying squirrel range shifts, but several hypotheses have been proposed, many with climate warming as a driving factor (Weigl, 1978; Bowman et al., 2005; Smith, 2007; Wood et al., 2016). Warm winter temperatures are currently thought to be the cause of recent ranges shifts of flying squirrels in eastern North America (Garroway et al., 2010; Wood et al., 2016). This hypothesis explains northward movement of southern flying squirrels (*Glaucomys volans*) but does not take into consideration both abiotic and biotic factors that may explain the shift of northern flying squirrels (*Glaucomys sabrinus*).

In North America, the southern flying squirrel's northern range boundary is quickly moving northward (Bowman et al., 2005; Garroway et al., 2011) and the range sympatry of the two species is expanding in some areas, while in others there is a complete species turnover (Olson, 2016; Wood et al., 2016). The first report of hybridization between North American flying squirrel species was recently made through molecular analyses (Garroway et al., 2010). The two species of flying squirrel are rarely syntopic as the southern species are competitively dominant and outcompetes the northern flying squirrel for nest cavities (Wood et al., 2016;

Weigl, 1978; Weigl, 2007). Southern flying squirrels carry an intestinal parasite, *Strongyloids robustus* (Garroway et al., 2010; Krichbaum et al., 2010), and where the two flying squirrel species co-occur, transmission of the parasite happens by skin contact with infected feces and soil (Wetzel and Weigl, 1994; Weigl, 2007). Transmission happens primarily during summer months at nest sites, as *S. robustus* has a poor tolerance for cold temperatures (Wetzel and Weigl, 1994; Weigl, 2007). *G. volans*, a common host for *S. robustus*, shows no sign of disease when infected by the parasite, while *G. sabrinus* may be killed if infected (Weigl et al., 1999; Krichbaum et al., 2010). Some populations of *G. sabrinus* in the U.S are listed as endangered and declines in population are due to several factors including climate change, habitat loss, and infection with *S. robustus* (O'Brien et al., 2022). However, it remains unclear if parasite-mediated competition in flying squirrels occurs at higher latitudes, in areas where southern flying squirrels have expanded their range in response to climate warming.

As they are nocturnal and tree nesting, flying squirrels may be particularly susceptible to climate change (Lovegrove et al., 2014), as they will experience the highest daily ambient temperatures during their day-time resting phase. Energy is devoted to cooling when temperatures are high, which can be costly. While other studies have focused on the impacts of warming winter climates on range shifts, I examined the rising summer temperatures as a driver of observed range shifts and focused on the northern flying squirrel (*G. sabrinus*) to determine the effect of high temperatures on thermoregulation and energy use. It is not yet known how northern flying squirrels will cope with increasing temperatures, or how much of a role climate warming will play in their movement and distributions. This information would provide insight on small mammal responses to climate warming and may be helpful for planning effective

conservation strategies for species facing range shifts with increasing temperatures in North America.

### **1.3 Thesis Chapters**

The research component of this thesis is comprised of two chapters: Chapter 2, titled “Northern flying squirrel thermal physiology”, focuses on examining the relationship between energetics and the environment of *G. sabrinus* to better understand the effects of rising ambient temperatures on endotherms. Although the physiology and heat tolerance of the southern species of flying squirrel in Maine has previously been studied, little information exists on the northern species. I used *G. sabrinus* as a study species to determine the effect of high temperatures on thermoregulation and energy use to test if thermal limits will cause range shifts. The specific objectives of this chapter are 1) to determine metabolic rate and evaporative water loss of *G. sabrinus* under different ambient temperatures using flow-through respirometry and (2) to estimate the responses of *G. sabrinus* to high ambient temperatures in the field by measuring body temperature. I predicted that northern flying squirrels would be more sensitive to heat than the southern flying squirrels, and that daytime body temperature ( $T_b$ ) would follow ambient temperatures ( $T_a$ ) in free-ranging northern flying squirrels.

Chapter 3, titled “The response of northern flying squirrels to rising ambient temperatures”, focuses on the recently documented range shift of the two flying squirrel species in the Holt Research Forest. I looked at climate data from regions across Maine over recent decades to determine if difference of temperature over time influenced the movement of *G. volans* and *G. sabrinus*. I predicted that temperature increases from 1981-2021 corresponded to the northward movement of flying squirrels in Maine.

## CHAPTER 2

### NORTHERN FLYING SQUIRREL THERMAL PHYSIOLOGY

#### 2.1 Introduction

As climate warming presents major challenges to many different species and ecosystems, it is important to find ways to evaluate the vulnerability of organisms to climate warming so that we can develop appropriate management strategies (Weiskopf et al., 2020). Attempts to evaluate vulnerability to climate warming should be based on biological and physiological foundations (Huey et al., 2012, McKechnie and Wolf, 2019). Ectotherms rely on external sources of heat production and have a more consistent and predictable relationship between body temperature ( $T_b$ ) and ambient temperature ( $T_a$ ) (Buckley et al., 2012; Tattersall et al., 2012), while endotherms have a more complicated relationship between the two as they maintain relatively high  $T_b$  within a narrow range and metabolic heat production is regulated in response to environmental temperatures (Boyles et al., 2011; Lowell and Spiegelman, 2000). Although endotherms like birds and small mammals are usually thought to have well-developed abilities for metabolic and osmotic regulation, increasing temperatures are challenging these thermoregulatory abilities (McKechnie and Wolf, 2019; McKechnie and Wolf, 2010; Ratnayake et al., 2019). As climate warming progresses, endotherms may need to adapt to changes in their thermal environment (Tattersall et al., 2012; Boyles et al., 2011). In endotherms, internal body temperatures remain relatively constant and independent of ambient temperature (Bartholomew, 1972). Therefore, controlling  $T_b$  accounts for a large portion of an endotherm's energy when  $T_a$  deviates from the thermoneutral zone (TNZ), the range of ambient temperatures in which an endotherm can maintain its desired body temperature while keeping metabolism at a minimum (Figure 1; Boyles et al., 2011; Withers et al., 2016). Limits of the thermoneutral zone (termed the

upper and lower critical temperatures) are species specific and can vary. The Scholander-Irving curve, or thermal profiles, is a common way to illustrate the relationship metabolic rate and ambient temperature in endotherms (Scholander, 1955). Oxygen consumption ( $VO_2$ ), a proxy for metabolic rate, is measured over a series of environmental temperatures for resting animals using flow-through respirometry. At temperatures below the lower critical temperature of the thermoneutral zone, endotherms will use shivering and non-shivering thermogenesis to maintain body temperature ( $T_b$ ) when thermal conductance is at minimum (Withers, 1992). As temperatures rise above the upper critical temperature, endotherms will use means of evaporative cooling, such as panting and sweating to maintain a desired  $T_b$  (Withers, 1992). The Scholander-Irving model can be overly simplistic when it comes to climate predictions (Mitchell et al 2018) as it does not consider other variables such as activity levels, microclimate use, or reproductive status. However, the Scholander- Irving model provides a useful estimate of the cost of warming during rest, as it illustrates the relationship between metabolic rate and ambient temperatures in resting endotherms. To predict an animal's vulnerability to climate warming, it is important to improve our overall understanding of endothermic physiology by including measures such as metabolic rate and body temperature as well as activity levels, microclimate, and reproductive status (Levesque et al., 2016; Levesque and Marshall, 2021; Kearney and Porter, 2009).

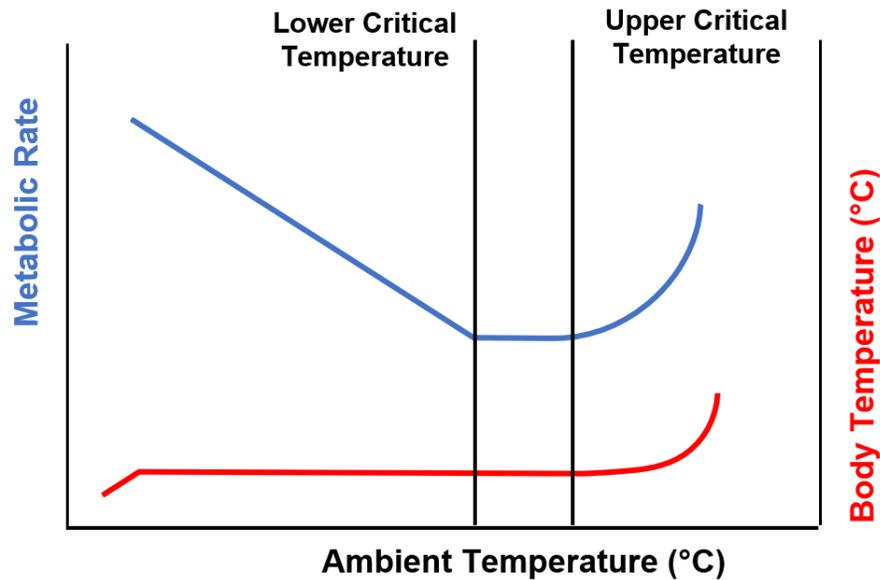


Figure 1: A depiction of the Scholander-Irving Curve which shows the relationship between ambient temperature, body temperature and resting metabolic rate of an endotherm.

Northern flying squirrels are nocturnal and arboreal, and as they rest at the hottest parts of the day, often buffering themselves in tree holes, they may be particularly susceptible to climate change (Lovegrove et al., 2014; Youngentob et al., 2021). Ambient temperature can influence physiological responses in animals, mainly due to effects on biochemical reaction rates, such as metabolic rate (Tattersall et al., 2012), which is an index of energy expenditure (Olson et al., 2017). The Scholander-Irving model can be used for resting flying squirrels to understand the relationship between metabolic rate and ambient temperatures. Increasing ambient temperatures may affect flying squirrel body temperature, which can serve as an important variable when looking at the locomotor activity for small mammals. The energetic costs of different types of locomotion vary and can increase maximum body temperature during the active phase differently (Bieber et al., 2017). For instance, the increase in metabolic rate during active phases is more pronounced in arboreal mammals compared to terrestrial mammals

(Bieber et al., 2017; Karasov, 1992). Night-active lifestyles can be helpful for thermoregulation, as animals do not need to regulate body temperature during the hottest hours of the day, saving both energy and water (Van der Vinne et al., 2015; Levesque et al., 2016). However, with climate warming, average night temperatures will continue to increase (NOAA, 2018). Climate warming will also cause daytime temperatures to increase, which is when northern flying squirrels are resting and may be more vulnerable to the heat.

There has been little research on the thermoregulation of northern flying squirrels, although previous studies have provided insights on that for southern flying squirrels. Southern flying squirrels in Maine show a clear pattern of body temperature matching their daily activity cycle (Hensley, 2019). There is no clear upper critical limit to the thermoneutral zone for metabolic rate in southern flying squirrels, the evaporative water loss upper limit has been quantified as 36.2°C. Although it has been documented that high temperatures are unlikely to cause an energetic strain on the southern species (Hensley, 2019), those high temperatures may impact the northern species, and may cause them to move further north to escape the heat. To determine if increasing temperatures play in the northward expansion of northern flying squirrels, I studied the effects of high ambient temperatures on the thermoregulation and energy of the species. I (1) measured metabolic rate and evaporative water loss to determine if these measurements would increase after surpassing the upper critical limit. I also (2) measured body temperatures of free-ranging northern flying squirrels during the active (nighttime) and resting (daytime) phases and hypothesized that body temperature would be higher during the active phases. Better understanding the upper critical limit and body temperature patterns of northern flying squirrels will help to provide insight on how rising temperatures will affect species' range

shifts. If northern flying squirrels cannot take the heat, or adapt to it, they may need to move northward.

## **2.2 Methods**

### **2.2.1 Animal Capture and Handling**

Northern flying squirrels were live captured from Aroostook State Park (46.604° N, -68.010° W) in Presque Isle, Maine from July-November 2019 and capture was attempted from August-September 2021. The park is adjacent to Echo Lake and the forest is a mixture of spruce (*Picea*), cedar (*Cedrus*), fir (*Abies*), beech (*Fagus*), and maple (*Acer*) trees. Individual Sherman Sheet Metal Traps (LFA 9 x 3 x 3.5 Solid, Aluminum Trap) and Tomahawk Live Traps (Tomahawk Live Trap, Hazelhurst, WI) were placed on the forest floor at large trees and near snags and were opened at dusk. Cotton balls were placed in each Sherman trap for nesting material, and a mixture of peanut butter, rolled oats, and bird seed was used for bait. Each tomahawk trap was covered in tarp to exclude rain and solar radiation from the trap. Traps were checked in the morning at sunrise and were either closed or collected to prevent small animal capture throughout the day.

Captured flying squirrels were transferred from the trap into a mesh handling bag and were then sexed and measured. Morphometric measurements included ear length, hindfoot length with toes, hindfoot length without toes, and forearm length using an electronic caliper. Flying squirrels were weighed to the nearest 0.1g using a digital scale (UNIWEIGH Digital Pocket Scale, Cochin, India). Squirrels were fitted with an ear tag (Mouse Ear Tags, National Band and Tag Company, Newport, KY) for identification upon recapture and a 3mm biopsy tissue punch was taken from the opposite ear for genotyping. In addition, subcutaneous temperature was monitored using an integrated transponder (PIT) tag (BioThermo13, Biomark,

Boise, ID). Reproductive status was also recorded. All capture and handling procedures were approved by the University of Maine's Institutional Animal Care and Use Committee Protocol #A2017-03-02 and Wildlife Scientific Collection Permit #2019-516 issued by the State of Maine Department of Inland Fisheries and Wildlife to D. Levesque.

### **2.2.2 Respirometry Experiments**

Adult male northern flying squirrels, previously captured for core body temperature data (see below), were recaptured, and used for resting metabolic rate experiments in the University of Maine at Presque Isle lab. Squirrels were placed in a plastic container prior to experiments and where they would then be placed in a respirometry chamber. All squirrels were fasted before experiments and were weighed before and after experiments. During experiments, squirrels were monitored for stress behaviors and activity. Squirrels were given apple slices after the completion of an experiment and were returned to the location they were originally captured.

### **2.2.3 Flow-Through Respirometry Set-up**

The metabolic chamber used was an airtight, plastic container (Lock and Lock Co., Seoul, South Korea) with a port for incurrent airstream, a port for the excurrent airstream, and one for a temperature sensor (Compression Push Fittings, BrassCraft, Masco, Michigan). A mesh wire grate was used to keep the squirrel elevated above the temperature sensor and ports. Mineral oil was placed at the bottom of the container to absorb urine or feces.

A thermocouple and meter (TC-2000 Type-T Thermocouple Meter, Sable Systems, North Las Vegas, NV) were used to monitor temperature inside the chamber. The chamber temperature was recorded using temperature data loggers (BlueMastero Tempo Disc, DSCTHD001). The squirrel's activity was monitored via a USB camera. The squirrel in the metabolic chamber was

placed into a temperature controlled-cooler (Pelt-5, Sable Systems, North Las Vegas, NV). The incurrent air flow was pulled from an air vent and through a column with desiccant ( $\geq$  CaSO<sub>4</sub>,  $<2\%$  CoCl<sub>2</sub>, W.A. Hammond Drierite Co. Ltd., Xenia, OH) by a 1.0 L/min pump. Once dried, the incurrent air was divided and pushed into two airstreams. Incurrent airflow was regulated by Gas Mass Flow Controllers (MC-Gas Mass Flow Controllers, Alicat Scientific, Tucson, AZ). Incurrent air then entered the cooler box and passed through a coil of copper tubing to equilibrate its temperature to the cooler's. Air went through the chamber and exited through the excurrent port and then entered a gas flow switcher (BL-2 Baseline System or RM-8 Flow Multiplexer, Sable Systems, North Las Vegas, NV). The outlet air from the BL-2 is pulled through gas analyzers, carbon dioxide and oxygen using a subsampler pump (SS-4 Subampler, Sable Systems, North Las Vegas, NV). A water and carbon dioxide analyzer were used for all respirometry experiments (LI-840A CO<sub>2</sub>/H<sub>2</sub>O Gas Analyzer, LI-COR, Lincoln, NE).

Flying squirrels were exposed to a maximum of four temperatures during an experiment. The first temperature the squirrels were exposed to during experiments was 20 °C and the squirrel remained at this temperature for approximately 2 hours. Once a squirrel was resting, chamber temperature was increased by no more than 5° increments between 20-35°C. Each temperature trial lasted for a minimum of 1 hour.

#### **2.2.4 Core Body Temperature**

Temperature sensitive data loggers weighing 2.5g and 2.6g (Gerhard Fluch, University of Veterinary Medicine, Vienna, Austria), coated in a layer of surgical wax, were used to collect core body temperature. Data logger implantation surgeries took place in July of 2019 at the University of Maine at Presque Isle's Biology Lab. Only adult male northern flying squirrels were used in body temperature experiments so that reproductive females would not be negatively

impacted. The data loggers were programmed to record the core body temperature every 2 minutes and were sterilized in Isopropyl alcohol for 4 hours prior to surgery. Squirrels were weighed, placed in a ventilated plastic Kritter Keeper and fed a slice of apple coated with about 0.01g of Capofran analgesic 2 hours before surgery. Sedation took place in an air-tight, plastic container with 5% vaporized Isoflurane (Isoflurane, USP, Piramal Healthcare, Inc., Bethlehem, PA). A 2-3% concentration of Isoflurane was delivered by mask during the surgery. The abdomen was shaved and disinfected with a betadine and isopropyl alcohol. A small incision was made in the skin and abdominal muscle tissue along the linea alba and the data logger was placed in the intraperitoneal cavity. The incision was closed with dissolvable sutures (Coated VICRYL (Polyglactin 910) Suture, Ethicon, Inc., Bridgewater, NJ). The squirrels were fitted with radio collars (~4g, PD-2C Transmitters, Holohil Systems Ltd., Ontario, Canada). After the surgery, the squirrels were weighed and fed apple slices and were kept in the lab overnight. They were released at their capture sight the next morning. Radio telemetry was used throughout the summer to locate nests of collared squirrels, and squirrels were recaptured in November 2019 to collect respirometry data and recover data loggers through surgery. Implanted squirrels were euthanized through Isoflurane overdose and cervical dislocation.

## **2.3 Statistical Analysis**

### **2.3.1 Respirometry Data Analysis**

Carbon dioxide, water vapor, and analyzer cell pressure measurements were recorded every second using LI-840A Software (LI-COR, Lincoln, NE). Using a custom code following Breit et al. (in prep), I isolated the most stable carbon dioxide production values by determining the lowest standard deviation in a 20-minute period. From that, the most stable 10-minute period was isolated, and the mean carbon dioxide, water vapor, and cell pressure was calculated. This

10-minute period was matched with flow rate, chamber temperature, and subcutaneous temperature.

All measurements of carbon dioxide production were transformed into  $VCO_2$  using equation 10.4 and oxygen consumption was calculated as  $VO_2$  (Lighton, 2018). The respiratory quotient was set at 0.7 as determined from similar measurements of the southern species (Hensley, 2019). Evaporative water loss (EWL) and evaporative heat loss (EHL) were calculated using equations from Withers (2001; 1992). Metabolic heat production (MHP) was transformed into energy equivalent of Watts using an oxycaloric equivalence equation 9.13 ( $5.164 \cdot RQ + 16 \text{ J} \cdot \text{mlO}_2^{-1}$ ) from Lighton (2018). EWL was converted to evaporative heat loss (W) using Withers (1992) and used in the calculation of the ratio of EHL to MHP.

Resting metabolic rates and ambient temperatures were plotted using R Packages “ggplot2” and “cowplot” (Wickham, 2016; Wilke, 2018). Evaporative water loss, subcutaneous temperature, and the ratio of evaporative heat loss to metabolic heat production were all plotted in relation to ambient temperature.

### **2.3.2 Core Body Temperature Data Analysis**

Free-ranging body temperature ( $T_b$ ) was recorded every 2 minutes from July-November 2019. Data from the first week after surgical implantation were excluded from the analyses. Analyses were performed in R using code developed by Hensley (2019). Each body temperature was determined to be “daytime” or “nighttime” using sunrise and sunset times from the R package “maptools” (Bivand and Lewin-Koh, 2018). Average minimum and maximum  $T_b$  for daytime and nighttime periods were calculated with the “ddply” function in the R package “plyr” (Wickham, 2011). Additionally, body temperature data were divided into an active phase, resting phase, and transitional phase, and the mode and the 10<sup>th</sup>-90<sup>th</sup> quantiles of distribution were

calculated using code from Levesque et al. (2021). The Heterothermy Index (HI) (Boyles et al., 2011a/b) and the non-stationary waveform analysis were both used to quantify the variability in the body temperature and look at patterns of heterothermy and the period and amplitude of the Additive Quantile Regression Analysis.

## **2.4 Results**

Between June and September of 2019, 17 northern flying squirrels were captured at Aroostook State Park (Appendix 1). Only adult male squirrels were used in experiments to avoid negatively impacting reproductive females, and although several males were caught, experiments were limited to when trained personnel were available. Two were implanted with data loggers in July 2019 once the surgical equipment became available, and both were recaptured in November 2019. A short trapping season, due to a delay of permits during COVID-19, in the summer of 2020 took place at Hirundo Wildlife Refuge, Old Town, ME, but no squirrels were captured. Trapping took place at Aroostook State Park in August 2021, but only one lactating female flying squirrel was captured and released.

### **2.4.1 Resting Metabolic Rate**

The two squirrels that were implanted with data loggers for core body temperature analyses were used in respirometry experiments upon recapture in 2019. Oxygen consumption decreased as ambient temperature increased from 20-36°C (Figure 2a), but the sample size was insufficient to be able to identify inflection points in either metabolic rate or evaporative water loss, which would be indicative of the limits of the thermoneutral zone. The ratio of evaporative heat loss to metabolic heat production (EHL/MHP) (Figure 2d) and the dry thermal conductance increased with increasing ambient temperatures (Figure 2b), and a corresponding increase in

subcutaneous skin temperature ( $T_{\text{sub}}$ ) (Figure 2e). The values of body temperature were an average of  $1.3^{\circ}\text{C}$  higher than  $T_{\text{sub}}$  temperatures.

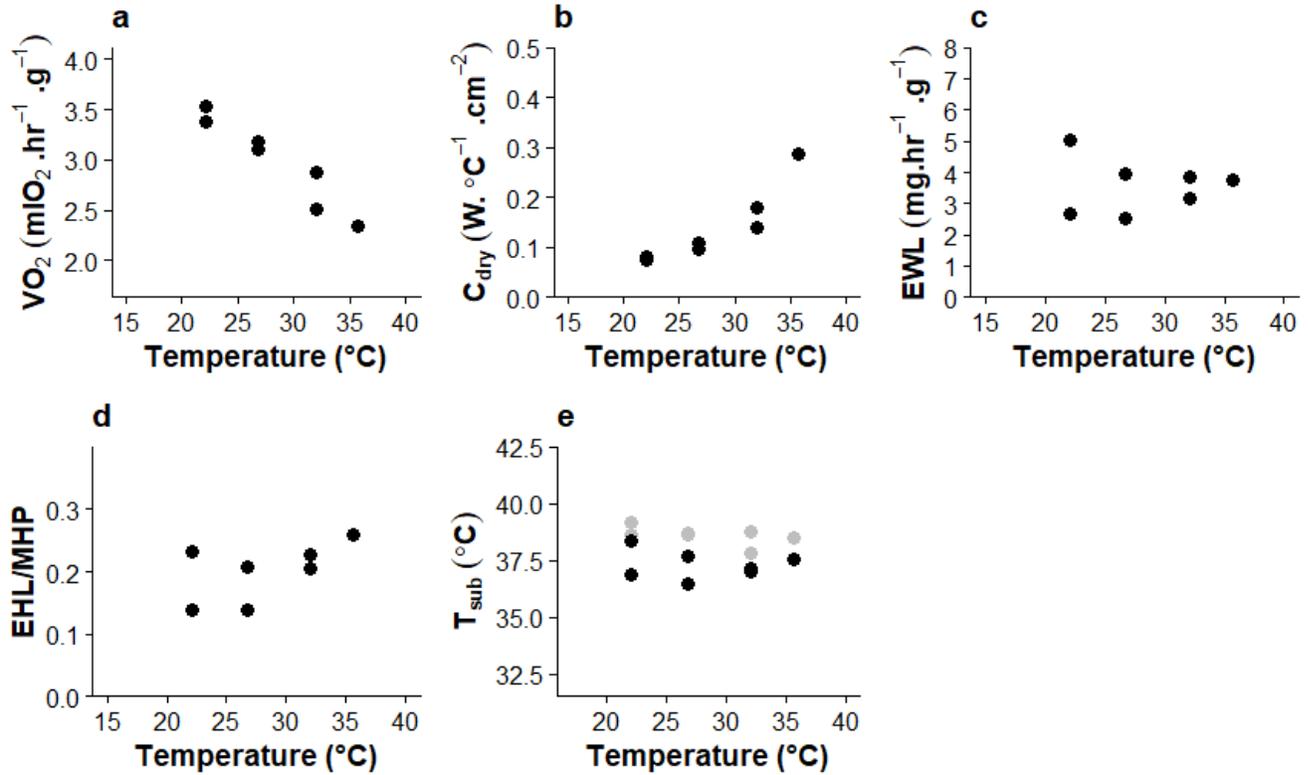


Figure 2: Respirometry measures of two *G. sabrinus* exposed to increasing ambient temperature including a) Oxygen consumption rate, b) Dry thermal conductance, c) Evaporative water loss d) Ratio of evaporative heat loss (EHL) to metabolic heat production (MHP), e) Subcutaneous temperature. Grey circles are core body temperature ( $T_b$ ) and black circles are subcutaneous skin temperature ( $T_{\text{sub}}$ ).

### 2.4.2 Core Body Temperature

Core body temperatures retrieved from the free ranging squirrels show a clear difference between the active and resting phases (Table 1 and 2). In total the loggers recorded 104 days. Body temperature was clearly distinct between daytime and nighttime values (Figure 3). The mean daytime  $T_b$  (resting phase) was  $38.3^{\circ}\text{C}$ , and the mean nighttime  $T_b$  (active phase) was

39.4°C. For *G. sabrinus*, the average daytime body temperature varied more than the average nighttime body temperature over months from August-November. The mean daytime mode  $T_b$  was 38.3°C and the mean nighttime mode  $T_b$  was 39.7°C (Figure 4 and 5). The mean amplitude of wavelets identified using the additive quantile regression wavelet analysis was 2.4°C. This is an indication of the variation between the high active phase body temperature and lower resting phase body temperature.

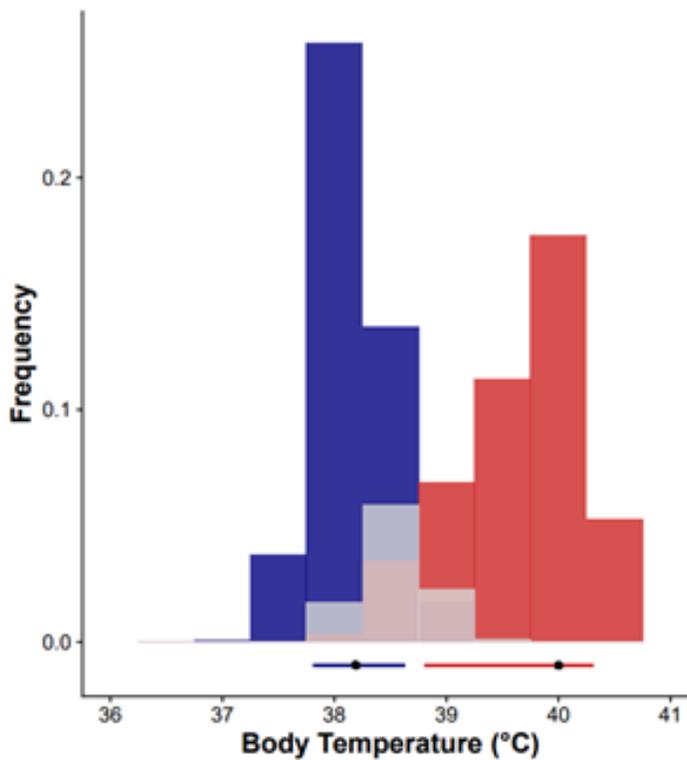


Figure 3: Histogram of core body temperature for *G. sabrinus*. Generated by dividing the body temperature from both northern flying squirrels into active (red), resting (blue), and transitional (gray) following code from Levesque et al., (2021). Black dots indicate the mode, and the lines indicate the 10<sup>th</sup>-90<sup>th</sup> quantiles of distribution.

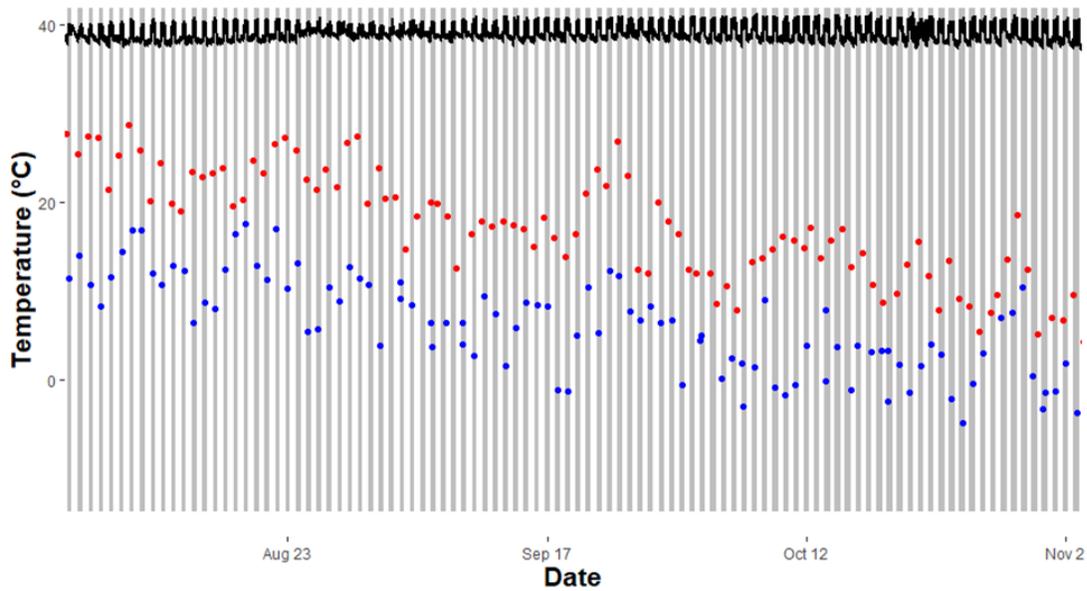


Figure 4: Free-ranging body temperature for a northern flying squirrel (*Glaucomys sabrinus*) from August-November 2019. Gray bars indicate the night phase as the species is nocturnal. Points indicate the 24-hour maximum (red) and minimum (blue) ambient temperatures collected from Caribou, Maine weather station data.

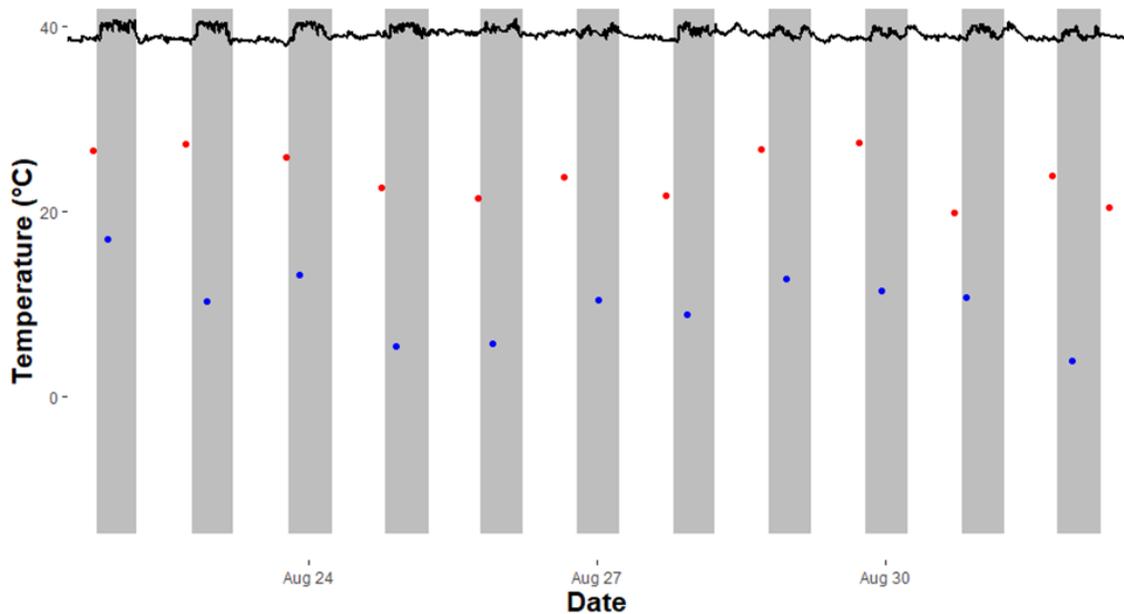


Figure 5: Free-ranging body temperature for a northern flying squirrel (*Glaucomys sabrinus*) for August 2019. Gray bars indicate the night phase as the species is nocturnal. Points indicate the 24-hour maximum (red) and minimum (blue) ambient temperatures collected from Caribou, Maine weather station data.

Table 1: Body temperature parameters for northern flying squirrel ID#81. Means  $\pm$  standard deviations of various parameters of  $T_b$  of free-ranging northern flying squirrel ID#81.

<b>ID#81</b>				
	Aug. 2019	Sep. 2019	Oct. 2019	Nov. 2019
<b>Mean <math>T_b</math> (<math>^{\circ}</math>C)</b>				
Day mean	$38.5 \pm 0.2$	$38.6 \pm 0.2$	$38.0 \pm 0.3$	$38.0 \pm 0.3$
range	37.9 - 39.6	38.3 - 39.1	37.6 - 38.4	37.6 - 38.4
Night mean	$39.5 \pm 0.6$	$39.6 \pm 0.6$	$39.3 \pm 0.8$	$39.3 \pm 0.8$
range	39.1 - 39.9	38.5 - 40.2	38.9 - 39.6	38.9 - 39.6
<b>S.D. <math>T_b</math> (<math>^{\circ}</math>C)</b>				
Day mean	$0.5 \pm 0.1$	$0.2 \pm 0.1$	$0.2 \pm 0.1$	$0.2 \pm 0.1$
range	0.1 - 0.5	0.1 - 0.4	0.1 - 0.9	0.2 - 0.4
Night mean	$0.2 \pm 0.1$	$0.3 \pm 0.1$	$0.3 \pm 0.1$	$0.2 \pm 0.2$
range	0.4 - 0.8	0.1 - 0.8	0.4 - 1	0.4 - 1.0
<b>Mode <math>T_b</math> (<math>^{\circ}</math>C)</b>				
Day mean	$38.4 \pm 0.4$	$38.8 \pm 0.2$	$38.2 \pm 0.2$	$37.9 \pm 0.3$
range	37.9 - 39.3	38.3 - 39.3	37.7 - 38.6	37.5 - 38.3
Night mean	$39.8 \pm 0.4$	$39.7 \pm 0.7$	$40.2 \pm 0.6$	$39.8 \pm 0.7$
range	38.7 - 40.3	38.4 - 40.6	38.1 - 40.7	38.5 - 40.3
<b>Min <math>T_b</math> (<math>^{\circ}</math>C)</b>				
mean	$37.9 \pm 0.4$	$38.1 \pm 0.2$	$37.7 \pm 0.2$	$37.5 \pm 0.3$
range	37.4 - 38.7	37.7 - 38.6	37.3 - 38.2	37.2 - 38.1
<b>Max <math>T_b</math> (<math>^{\circ}</math>C)</b>				
Mean	$40.4 \pm 0.2$	$40.6 \pm 0.4$	$40.9 \pm 0.2$	$40.6 \pm 0.3$
Range	40.0 - 40.7	38.9 - 40.9	40.5 - 41.2	39.9 - 41.0
<b>Max <math>T_b</math>- Min <math>T_b</math> (<math>^{\circ}</math>C)</b>				
Mean	$2.5 \pm 0.4$	$2.4 \pm 0.4$	$3.1 \pm 0.3$	$3.0 \pm 0.5$
range	1.6 - 3.1	0.7 - 3.2	2.3 - 3.9	2.0 - 3.5
<b>Heterothermy Index</b>				
mean	$1.3 \pm 0.3$	$1.5 \pm 0.2$	$1.6 \pm 0.1$	$1.7 \pm 0.2$
range	0.6 - 1.7	1.2 - 1.7	1.4 - 1.9	1.4 - 2.0
<b>Additive Quantile Regression Wavelet Analysis</b>				
Period (days)	$0.7 \pm 0.4$	$0.7 \pm 0.4$	$0.9 \pm 0.3$	$0.7 \pm 0.4$
Amplitude ( $^{\circ}$ C)	$2.0 \pm 0.8$	$2.2 \pm 0.6$	$3.1 \pm 0.3$	$3.0 \pm 0.8$

Table 2: Body temperature parameters for northern flying squirrel ID#83. Means  $\pm$  standard deviations of various parameters of  $T_b$  of free-ranging northern flying squirrel ID#83.

<b>ID#83</b>				
	Aug. 2019	Sep. 2019	Oct. 2019	Nov. 2019
<b>Mean <math>T_b</math> (<math>^{\circ}</math>C)</b>				
Day mean	$38.2 \pm 0.2$	$38.3 \pm 0.2$	$38.3 \pm 0.2$	$38.0 \pm 0.3$
range	37.8 - 38.7	37.9 - 39.2	37.9 - 38.9	37.8 - 38.5
Night mean	$39.4 \pm 0.6$	$39.6 \pm 0.6$	$39.7 \pm 0.7$	$39.2 \pm 0.9$
range	39.0 - 39.7	39.0 - 40.0	38.3 - 40.0	38.9 - 39.5
<b>S.D. <math>T_b</math> (<math>^{\circ}</math>C)</b>				
Day mean	$0.2 \pm 0.1$	$0.3 \pm 0.1$	$0.3 \pm 0.1$	$0.2 \pm 0.1$
range	0.1 - 0.3	0.1 - 0.3	0.1 - 0.4	0.1 - 0.6
Night mean	$0.2 \pm 0.1$	$0.2 \pm 0.1$	$0.2 \pm 0.1$	$0.2 \pm 0.1$
range	0.4 - 0.8	0.2 - 0.7	0.5 - 0.9	0.8 - 0.9
<b>Mode <math>T_b</math> (<math>^{\circ}</math>C)</b>				
Day mean	$38.2 \pm 0.3$	$38.3 \pm 0.3$	$38.3 \pm 0.3$	$38.0 \pm 0.2$
range	37.7 - 38.6	37.9 - 39.1	37.9 - 39.1	37.8 - 38.3
Night mean	$39.6 \pm 0.5$	$39.9 \pm 0.4$	$39.9 \pm 0.5$	$39.2 \pm 0.8$
range	37.7 - 40.0	38.2 - 40.3	38.3 - 40.3	38.1 - 40.8
<b>Min <math>T_b</math> (<math>^{\circ}</math>C)</b>				
mean	$37.7 \pm 0.3$	$38.0 \pm 0.4$	$37.8 \pm 0.3$	$37.6 \pm 0.3$
range	37.0 - 38.1	37.6 - 39.4	37.3 - 38.3	37.1 - 38.3
<b>Max <math>T_b</math> (<math>^{\circ}</math>C)</b>				
Mean	$40.3 \pm 0.2$	$40.5 \pm 0.2$	$40.7 \pm 0.2$	$40.6 \pm 0.1$
Range	39.9 - 40.7	38.9 - 40.9	40.3 - 41.1	40.4 - 40.8
<b>Max <math>T_b</math>- Min <math>T_b</math> (<math>^{\circ}</math>C)</b>				
Mean	$2.6 \pm 0.4$	$2.5 \pm 0.4$	$2.9 \pm 0.3$	$3.0 \pm 0.3$
range	1.9 - 3.8	0.9 - 3.2	2.2 - 3.5	2.05- 3.5
<b>Heterothermy Index</b>				
mean	$1.2 \pm 0.2$	$1.2 \pm 0.2$	$1.3 \pm 0.2$	$1.7 \pm 0.2$
range	0.8 - 1.5	0.6 - 1.4	0.9 - 1.7	1.4 - 1.9
<b>Additive Quantile Regression Wavelet Analysis</b>				
Period (days)	$0.9 \pm 0.2$	$0.9 \pm 0.3$	$0.9 \pm 0.3$	$0.8 \pm 0.3$
Amplitude ( $^{\circ}$ C)	$2.7 \pm 0.5$	$2.5 \pm 0.5$	$2.9 \pm 0.4$	$2.8 \pm 0.7$

## 2.5 Discussion

The northern flying squirrel, *G. sabrinus*, a small arboreal and nocturnal mammal, did not show visible or physiological signs of heat stress under high ambient temperatures during respirometry experiments. Body temperatures recorded from free-ranging individuals between August-November matched those observed during respirometry experiments. Consistent daily rhythms in body temperature were observed in free-ranging flying squirrels, as predicted, and increased as ambient temperatures decreased. Contrary to my prediction an upper critical temperature would be found for metabolic rate and evaporative water loss, the upper critical limit was not reached at temperatures tested.

In my respirometry experiments, northern flying squirrels did not appear to be heat challenged as no increase in oxygen consumption was detected at the higher temperatures (Fig 1). One reason the northern species may not have been heat challenged could be they were not exposed to high enough temperatures during respirometry experiments. Another indication of heat stress is increases in evaporative water loss (Tattersall, 2012). To cope with high ambient temperatures, endotherms regulate a constant body temperature by increasing the rate of heat loss, and evaporative water loss is the only physiological mechanism they can use to dissipate heat (Tattersall, 2012). The evaporative water loss inflection point for *G. volans* was 36.2°C, while in the current experiments evaporative water loss was stable other than a slight increase at about 33.9°C (Fig 2) but there was an increase in the ratio of evaporative cooling to metabolic heat production at the highest temperature (36°C).

The thermoneutral zone for *G. volans* has previously been measured to be 30-36°C (Neumann, 1967), although recently, the lower critical temperature has been estimated to be

26.9°C (Olson et al., 2017) and the upper at >40°C (Hensley, 2019). Olson found that the lower critical temperature for the thermoneutral zone was 28.1°C for *G. sabrinus*, which was similar to what was found for *G. volans* (26.9°C) in the same study (Olson et al., 2017) but upper limits were not measured in that study. Similarly, the present study was unable to identify the upper critical temperature due to a low sample size and ambient temperature was not increased as high as it could have been. Northern flying squirrels are larger than the southern species and the basal metabolic rate would be expected to be higher, as metabolic rate depends on size (Clarke et al., 2010). In my respirometry analyses, metabolic rates of *G. sabrinus* (at 22°C, 3.5 mlO<sub>2</sub>.hr<sup>-1</sup>.g<sup>-1</sup>, at 36°C, 2.3 mlO<sub>2</sub>.hr<sup>-1</sup>.g<sup>-1</sup>) were slightly above the values found for *G. volans* (at 22°C, 2-3 ml O<sub>2</sub>.hr<sup>-1</sup>.g<sup>-1</sup>, at 36°C, 1-2 mlO<sub>2</sub>.hr<sup>-1</sup>.g<sup>-1</sup>) at corresponding ambient temperatures (Hensley, 2019), as they are larger. A study focused on *G. sabrinus* in Ontario, Canada in 2017 reported similar results for values of oxygen consumption at corresponding ambient temperatures of 20-36°C (Olson et al., 2017). Further research is needed to explore thermal limits of northern flying squirrels to fully determine the range of ambient temperatures they are able to tolerate. One clue might be to look at what body temperatures they experience in the wild, where temperatures are not controlled, and where their microclimates, such as insulated nests, will have different effects on body temperature.

Northern flying squirrels showed a clear pattern in body temperature variation between the active (daytime) and resting (nighttime) phases from August to November (Fig 4). As ambient temperatures decreased from mid-September to November for *G. sabrinus* (Fig 4), the amplitude of the squirrel's body temperature, as quantified by the heterothermy index and non-stationary waveform analysis, increased (Table 1 and 2). The average heterothermy index was 1.3 and the average amplitude of the wavelets was 2.4°C in August and September, while the

average heterothermy index was 1.6 and the average amplitude of the wavelets was 2.9°C in October and November, indicating that there was a higher heterothermic response when the average ambient temperatures were lower. It has been found for many nocturnal animals that the body temperature during the day-time resting phase decreases with decreasing ambient temperature (Lovegrove and Genin, 2008; Humphries and Careau, 2011). For *G. sabrinus*, the daytime body temperatures decreased in colder months and the nighttime temperatures varied little. In a study looking at the heterothermy of free-ranging eastern chipmunks, the heterothermy index increased in early winter and had a maximum in midwinter (Dammhahn, et al., 2017). In free-ranging chipmunks it was found that the increase of heterothermy index with colder temperatures was not just dependent on temperature, but possibly an endogenous circannual clock and food availability (Dammhahn et al., 2017) which may also be true for *G. sabrinus*. The Heterothermy Index provides an indicator of heterothermy, which can be used to compare body temperature variability between species. The average HI value for both northern flying squirrels is 1.4 (Table 1 and 2), which is similar to other squirrel species in the summer, *Spermophyllis parryii* (HI= 1.43), *Xerus inauris* (HI= 1.89) (Boyles et al., 2013), and *G. volans* (1.93) (Hensley, 2019).

The control of metabolic heat production is an essential process of body temperature rhythm (Refenetti, 2020). An increase in amplitude of body temperature rhythm when ambient temperatures are low may be due to how the circadian rhythm of body temperature is produced by modifying heat loss, which is favored in colder temperatures (Refinetti and Menaker, 1992). Another study proposed that lower ambient temperatures lead to lower body temperatures during the resting phase, as body temperature control is relaxed during sleep (Heller et al., 1983). Similar patterns were observed in free-ranging southern flying squirrels (Hensley, 2019) which

also showed a clear pattern in body temperature variation between the active and resting phases. My results that *G. sabrinus* had a higher body temperature at night and lower during the day are comparable to results of a study examining the relationship between daily rhythms of locomotor activity and body temperature in different mammalian species, including flying squirrels, where body temperature and activity level were higher during the active (nighttime) phase (Refenetti, 1999).

In this chapter, I demonstrated that northern flying squirrels may be able to tolerate high ambient temperatures. They had a clear body temperature pattern that was higher during active nighttime phases than during resting daytime phases, and the amplitude of body temperature rhythm increased as ambient temperature decreased. Neither the laboratory experiments (including temperatures as high as 36°C) nor the free-ranging data (including temperatures as high as 28.6°C) resulted in any significant signs of heat stress. Although I could not draw concrete conclusions from these results due to the low sample size, the free-ranging body temperature, HI, and amplitude from my study can be used to compare to other endotherms, and can provide preliminary results for future studies on endotherm physiology. This leads me to my next question, if northern flying squirrels can tolerate high ambient temperatures, will heat play a role in their range changes?

## CHAPTER 3

### THE RESPONSE OF NORTHERN FLYING SQUIRRELS TO RISING AMBIENT TEMPERATURES

#### 3.1 Introduction

##### 3.1.1 Climate Background

Since 1990, we have learned about the consequences of increasing greenhouse gases in Earth's atmosphere and 2019 was a notable year of global heat, with the most recent highest average global temperatures (WMO, 2019). On average worldwide, 18 of the 19 warmest years on record have occurred since 2001, with the five hottest years being most recent (NASA, 2019). 2021 culminated as the sixth warmest year on record with a temperature  $0.84^{\circ}\text{C}$  above the 20<sup>th</sup> century average, and overall, the global annual temperature has increased at an average rate of  $0.08^{\circ}\text{C}$  per decade since 1880 and over twice that rate since 1981 (NOAA, 2022). Effects of climate warming can be observed on a wide range of ecosystems and species across the world (Warren et al., 2011) in response to rising temperatures. Global climate is quickly changing, as in the last 90 years Earth's mean temperature rose  $0.6^{\circ}\text{C}$ , which is a rate of increase that has not been observed in 10,000 years (Parmesan and Matthews, 2006). The consensus of many climate scientists, including those in the Intergovernmental Panel on Climate Change (IPCC), is that "global rises in anthropogenically produced greenhouse gases and the rate of temperature change will accelerate over the coming century" (IPCC, 2001a). However, this global consensus has led to many new questions, such as the speed of emergence of new weather patterns and the effect of climate change on species and ecosystems.

In Maine, temperatures continue to increase statewide. Average annual temperature has increased  $3.2^{\circ}\text{C}$  in the last 124 years, and the rate of warming has increased since 1960 (Fernandez et al., 2020). The northeast is the fastest warming region in the U.S. and is projected

to warm 3°C when the rest of the world reaches 2°C (Fernandez et al., 2020). For the 21<sup>st</sup> century, models show a strong trend in warmer conditions with a higher amount of precipitation in all four seasons in Maine (Jacobson et al., 2009). Climate change will lead to significant changes in Maine’s plant and animal populations, and the state list of endangered and threatened species with likely grow (Jacobson et al., 2009).

The state of Maine contains three distinct climate zones: Northern, Southern Interior, and Coastal, and each are expected to react differently to climate change (Fernandez et al., 2020; Jacobson et al., 2009). Recent studies identified northern flying squirrels in the Northern zone, and southern flying squirrels in both the Southern Interior and Coastal zones. The two species of flying squirrels are currently undergoing range shifts in Maine where ranges of both species overlap. Maine is expected to have higher average temperatures due to the lengthening of summer and the increased incidence of high heat days (Fernandez et al., 2015), and as temperatures rise, southern flying squirrels may be more tolerant of the heat and northern flying squirrels less so, shifting the boundaries of each species’ range.

### **3.1.2 Shifting ranges in a changing climate**

As there is now undeniable evidence for contemporary climate change (IPCC, 2007), it is thought that most species will respond to this temperature increase through shifts in their geographic range (Garroway et al., 2011; Parmesan, 2006). For many species, they have sufficient resources to support populations beyond their originally occupied range, however, they are limited by other factors like climate (Parmesan et al., 1999). In these circumstances, climate warming can make new areas available by shifting climate systems, leading to species range shifts. A range shift can be defined as “the geographical expansion of a species into an area not previously occupied by that species” (Wingfield et al., 2015). However, this these range changes

may lead to increased sympatry between recently separated species (Garroway et al., 2011; Parmesan, 2006). For example, in Ontario, Canada, the northward expansion of *G. volans* into the range of *G. sabrinus* has resulted in the formation of a new hybrid zone (Garroway et al., 2010). Molecular analyses show that interbreeding has not taken place, and that hybridization was recent, corresponding to the increase in sympatry between the two species (Garroway et al., 2010).

In North America, the southern flying squirrel has expanded its range northward likely because of climate warming and the northern flying squirrel has also shifted north, likely from competition with the southern species (Wood et al., 2016). In a study conducted by Wood et al. (2016) at the Holt Research Forest (HRF), a pine-oak forest in Arrowsic on the south-central coast of Maine, *G. volans* and *G. sabrinus* species abundances, climate, food availability, and forest structure were tracked over a 30-year period. Researchers used weather data from the Brunswick, Maine station from 1983 to 2006, and from the Portland, Maine station from 2007-2013. Trapping occurred in early August from 1983-2013 and at the end of April from 1983-2002. From 1986 to 2004 there was a complete species turnover from *G. sabrinus* to *G. volans* at the HRF. In a study from 2017-2018 at the Dwight B. Demeritt Forest in Orono, Maine, only *G. volans* was found indicating further northward movement of that species (Hensley, 2019). Only *G. sabrinus* was found farther north in Presque Isle, Maine, where my research took place.

Species' ranges are fluid and can shift in response to both abiotic and biotic factors (Wood et al., 2016, Sexton et al., 2009), and recent anthropogenic climate change has resulted in northward range expansion in many taxonomic groups (Wood et al., 2016). A species' abundance near its range limit can often be determined by abiotic factors such as temperature, as well as interactions with biotic factors like competition (Wood et al., 2016, Sexton et al., 2009).

Although multiple studies have found northward movements of *G. volans* in response to climate warming (Wood et al., 2016; Bowman et al., 2005; Myers et al., 2009), climate-driven change was not as clear in the HRF study. For *G. sabrinus*, disease, food, and changes to forest structure may have shaped population dynamics, and competition most likely played a critical role (Wood et al., 2016) as *G. sabrinus* faced a species that could directly outcompete it (Wood et al., 2016; Weigl, 2007; Smith, 2007). The transmission of *S. robustus* occurs during summer months, and minimum summer temperature temperatures rose significantly over the course of the HRF study (Wood et al., 2016), increasing the opportunity for infection (Wetzel and Weigl, 1994). This pathogen outbreak of *S. robustus* in northern New England likely contributed to range expansion and hybridization. Pathogen outbreaks due to climate warming pose a major threat to biodiversity across the world (Harvell et al., 2002) and may have caused range changes of flying squirrels observed in Maine (Wood et al., 2016).

Species range shifts are often more complex than simple climate-driven changes; climate warming is only one factor that drives range shifts does not lesson its importance. Understanding how local conditions and large spatial scales can affect organisms can help to broaden our understanding of the effect that climate warming has on species distributions (Helmuth et al., 2005). I evaluated climate data from three regions in Maine over a 40-year period and attempted to determine if maximum and minimum environmental temperatures contributed to flying squirrel range shifts. My objective was to understand if northern flying squirrel extirpation from the HRF related to increased maximum environmental temperatures. Further I explored how maximum environmental temperatures have changed in the species' current and former ranges in Maine.

### 3.2 Methods

To assess if increases in environmental temperatures have influenced the movement of *G. volans* and *G. sabrinus* in Maine, I looked at climate data from weather stations near Arrowsic, Bangor, and Presque Isle, Maine. I used data collected from the PRISM climate group (<https://prism.oregonstate.edu/>) and the R package “prism” (Hart and Bell, 2015) to download and graph average yearly maximum and minimum temperatures for every day of the year from 1981-2021 for the three locations across Maine. Temperatures were plotted using “ggplot2” and an annual average trend line was added. I also compared maximum and minimum temperatures from summer months (June-August) and winter months (December-February).

### 3.3 Results

The Holt Research Forest (HRF, Arrowsic) regional temperatures were most similar to the Bangor region, with an average daily maximum temperature of 12.7°C for Arrowsic and 12.5°C for Bangor from 1981-2021 (Fig 6a). The average minimum temperature for Arrowsic was 2.7°C and Bangor 2.1°C from the same years (Fig 6b). The Presque Isle region’s average maximum was 10.0°C and minimum was -1.1°C. Both maximum and minimum temperatures trended upward in each region over the 40-year period (Fig 7). Summer (June-August) maximum daily average temperatures for Arrowsic over the 41-year period was 24.2°C, and from 1981-2004 (the years when *G. sabrinus* were captured) it was 24.1°C (Fig. 8). In the years prior to *G. volans* arrival from 1981 to 1986, the daily average minimum winter (December-February) temperature was -9.4°C, while the minimum daily average temperatures over the 40-year period was -9.0°C (Fig 8).

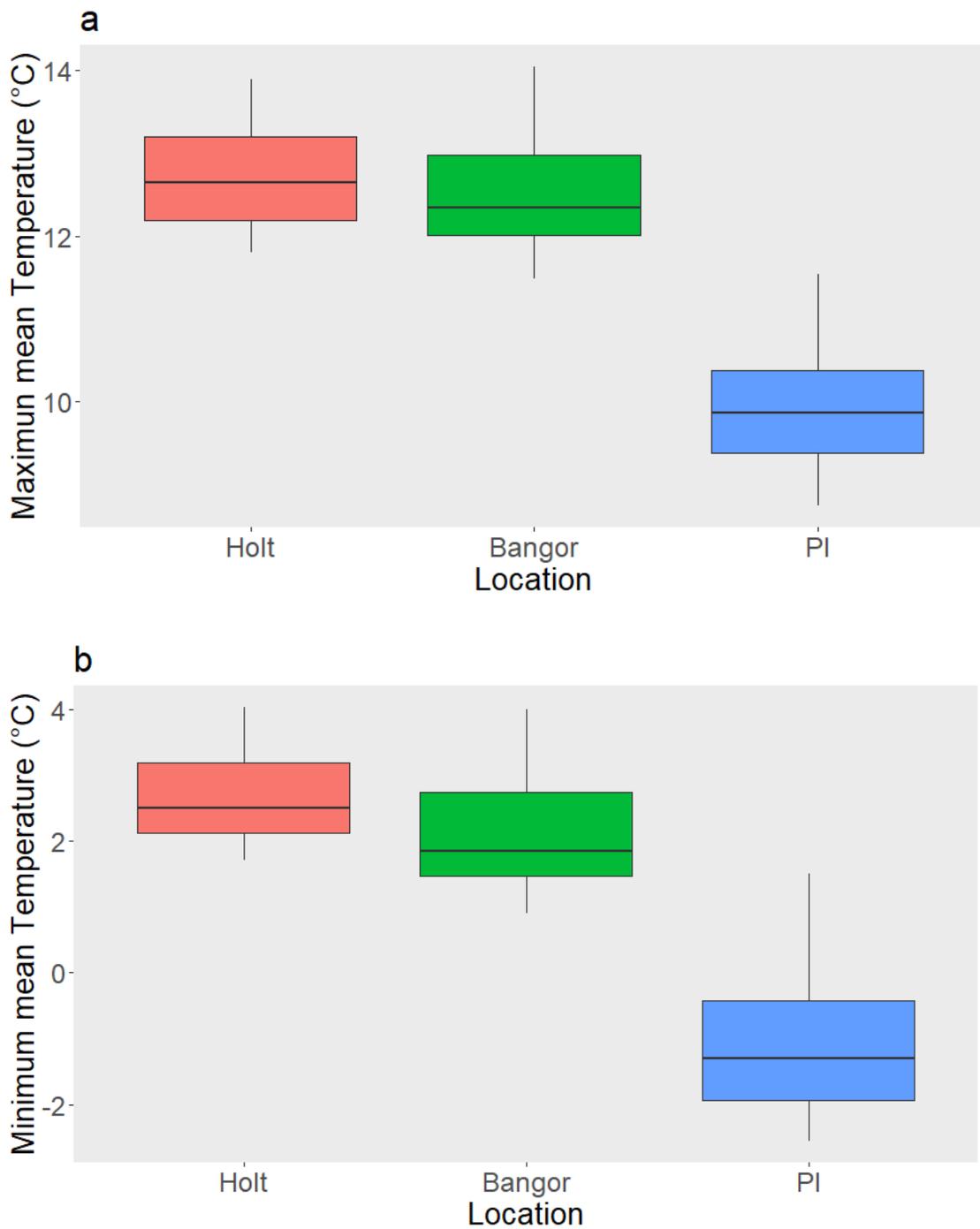


Figure 6: Boxplots of average maximum and minimum temperatures for every day of the year from 1981-2021 for Arrowsic (red), Bangor (green), and Presque Isle (blue) a) maximum mean temperatures; b) minimum mean temperatures. The middle line represents the median and the vertical lines represent the maximum and minimum temperatures.

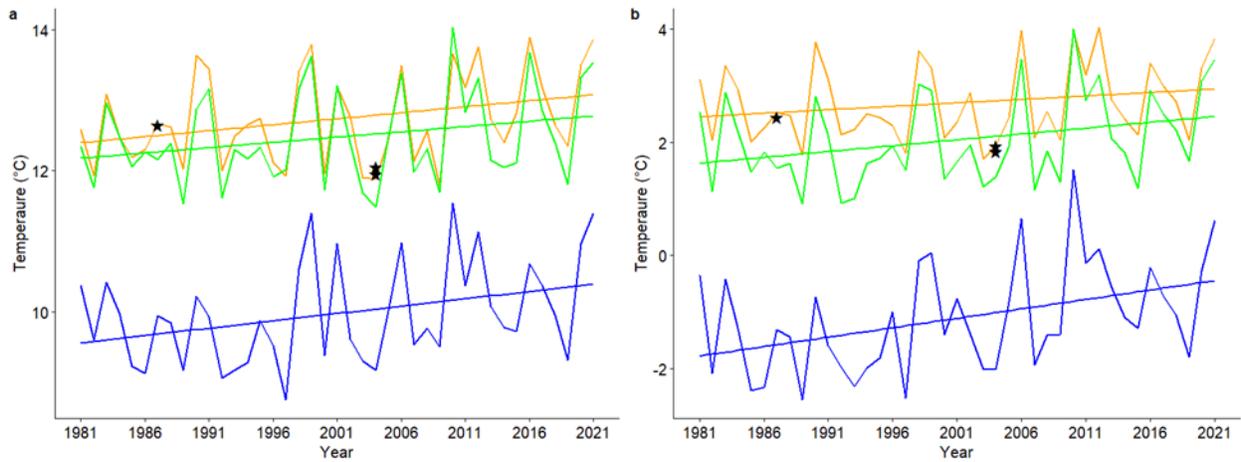


Figure 7: Average yearly maximum and minimum temperatures for every day of the year from 1981-2021 a) Annual average maximum; b) annual average minimum. Orange lines are Arrowsic, green lines are Bangor, and blue are Presque Isle. One star indicates when southern flying squirrels arrived in Arrowsic, and two stars indicate when northern flying squirrels disappeared from Arrowsic.

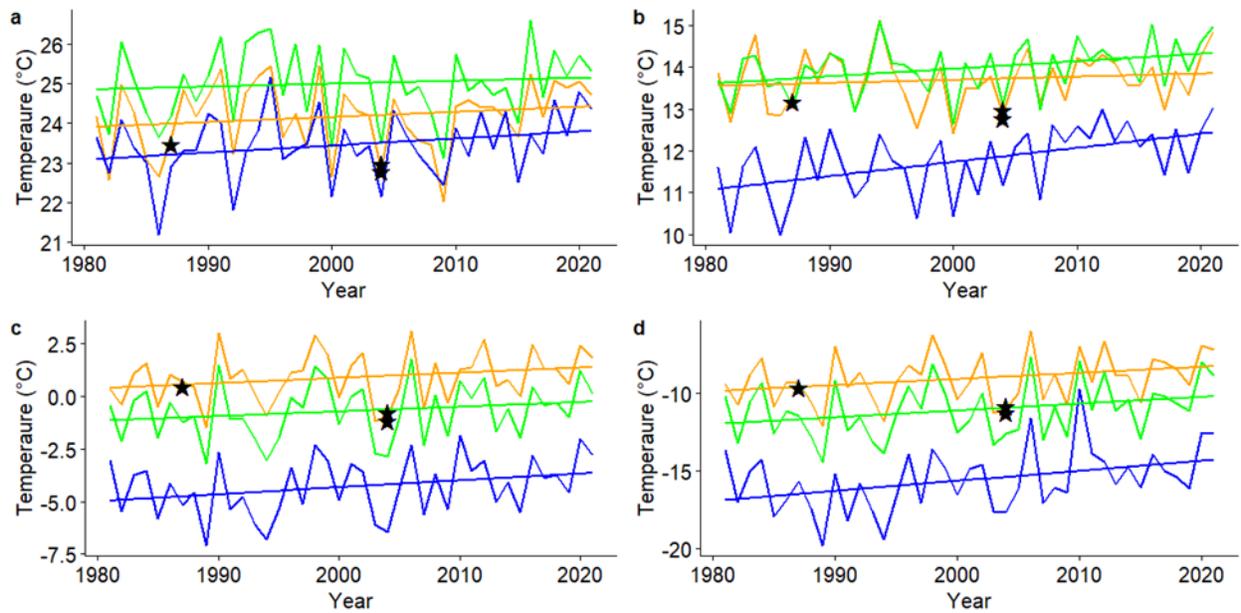


Figure 8: Summer (June-August) and winter (December-January) daily average temperatures from 1981-2021. Orange lines are Arrowsic, green lines are Bangor, and blue are Presque Isle. a) maximum summer temperatures; b) minimum summer temperatures; c) maximum winter temperatures; d) minimum winter temperatures. One star indicates when southern flying squirrels arrived in Arrowsic, and two stars indicate when northern flying squirrels disappeared from Arrowsic.

### 3.4 Discussion

For the past century, the rate of warming in Maine has been increasing, and temperatures have increased in all three of Maine's climate zones (Jacobson et al., 2009). I found that Arrowsic, Bangor, and Presque Isle had increasing maximum and minimum temperatures from 1981-2021. Bangor and Arrowsic regions had similar temperatures that were generally warmer than Presque Isle, where only northern flying squirrels occur. While Bangor and Arrowsic regions had similar maximum summer temperatures, Presque Isle's temperatures varied from the other two regions in both the summer and winter. If climate warming is the driving factor of flying squirrel range shifts, increasing temperatures in Maine could be indirectly contracting *G. sabrinus*' range northward.

Climate warming is increasingly affecting species and their ecosystems, causing species from many taxonomic groups to move toward higher latitudes due to physical limitations and species interactions (Wallingford et al., 2020; Parmesan and Yohe, 2003). Colonizing new habitats helps species persistence, however, as climate quickly changes, some range-shifting species may alter community structures and ecosystems (Wallingford et al., 2020). The southern flying squirrel is currently displacing northern flying squirrel as the southern species expands its range in response to climate warming (Wood et al., 2016). Based on my results, I could not conclude that high temperatures caused northern flying squirrels to disappear from the Holt Research Forest. However, between the time when *G. volans* arrived in 1987 and when *G. sabrinus* disappeared in 2004, there were high peaks ( $T_a > 13.6^\circ\text{C}$ ) in annual maximum temperatures (Fig 8) and  $T_a > 25^\circ\text{C}$  in the summer maximum temperatures. The disappearance of the northern species from the Holt Research Forest was likely not directly due to high temperatures, but that increasing temperatures are causing southern flying squirrels to expand their range and outcompete the

northern flying squirrels. In the years prior to the arrival of *G. volans*, the average minimum winter temperatures were lower than years when *G. volans* occupied the Holt Forest (Fig 8) indicating that the winter temperatures may be the driving factor of range shifts. When *G. volans* populations peaked in 2001-2002 and 2010-2011 (Wood et al., 2016), average maximum winter temperatures were 1.8°C, and 1.3°C, which was higher than the 40-year average of 0.9°C. Similarly, in the Holt Forest research study conducted by Wood et al. (2016), it was found that while there was no single cause for flying squirrel range shifts, climate warming was one factor, but maximum winter temperatures were the best indicator of *G. volans* abundance (2016).

Although there is overwhelming evidence that a species' physiological tolerance to climate warming influences the location of their ranges (Sexton et al., 2009), we should also consider factors other than climate (HilleRisLambers et al., 2013). Biotic processes such as predation, competition and the transmission of new parasites or pathogens, can have significant impacts on communities. Competition is a driving factor when it comes to shaping ecological communities. Southern flying squirrels carry an intestinal parasite that is fatal to northern flying squirrels (Garroway et al., 2010; Krichbaum et al., 2010). If biotic interactions affect populations at upper range limits, they may also influence the rate of climate-induced range shifts (HilleRisLambers et al., 2013).

Further research on flying squirrel microclimates will be important for mechanistic modeling and explanations of driving factors for observed range shifts. Ecological processes that are important to an animal's survival have energetic costs, and the need for an animal to meet these costs within the constraints of the environment has been overlooked in ecological models (Tomlinson et al, 2014). As climate warming has the potential to present many global challenges for animals and their environment, it is important for research to focus on more predictive,

process-based approaches to modelling species' responses to climate warming (Tomlinson et al, 2014). One of the most neglected factors in endotherm research is a mechanistic understanding of the energetic requirements of an animal. Mechanistic models can be used to incorporate the relationships between environmental conditions and organismal performance, and mechanistic approaches include models that translate environmental conditions into relevant metrics (Buckley et al., 2010). Most modelling approaches are correlative, as they link spatial data to species distribution records, but alternative strategies incorporate mechanistic links between functional traits and their environments into species distribution models (SDMs) (Kearney & Porter, 2009). Recent advances have been made in the developing of niche envelope models that provide powerful tools to make connections between physiological energetics, changing environmental conditions, and species distribution patterns (Tomlinson et al, 2014). To properly evaluate an organisms' vulnerability to climate warming, future endotherm research should include the use of mechanistic models to incorporate relationships, environmental conditions, and an organism's performance. Microclimatic variables are needed for a wide range of questions in environmental sciences. Microclimates are the physical conditions experienced by organisms, and they are relevant to the process of heat and mass exchange (Kearney and Porter, 2017). They include air temperature, wind, humidity, substrate temperature, and soil moisture, and represent the interaction between climate conditions and habitat (Kearney and Porter, 2017). Because weather stations measurements are meant to provide a regionally relevant measure, they do not account for these microclimate conditions. In ecology, microclimatic conditions are necessary for modelling the heat and water budgets of organism, and then climatic constraints on distribution and abundance can be inferred (Kearney and Porter, 2017). Climate and weather are critical to the distribution and abundance of organisms and although ecologists have access to

vast climate and weather data from across the globe, for many applications this is not enough, and microclimate conditions are necessary (Kearney and Porter, 2017).

Despite the importance of microclimates in ecological studies, there has been no accessible microclimate model available for use until recently; NicheMapR is an R package that allows for mechanistic modelling of heat and mass exchange between organisms and their environments (Kearney and Porter, 2017). The microclimate model includes programs to compute solar radiation, effects of shading, and slope. The model also allows for a global, monthly long-term average dataset with environmental inputs and to run the model with the global climate database. For further research on the response of flying squirrels to rising ambient temperatures, using the NicheMapR microclimate model will allow for a more mechanistic understanding of species range shifts.

## GENERAL CONCLUSIONS

My findings show that northern flying squirrels may be able to tolerate high ambient temperatures and although rising temperatures may not have directly impacted their disappearance from the Holt Research Forest, climate warming may have an indirect effect on their range expansion northward. As temperatures continue to rise, southern flying squirrels may move north as they will not be limited by cold winter temperatures, and competitively drive out northern flying squirrels.

Changes in ecosystems are increasingly apparent because of climate warming (Pecl et al., 2017; Weiskopf et al., 2020) and worldwide, species are experiencing range shifts as rising temperatures cause species range boundaries to move into high latitudes (McCarty, 2001). Northern flying squirrels may need to make behavioral changes as temperatures continue to rise with climate warming. However, although a warming climate has driven many species range shifts northward (Parmesan, 2006), it must be looked at as only one factor of many others including food (Bowman et al., 2005), habitat transformation, competition, and disease (Wood et al., 2016). Additional research on defining the upper critical temperature for northern flying squirrels will help to determine if range shifts of flying squirrels in Maine are climate warming induced. Further research that would include both male and female flying squirrels of all ages would provide more information on variation of sex and age. Further research should also include additional variables such as humidity, precipitation, extreme weather frequency and intensity. Land use changes, forest composition, availability of food, and selection of nest cavities would also be important to evaluate climate warming's contributions to flying squirrel behavior. Understanding physiologically inclined mechanisms of species behavioral changes will be critical for the conservation of species facing climate-driven range shifts.

As northern flying squirrels did not appear to be heat challenged during physiological experiments, and as their movement did not appear to be caused directly from rising temperatures, this brings us back to my original question: northern flying squirrels are moving north- how much of it is because of the heat? Although northern flying squirrels did not seem heat stressed, I did not test temperatures higher than 36°C in my respirometry experiments. Perhaps higher temperatures would challenge them more, and we may see these temperatures as climate change progresses. Maybe, peaks in maximum temperatures before northern flying squirrels disappeared from the Holt Forest impacted their movement, or maybe, other indirect factors such as competition with southern flying squirrels caused their range to change. Furthermore, did northern flying squirrels experience an actual range shift, or were local population dynamics observed? While my research cannot provide the answers to these questions, it can provide support in future studies to examine flying squirrel physiology and climate warming.

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## APPENDIX

Table 3: Morphometric measurements for captured *G. sabrinus* in 2019. Only records from first capture are included. \* indicate questionable sex identification.

Date	Sex	Weight (g)	Forearm (mm)	Hindfoot w/o Toes (mm)	Hindfoot w/ toes (mm)	Ear (mm)
06/19/19	-	56.9	-	-	-	-
06/27/19	F	61.5	-	20.84	25.91	10.45
07/02/19	M	95.9	-	-	-	-
07/02/19	F	89.0	-	22.10	-	-
07/04/19	M	98.2	27.59	22.72	30.4	12.73
07/24/19	F	99.0	23.79	28.01	-	16.12
07/25/19	M	98.1	24.17	21.15	36.05	12.85
07/25/19	M	57.0	-	-	26.93	14.63
07/26/19	M	55.1	21.59	21.43	31.51	10.20
07/29/19	F*	64.9	23.41	21.35	30.47	14.93
07/29/19	M*	53.8	18.76	19.64	30.78	13.67
07/31/19	M	118.5	21.41	23.14	33.53	15.00
07/31/19	M	86.6	27.31	21.81	30.34	16.29

Table 3 Cont.

08/14/19	F*	-	-	-	-	-
08/14/19	F*	79.7	22.42	15.84	20.06	13.73
08/16/19	M*	60.4	20.90	19.62	28.54	15.26
08/22/19	F*	74.4	-	25.26	27.88	14.92

## **BIBLIOGRAPHY OF THE AUTHOR**

Elise Gudde was born in Caribou, Maine on December 16, 1996. She was raised in Caribou, Maine and graduated from Caribou High School in 2015. She attended the University of Maine at Presque Isle and graduated in 2019 with a Bachelor's degree in Environmental Science and Sustainability. During her time at the University of Maine at Presque Isle, she worked as an undergraduate research assistant from 2017-2019 where she collected data on migratory songbirds and their food use. She entered the Ecology and Environmental Sciences graduate program at the University of Maine in the summer of 2019 to work with Dr. Danielle Levesque on the physiology of flying squirrels. After receiving her degree, Elise will continue to work as a life science teacher at the Maine Academy of Natural Sciences. Elise is a candidate for the Master of Science degree in Ecology and Environmental Sciences from the University of Maine in August 2022.