USING PHYSIOLOGICAL CONDITIONS TO ASSESS CURRENT AND FUTURE
WOOD FROG (RANA SYLVATICA) HABITAT USE IN THE SUBARCTIC

A Thesis
by
THOMAS PATRICK HASTINGS

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Abstract

USING PHYSIOLOGICAL CONDITIONS TO ASSESS CURRENT AND FUTURE
WOOD FROG (RANA SYLVATICA) HABITAT USE IN THE SUBARCTIC

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Arctic regions are already experiencing the disproportionate impacts of climate

change due to rapid surface warming rates. Changes in environmental variables as a result of

climate change can influence the habitat suitability of wildlife. Animals that are dependent on

the environment to meet physiological requirements, such as terrestrial amphibians, are

highly susceptible to the impacts of climate change. *Rana sylvatica* (wood frogs), for

example, must use habitats that balance thermal and hydric physiological requirements.

However, there is a lack of knowledge on amphibian physiological conditions in Arctic and

Subarctic landscapes. I investigated how environmental conditions and habitat characteristics

influence physiological conditions and habitat use of wood frogs near Churchill, Manitoba. I

used lab validated plaster models to estimate water loss rates (ug $*$ min^{-1} $*$ cm^{-2}) and body

temperature (°C) at specific radio-tracked frog and random locations (microhabitat scale) and

among different habitat types (macrohabitat scale) throughout the Subarctic. At the

macrohabitat scale, water loss rates are greater in the tundra than in the boreal forest and

ecotone habitats. At the microhabitat scale, wood frogs are 0.5% more likely to use any given

location for every 1 ug $*$ min^{-1} $*$ cm^{-2} decrease in water loss rates and are 329% more likely to use locations with surface moisture. In addition, frogs are more likely to frequent locations with increased cover from vegetation and decreased exposure to harmful environmental conditions, such as wind and sunlight exposure. I also found that physiological conditions predicted by measured environmental conditions can be used to explain up to 60% and 91% of the variation in measured water balance and body temperatures, respectively. My results indicate that maintaining water balance is important for frogs in Subarctic landscapes. However, maintaining physiological conditions may be particularly challenging for amphibians found in extreme environments that are vulnerable to future climate change. Rapidly increasing surface temperatures and resulting alterations (e.g. permafrost thaw and reduced wetland availability) make it unclear how wood frogs and other ectotherms will respond to shifting environmental conditions and habitat characteristics. Knowledge on the relationship between the environment and physiological conditions can help us make predictions about how these Arctic and Subarctic amphibians will respond to changes in habitat suitability.

Acknowledgments

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Foreword

This thesis will be submitted to the journal *Functional Ecology*, a peer-reviewed journal owned by the British Ecological Society and published by Wiley-Blackwell on behalf of the British Ecological Society; it has been formatted according to the style guide for that journal.

Introduction

Climate change has altered temperature and precipitation patterns at a global scale. At the Earth's surface, temperatures have been warming at an average rate of 0.12 °C per decade since 1951 (IPCC, 2014). The global mean surface temperature is predicted to rise another 0.3 to 0.7 °C from 2016 to 2035. Although changes in the amount of precipitation are more difficult to predict, extreme events such as increased variability in temperatures, more frequent heavy rains, and droughts are more likely (IPCC, 2014; Post et al., 2009; Prudhomme et al., 2014; Walther et al., 2002).

Arctic regions are especially susceptible to the impacts of climate change due to rapid surface warming (Post et al., 2009). Northern latitudes are already warming at twice the rate of lower latitudes in the Northern Hemisphere (Cohen et al., 2014), with Arctic surface air temperatures increasing 2-4 °C between 1963 and 2012 (Walsh, 2014). North America, West Siberia, and the Arctic Ocean are three regions within the Arctic that are contributing most to surface warming, especially during the fall and winter (Johannessen, Kuzmina, Bobylev, & Miles, 2016; Walsh, 2014). Furthermore, multiple processes contribute to the now well-documented poleward warming known as Arctic amplification (Serreze & Francis, 2006). For example, as warming increases, snow and ice layers that reflect solar radiation continue to retreat, exposing greater areas of dark vegetation that absorb heat from the sun (Serreze & Barry, 2011). Similarly, an increased length of the ocean's ice-free season and decreased

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thickness of sea ice allows more time for solar radiation to be absorbed by open waters (Cohen et al., 2014). Increased surface and water temperatures contribute to snow and ice melt and thus a positive albedo feedback loop (Serreze & Barry, 2011). Many other processes also interact to drive Arctic amplification and continue to alter Arctic and Subarctic landscapes at rates faster than other global regions (Kapsch, Graversen, Tjernström, & Bintanja, 2016; Pithan & Mauritsen, 2014; Shupe & Intrieri, 2004).

An important question remains regarding how climate change will affect animals and their habitats (Hickling, Roy, Hill, Fox, & Thomas, 2006). Environmental variables altered by climate change such as the composition of vegetation, wetland hydrology, and water availability can influence habitat selection by individuals at multiple spatial scales (Blaustein et al., 2010; Brooks, 2009; IPCC, 2014; Johnson, 1980; Post et al., 2009). In severe instances, habitat alteration and environmental variables (e.g. surface temperature and moisture) can be limiting factors that create boundaries for dispersal (Cunningham, Rissler, Buckley, & Urban, 2015).

Species can tolerate stressful habitat alterations and changing climatic conditions by adapting to changes or shifting their range to remain within suitable habitat boundaries (Chen, Hill, Ohlemüller, Roy, & Thomas, 2011; Cunningham et al., 2015; Walther et al., 2002). Indeed, species ranges are shifting towards higher latitudes and elevations to remain within suitable habitat and climates (Hickling et al., 2006; Walther et al., 2002). For example, during the 20th century, 63% of 35 non-migratory butterfly species shifted their range north by 35-240 km in response to European warming (Parmesan et al., 1999). However, failure to shift ranges when faced with warming conditions can lead to local reductions in reproductive output and body condition, as observed with *Ursus maritimus* (polar bear) in Baffin Bay

(Laidre et al., 2020). Due to the potential impacts of habitat alteration, there is an increasing urgency to study how wildlife will respond to climate change (Buckley, 2008).

Species have differing physiological tolerances and therefore must select habitats with appropriate environmental conditions (Martin, 2001). Species distribution modeling can help predict environmental conditions and habitats that will be most suitable to sustain various species (Shabani, Kumar, & Ahmadi, 2016). As an example, four ground-nesting bird species shifted their microhabitat nest selection between two vegetational habitat types in response to shifting precipitation patterns (Martin, 2001). Microhabitat shifts by the birds altered temperature conditions and increased nest predation as a result of new species interactions (Martin, 2001). Additionally, mechanistic models predict how species-specific traits (e.g. energy and mass balance, survival, and surface activity time) are influenced by the relationship between specific microclimatic environmental conditions and physiological mechanisms (Buckley, 2008; Kearney, Phillips, Tracy, & Porter, 2008). The physiological conditions of an animal can be measured using physical models that simulate animal conditions under natural environments (Bartelt & Peterson, 2005). This process-based modeling approach predicts which habitats will be most suitable to sustain specific traits (Shabani et al., 2016). Incorporating species' physiological tolerances and microclimatic conditions improves insight to habitat suitability, habitat selection, and impacts to wildlife in the predicted distributions (Kearney & Porter, 2009).

Ectotherms are especially susceptible to environmental variation and habitat alteration and must use habitats with particular moisture and microclimate conditions in order to sustain a positive water balance and optimal body temperature (Berven, 1982; Groff, Calhoun, & Loftin, 2017). For instance, terrestrial amphibians rely on environmental

moisture as their permeable skin allows evaporative water loss in the absence of external moisture (Adolph, 1932; Peterman & Semlitsch, 2014; Riddell, McPhail, Damm, & Sears, 2018; Toledo & Jared, 1993; Tracy, Tixier, Le Nöene, & Christian, 2014). Water loss rates are also influenced by body temperature, and amphibians tend to select habitats that appropriately balance thermal and hydric physiological requirements (Seebacher & Alford, 2002). Furthermore, when exposed to warmer temperatures during active and hibernation periods, animals like *Bufo bufo* (common toad) expend more energy as metabolic rates are greater for extended periods of time. Energy loss decreased size at sexual maturity and body condition and increased the mortality of female common toads (Reading, 2007). Impacts of the environment on terrestrial amphibians reinforces the link between body temperature, water balance and habitat selection (Bartelt, Klaver, & Porter, 2010; Seebacher & Alford, 2002).

For terrestrial amphibians to maintain a positive water balance, they must have rehydration rates that are greater than dehydration rates from evaporative water loss (Dabes, Bonfim, Napoli, & Klein, 2012; Navas, Antoniazzi, & Jared, 2004). Morphological adaptations, including specialized ventral and dorsal skin to increase water uptake rates, aids amphibians in enhancing their rehydration (Navas et al., 2004; Ogushi et al., 2010). Other behavioral adaptations, such as entering water conserving postures and selecting habitats with available moisture, help prevent greater evaporative water loss rates (Dabes et al., 2012; Navas et al., 2004). *Rana sylvatica* (wood frog) at southern latitudes, for example, migrate to ephemeral ravines to remain within suitable soil moisture levels (Rittenhouse & Semlitsch, 2007), suggesting that specific microhabitat selection can decrease chances of mortality via desiccation (Peterman & Semlitsch, 2014; Rittenhouse, Harper, Rehard, & Semlitsch, 2008;

Seebacher & Alford, 2002). Furthermore, 11 species of anurans found in a coastal sand dune habitat of Brazil, had rehydration rates that were faster than dehydration rates, with *Rhinella jimi* (Jimi toad) having the fastest rehydration rate (Dabes et al., 2012). Thus, as long as water is readily available, amphibians can remain hydrated and avoid desiccation.

Maintaining physiological requirements in extreme environments may require both behavioral and physiological adaptations. Terrestrial amphibians have evolved a variety of adaptations that have allowed them to maintain physiological requirements and occupy extreme environments ranging from hot, dry deserts to the cold, dry Arctic (Navas, Antoniazzi, Carvalho, Suzuki, & Jared, 2007; Storey & Storey, 1988). For example, juvenile *Rhinella granulosa* (granular toad) can remain surface active while dispersing or foraging during the day under dry, high temperature conditions (soil temperatures up to 40 °C) due to a high critical maximum body temperature (44.2 °C) and selection for shade and increased humidity. In the same environment, adult granular toads avoid these same harsh conditions by remaining inactive during the day (Navas et al., 2007).

Similar to amphibians in xeric environments, terrestrial amphibians in extreme Subarctic environments also use shelter and wetland characteristics to maintain favorable body conditions. In one of the only Subarctic habitat use studies conducted during the active period, wood frogs, my study species, were found primarily in tundra habitat and were more likely to use microhabitat locations with standing water, shrubs and sedge cover than random locations with less cover (Bishir, Hossack, Fishback, & Davenport, 2018). However, most active period research for this species has taken place in temperate regions, and less is known about wood frog activity in higher latitudes where habitat characteristics and conditions are notably different (see study area section in the methods below). Although there seems to be a

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consistent pattern that amphibian habitat use in a terrestrial environment is influenced by thermal and hydric body conditions, such as with wood frogs, there is still little known about amphibian habitat use or how specific habitats are related to physiological requirements in Arctic/Subarctic regions (Churchill & Storey, 1993; Rittenhouse & Semlitsch, 2007).

The lack of knowledge on amphibian physiological conditions in Arctic/Subarctic regions is important because Arctic amplification is already altering Subarctic landscapes used by wood frogs (Lantz, Marsh, & Kokelj, 2013; Mamet & Kershaw, 2012; Wolfe et al., 2011). Longer ice free seasons have resulted in greater evaporative water loss rates and increased drainage from permafrost thaw has reduced hydroperiods for Subarctic wetlands (Wolfe et al., 2011). In addition, warming has facilitated the expansion of shrubs into tundra habitat (Lantz et al., 2013). During the summer, increased evapotranspiration and canopy cover from shrubs decreases soil moisture and cools ground temperatures, respectively (Myers-Smith et al., 2011). Decreasing moisture availability and a shift towards a drier landscape will reduce the amount of habitat suitable for wood frogs. Identifying important microclimatic conditions and the physiological responses of wood frogs will help identify factors driving the habitat use of this Subarctic ectotherm.

The goal of my research was to investigate how environmental conditions and habitat characteristics influence the physiology and habitat use of wood frogs at the northern edge of their range. I used lab-validated plaster models to estimate water loss rates (ug $* $ min^{-1} $*$ cm^{-2} body surface area) and body temperature (°C) of wood frogs at specific radio-tracked and random locations near Churchill, Manitoba. I hypothesized that estimated water loss rates and water balance of frogs would differ among three macrohabitat types found in the Subarctic (tundra, boreal forest, and tundra/forest ecotone) due to differences in habitat

associated environmental differences. I predicted that water loss rates would be greater in the

tundra macrohabitat with less vegetational complexity due to warmer temperatures (air and

ground) and greater wind speeds. Similarly, I hypothesized that water loss rates would be

lower at specific frog locations than at random points due to their use of habitat

characteristics that reduce exposure to harsh environmental conditions. In addition, I used

empirically determined relationships between measured environmental conditions and the

physiological status of the wood frogs to make predictions about their water balance and

body temperatures at all studied locations. With the ability to predict the physiological

conditions of these frogs, we can now monitor how future climatic changes will affect their

habitat suitability at multiple scales.

Materials and Methods

Study area

My study area was located in the Hudson Bay Lowlands of Subarctic Manitoba

(58.7375° N, -93.8190° W, 17 m). This landscape is unique because about 25% of it is

covered in shallow wetlands that remain filled year round due to a continuous layer of

permafrost that prevents drainage (Macrae, Brown, Duguay, & Petrone, 2014). My study

area is located close to the boreal treeline, allowing comparisons among multiple

macrohabitat types (tundra, boreal forests, and boreal forest/tundra ecotone) located closely

to each other (Wolfe et al., 2011; Fig. 1). Studying boreal forest and ecotone macrohabitats

may also provide insight into future landscape conditions as Subarctic vegetation shifts past

historic treelines in response to warming (Mamet, Brown, Andrew, Trant, & Laroque, 2019;

Mamet & Kershaw, 2012). In addition, the small elevational change and coastal location of

the Hudson Bay Lowlands exposes this study area to consistent onshore and offshore winds,

with warm, dry offshore winds causing greater evapotranspiration (Rouse, 1991). Last, summer temperatures near Churchill are mild with an average daily air temperature around 12 °C in July (Brandson, 2011; Macrae et al., 2014).

Study species

The wood frog (*Rana sylvatica*; LeConte, 1825) is a widespread and locally abundant North American amphibian (Dodd, 2013). The geographic range of this species extends from the southern Appalachian Mountains to the northeastern United States, as well as throughout Canada and into Alaska. Its range also extends north to above the Arctic Circle (Dodd, 2013; Larson et al., 2014).

Plaster models

I created plaster models to simulate the evaporative water loss rates, water balance, and body temperatures of wood frogs (Peterman et al., 2013; Tracy et al., 2007; Fig. 2). Plaster models are a useful tool for estimating amphibian physiological conditions under various environmental conditions as they can be deployed in the field at specific locations (Riddell, Apanovitch, Odom, & Sears, 2017). In addition, like the permeable skin of terrestrial amphibians, plaster models lose water in the absence of moisture (Peterman et al., 2013; Tracy et al., 2007). Plaster models are also advantageous because they do not shrink and alter water loss rates due to changes in surface area while being deployed in the field for extended periods of time (Tracy et al., 2007). While useful, physical models do differ from live animals in that they cannot simulate behavioral responses to changing weather conditions, and some do not have the same skin resistance to water loss as live animals (Riddell et al., 2017; Tracy et al., 2007).

To make the plaster models, rather than using a dead specimen, I made a mold of a 3-D printed generic ranid frog with dental alginate (Jeltrate Plus, DENTSPLY Caulk, Milford, Delaware, USA; Riddell, Apanovitch, Odom, & Sears, 2017; Tracy et al., 2007). I created molds for three different frog sizes, measured as the snout-vent length (SVL), to represent the size range (44-54 mm SVL) of adult wood frogs in the Subarctic study area. However, water balance was not strongly influenced by the size range I evaluated at the macro- (-1.18 ± 0.99 ug $*$ min^{-1} $*$ cm^{-2}, n=478) and microhabitat scale (0.32 ± 0.71 ug $*$ min^{-1} $*$ cm^{-2}, n=499), and therefore plaster model size class was not included in my statistical modeling. I filled each mold with Plaster of Paris to create 20 identical models for each size class (total of 60 models). All models included a 1.7 cm indent to retain an iButton temperature logger (model DS1921G, Maxim Integrated, San Jose, California, USA) at the base (Hossack et al., 2009). Last, I mixed each model with an equal amount of brown paint (Beauti-Tone Paint, Ontario, Canada) to mimic the surface color and solar absorbance of live wood frogs.

Prior to deploying the models, I used wind tunnel trials in a controlled lab space at the Churchill Northern Studies Centre to validate that plaster models can be used to estimate the thermal and hydric conditions of wood frogs (Peterman and Semlitsch, 2013; Tracy et al., 2007; Fig. 3). To do this, models and live frogs were soaked in water for 1 hour prior to all trials and excess water was removed with paper towel. Adult wood frogs were paired with a plaster model of a similar SVL for 2-hour water loss trials. Indoor trials consisted of two wind tunnels under controlled air temperature (°C), relative humidity (%), and wind speed (meters per second) conditions. Inside the wind tunnels, the model and frog were placed in a wire cage with a glass bottom to ensure position of the subjects remain constant during the trial (Fig. 3). At the start and end of all trials, frogs and models were weighed to the nearest

hundredth of a gram using a portable electronic scale (Ohaus NV4000 Precision Balance, Hogentogler and Co. Inc., Columbia, MD, USA) and surface temperature (skin or plaster) was recorded using an infrared thermometer (FLUS IR-802, Shenzhen FLUS Technology Co. Ltd, Shenzhen, China). The change in weight of models and frogs was used to estimate evaporative water loss rates (Peterman et al., 2013; Seebacher & Alford, 2002; Tracy et al., 2007).

In addition to model validations trials, water loss trials were also conducted in the lab and field to determine how long evaporative water loss rates remained constant (Fig. 4). To accomplish this goal, models were weighed every 30 minutes for 6 hours in a controlled lab setting to determine when water loss rates were no longer constant. The same process was repeated with models deployed in tundra habitat during sunny and windy conditions to provide an estimate of water loss rates under a range of natural conditions (Fig. 4).

Field sampling – model use

During my data collection at both the macro- and microhabitat scale, two plaster models of the same size class were always deployed on the same ground cover type to ensure similarities in environmental conditions (Fig. 5). I also randomly determined which cardinal direction each pair of models would be deployed to reduce any effect of placement direction (M. Gifford, personal communication). One model was used to estimate evaporative water loss rates and one model estimated water balance of wood frogs. Studies have typically only used models to study evaporative water loss rates, and created a barrier between the model and the substrate surface to prevent any moisture absorbance (Peterman & Semlitsch, 2014; Tracy et al., 2007). However, I wanted to determine not only how evaporative water loss differs between locations, but also if frogs use microhabitats that support greater water

balance. To accomplish this, I placed one model in the field without any barrier to the substrate (water balance model; Fig. 2). Prior to deployment, I soaked each model in water for 1 hour to fully saturate the models (Peterman & Semlitsch, 2014).

All models were deployed in the field for ≤ 2 hours to ensure constant water loss rates among all models (Fig. 4). To account for differences in deployment time, all water loss estimates were standardized as the rate of water loss (ug/minute) divided by the surface area (cm^2) of each plaster model (Riddell & Sears, 2015). Surface area (cm^2) was calculated for the three different model size classes using the Mesh 3D Print Toolbox add-on in program Blender 2.79.0. To determine the surface area of live frogs, I entered the SVL of frogs into a linear regression equation calculated from the relationship between plaster model SVL (independent variable) and plaster model surface area (dependent variable). The regression equation and R^2 value that resulted from this analysis are as follows:

$$y = 2.01x - 51.37, \ R^2 = 0.99, n = 3, SE = 0.76$$

where y = surface area of wood frogs (cm^2) and x = SVL (mm) of wood frogs. Last, plaster model surface and body temperature were measured each time a model was deployed and collected using an infrared thermometer and iButton.

Field sampling

To compare the evaporative water loss rates, water balance, and body temperature of models among three Subarctic macrohabitat types (boreal forest, tundra, and forest/tundra ecotone), models were deployed along transects at four replicate sites (Fig. 5). The four sites were haphazardly chosen to represent the range of habitat conditions within the study area. Site selection was also contingent upon the presence of adult wood frogs and proximity of

the macrohabitat types. Two 40-meter transects were randomly placed within each habitat type at all sites (total of six transects per site; Fig. 6). Along each transect, paired models were deployed at 10-meter intervals. At each site, three teams of researchers and citizen scientists deployed models along transects in the three habitat types simultaneously to prevent differences in weather conditions. Between 30 June and 5 July 2018, models were deployed along transects at each of the four sites in the morning (approx. 09:00-11:00) and afternoon (approx. 14:30-16:30) for 2 hours to gain additional data on hydric and thermal physiological conditions that may be experienced by wood frogs throughout the day. Site order was randomly generated during both sampling periods to prevent bias. Last, the same 24 transects were resampled a second time during the summer between 25 July and 30 July 2018.

At a finer spatial scale, I compared hydric and thermal conditions at specific microhabitat locations selected by frogs near Churchill, Manitoba. In 2018, I captured and individually fit 33 adult frogs at four sites with a radio-transmitter belt (Bishir et al., 2018; Groff et al., 2015; Fig. 5; Appendix A). Each belt was made of stretch bead cord (Stretch Magic, Pepperell Braiding Company, Pepperell, Massachusetts, USA) and a 0.4 g radio transmitter (Blackburn Transmitters, Nacogdoches, Texas, USA). Adult frogs were tracked every three to four days using an R-1000 telemetry receiver (Communications Specialist Inc., Orange, California, USA) and a model RA-23K VHF antenna (Telonics, Mesa, Arizona, USA). Frogs were located to within 10 cm or until visually identified and coordinates for each new location were determined with a handheld GPS unit (Garmin Etrex 10, Olathe, KS, USA). Paired plaster models were placed 15 cm from the located frogs. However, if wood

frogs relocated immediately upon our arrival, models were deployed at the exact location the frog vacated.

All new frog relocations were paired with a random location where plaster models were also set. Random locations were positioned at half the distance of the previous movement and at a randomly generated compass bearing between 0 and 360 degrees (Bishir et al., 2018; Groff et al., 2017). This allowed me to determine if wood frogs were using microhabitats that influence their physiological status. Paired random locations also allowed me to characterize available environmental and associated physiological conditions that fall within the movement distances of each frog. During the summer of 2019, models were deployed at known wood frog and random point locations (240 total) generated from frogs tracked by Bishir et al. (2018) in 2015 and 2016. Placing models at these locations increased my sample size of known wood frog microhabitat locations and associated random points.

At all paired model locations (along transects, specific wood frog locations, and random locations) I collected environmental data to understand how each variable may influence the physiology of wood frogs. First, I measured relative humidity next to models with a portable meter. Ground temperature (°C) was recorded in three locations at the base of the models using an infrared thermometer. Air temperature was measured by deploying a shaded iButton attached to a post about 1 meter above the ground. One iButton was deployed at the center of each transect when comparing the conditions among the different macrohabitat types. For measuring conditions at specific wood frog locations, the iButton post was placed within the macrohabitat type of both the frog and random locations. If the frog and random locations fell within different macrohabitat types, then multiple iButton posts were positioned. Next, I used my air temperature measurements along with the

Clausius- Clapeyron equation and my relative humidity measurements to calculate saturation vapor pressure (es) and actual vapor pressure (ea), respectively (Riddell et al., 2017). Vapor pressure deficit (kPa) was calculated as the difference between ea and es. I determined wind speed (m/s) by holding an anemometer directly above the models. The greatest wind speed detected in a one-minute period was recorded. Microhabitat characteristics were estimated within a 1-meter radius plot surrounding all paired model locations. The ground cover types (soil, water, moss/lichen, sedge, shrub) were visually estimated to the nearest 5%. The sum of all ground cover type estimates did not need to equal 100% as several habitat characteristics could overlap within the plots. Finally, rainfall during a plaster model deployment, direct sunlight on plaster model surfaces, and the placement of plaster models on measurable surface moisture were recorded as binary variables (yes, no).

Data analysis

Lab validation trials

Linear regression analysis was used to validate that plaster models were sufficient for estimating the evaporative water loss rates and body temperature of live wood frogs. I calculated a regression equation using plaster model evaporative water loss rates as the predictor variable and live frog water loss rates as the dependent variable from the controlled, lab validation trials. The same analysis was repeated for the surface temperatures of plaster models and wood frogs as part of the validation process.

Macrohabitat scale analysis

I used linear mixed effects models (LMER, Package lme4, version 1.1-21) to estimate the effects of environmental conditions and habitat characteristics on the water balance and

body temperature of wood frogs at the macrohabitat scale. Coefficients in these models estimate the strength of a variable's effect on frog water balance and body temperature. Likewise, estimates represent the change in the dependent variable for every unit increase in a predictor variable while holding all other variables constant. LMER models at the macrohabitat scale were important for estimating differences in water balance and body temperature among the three habitat types, as well as determining which environmental conditions and habitat characteristics altered those physiological conditions at a broader Subarctic landscape scale. Water balance and body temperature of the plaster models were included as the dependent variables in separate LMER models. Macrohabitat type, environmental conditions, and habitat characteristics were included in the models as fixed effects. I nested plaster model locations within transect study site as random effects to account for the repeated measure of specific locations and any additional variation added by the sites.

First, all variables that were not recorded in at least 10% of the plaster model locations were removed from the variables list. An initial LMER model was performed with all fixed effect variables included. Backwards selection was applied to remove all non-significant variables ($p > 0.05$) beginning with highly correlated variables ($r \geq 0.7$) of least significance (greatest p-value). Final models presented include only significant predictor variables unless otherwise indicated by bold font.

Microhabitat scale analysis

I used conditional logistic regression (CLR, Package survival, version2.44-1.1) to identify predictors of habitat use by wood frogs at the microhabitat scale. This analysis also determined if water balance and body temperature are physiological conditions influencing

habitat use. The physiological status, environmental conditions, and habitat characteristics of the frogs were included as independent, predictor variables. A cluster ID function was used to account for repeated measures and lack of independence of locations used by a single frog. Therefore, each frog was assigned a unique ID to include in the CLR model. Backwards selection beginning with highly correlated variables was again applied to arrive at a final CLR model. Next, the odds of a frog using a particular location for every unit change in a predictor variable were derived from exponentiated coefficient estimates and calculated using the following equation:

$$\text{Odds} = (\text{Exp. coefficient} - 1) * 100$$

Physiological predictions

I predicted the water balance and body temperature of wood frogs by incorporating the relationships between environmental and physiological conditions learned from my statistical and plaster models. The ability to make such predictions allows researchers to monitor the physiological status of frogs in the future as environmental conditions continue to change with rapid warming. Using the same mixed effects modeling approaches as above, four additional models were performed including only environmental conditions as fixed effects. Each model included a physiological condition (water loss or body temperature) as the dependent variable at the macro- and microhabitat scale (four models total). At the microhabitat scale, many plaster models did not lose water and therefore at those locations, my dataset included zero values for the dependent variable. To account for the high number of zero values and to best predict the water balance of these frogs, I used a generalized linear mixed effects model (GLMER, Package lme4, version 1.1-21) with a Gamma family distribution (link=log) and bobyqa optimizer (Bolker et al., 2009). Physiological conditions

were predicted for all known model locations at both the macro- and microhabitat scale using the intercepts and coefficient estimates output from all LMER and GLMER models.

To determine how well the physiological conditions observed in the field (plaster model water balance and body temperature) can be predicted by measurable environmental conditions, I used linear regression analysis with observed physiological conditions as the dependent variable and predicted physiological conditions as the independent variable. I used nonlinear regression with a quadratic function to better fit my observed and predicted water balance values at the macrohabitat scale. Package lme4, version 1.1-21 was used to perform all LMER and GLMER models. All statistical analyses were completed in Program R, version 3.6.0 (R Core Team, 2019).

Results

Validation trials and macrohabitat scale

I was able to use the plaster models to study the effects of the environment on the physiological status of wood frogs. I found from the validation trials that the plaster models explained ~67% and ~80% of the variation in Subarctic frog water loss rates ($F_{1,29} = 57.74$, $p < 0.001$; Fig 7.) and body temperature ($F_{1,29} = 114.70$, $p < 0.001$; Fig. 8), respectively. At the macrohabitat scale, habitat type, environmental conditions, and habitat characteristics all influenced the water balance of wood frogs (Table 1). More specifically, water loss rates in the tundra were 90.11 ug $*$ min^{-1} $*$ cm^{-2} greater than in the ecotone habitat, when holding all other variables constant (Table 1). Water loss rates in the forest were 21.33 ug $*$ min^{-1} $*$ cm^{-2} lower than water loss rates in the ecotone, although this water loss rate should be interpreted with caution when considering the standard error of the estimate (Table 1). Water loss rates

decrease for every 1% increase in habitat cover characteristics, with water loss decreasing the most with increased soil cover (2.80 ug $* \text{min}^{-1} * \text{cm}^{-2}$) and the least with increased shrub cover (0.8 ug $* \text{min}^{-1} * \text{cm}^{-2}$). In contrast, water loss rates tended to increase with exposure to environmental conditions at the macrohabitat scale. For every 1 m/s increase in wind speed, 1 °C increase in ground temperature, and exposure to direct sunlight rather than shade, water loss rates increased (Table 1). However, for every 1% increase in relative humidity, water loss rates decreased by 2.15 ug $* \text{min}^{-1} * \text{cm}^{-2}$. While habitat type and habitat characteristics influenced water balance, only the same four environmental conditions mentioned above had an effect on body temperature at the macrohabitat scale (Table 2). In addition, environmental conditions had the same effect on body temperature as they did on water balance with the exception that for every 1 m/s increase in wind speed, body temperature decreased by 0.63 °C.

Microhabitat scale

Water loss rates and surface moisture availability are important predictors of microhabitat use by wood frogs within the Subarctic landscape (Table 3). Frogs were 0.5% less likely to use any specific location for every unit increase in water loss rate. Increased sedge and shrub cover increased the odds of a frog using a specific location while frogs were less likely to use locations with increased bare soil coverage. Importantly, frogs were 329% more likely to use any locations where saturated ground or standing water were available for

the frog to position itself in direct contact with. In addition, frogs were less likely to use specific locations with increased exposure to sunlight or greater wind speeds.

Macro- and microhabitat comparison

At the macrohabitat scale, relative humidity, wind speed, ground temperature, and sun exposure all altered body temperature and water balance (Table 2, 4). However, at the microhabitat scale, exposure to direct sunlight did not influence water balance or body temperature. Furthermore, water loss rates decreased and body temperature increased when surface moisture was available (Table 5, 6). Rainfall also decreased water loss rates (Table 5). Last, body temperature was not influenced by relative humidity at the microhabitat scale but did increase as vapor pressure deficit increased (Table 6). All remaining relationships between environmental and physiological conditions remained consistent between the macro- and microhabitat scale.

Physiological predictions

Using the environmental conditions determined to influence physiology from the LMER models (Table 2, 4), predicted physiological conditions were able to explain 82% and 70% of the variation in observed body temperature (Fig. 9) and water balance (Fig. 10) at the macrohabitat scale. However, it is worth noting that LMER models overestimated body temperature below around 10 °C, and therefore caution should be used if trying to predict body temperatures below that limit (Fig. 9). Similarly, using important environmental conditions from the microhabitat scale (Table 5, 6) predicted physiological conditions

calculated from the results of the LMER and GLMER model were able to explain 60% and 91% of the variation in observed water balance (Fig. 11) and body temperature (Fig. 12).

Discussion

Having the ability to predict the physiological conditions of terrestrial amphibians, like wood frogs, will be particularly important in rapidly warming landscapes where habitat use is threatened by shifting habitat characteristics and environmental conditions. In the Hudson Bay Lowlands near Churchill, Manitoba, I tracked the habitat use of wood frogs and estimated their physiological status with the use of lab validated plaster models. I also used environmental conditions to predict the water balance and body temperature of frogs at the macro- and microhabitat scales. Physiological conditions of wood frogs, including water balance and body temperature, are impacted by habitat type, environmental conditions, and habitat characteristics. At the macrohabitat scale, tundra habitat seems to be the least optimal for frogs given that water loss rates are greater there than in the forest or ecotone (Table 1). Water loss at the macrohabitat scale is driven by environmental conditions and habitat characteristics, while body temperature is influenced only by environmental conditions such as sun exposure, wind speed, and ground temperature (Table 2). At the microhabitat scale, habitat use by wood frogs is driven by water loss and surface moisture availability and is not influenced by differences in body or environmental temperature (Table 3). Such findings suggest that during the active period, water balance and the environmental conditions and habitat characteristics that influence the hydric condition of wood frogs, may be more important than body temperature in Subarctic landscapes. Regardless, we have the ability to predict both the water loss rates and body temperature of Subarctic frogs in the future by incorporating environmental conditions.

Macrohabitat diversity is important for some ectotherms, like wood frogs and *Ambystoma jeffersonianum* (Jefferson salamanders), that must complete complex annual life history requirements that often involve aquatic and terrestrial stages (Baldwin, Calhoun, & deMaynadier, 2006; Berven, 1990; Faccio, 2003). Yet, while wood frogs in Churchill, Manitoba are exposed to environmentally and structurally distinct macrohabitat types during their active period, they are primarily observed in shallow wetlands or other locations supporting standing water within the tundra macrohabitat type only (Bishir et al., 2018). Targeted use of wetland tundra habitat can be explained by the relationship between physiological conditions and habitat associated environmental conditions. For example, increased desiccation risk in tundra habitat outside of wetlands is likely driven by reduced vegetational complexity and exposure to greater wind speeds (Köhler et al., 2011). To increase water balance in Missouri, wood frogs moved to and congregated at ephemeral ravine macrohabitats (Rittenhouse & Semlitsch, 2007), which are wetter, more shaded, and have slower wind speeds than habits farther upslope. While wood frogs could likely maintain water balance in boreal forest or ecotone wetlands, additional, unmeasured factors may help explain frog presence in tundra habitats. For example, to consume enough food to sustain energy requirements during hibernation, prey preference by wood frogs and differences in invertebrate communities among habitat types may influence macrohabitat use (Cogălniceanu, Rusti, Plăiaşu, & Palmer, 2018; Costanzo et al., 2015). Although studying macrohabitats can provide useful information about broader landscape habitat suitability, evaluation of microhabitat is also crucial to better understand habitat use patterns.

Wood frogs use tundra habitat (Bishir et al., 2018) because of the unique combination of environmental conditions and habitat characteristics that result in a positive water balance.

Results similar to that of Bishir et al. (2018) suggest that tundra microhabitats provide adequate surface moisture to support a positive water balance. The vegetational complexity contributed by the sedges and shrubs increases cover and protects the frogs from excess sun and wind. Use of locations with greater relatively humidity also benefits water balance by decreasing the vapor pressure deficit surrounding an animal (Riddell et al., 2017). The choice of habitat and the resulting physiological condition of wood frogs is similar to that influencing terrestrial amphibians at lower latitudes. For example, the water loss rates of western slimy salamanders (*Plethodon albagula*) were increased by exposure to solar radiation, and in this study, I found that wood frogs were 57% less likely to use locations with exposure to direct sunlight (Peterman & Semlitsch, 2014). Yet, I did not find that exposure to direct sunlight under sunny conditions was an environmental condition influencing the water balance of the wood frogs. It is possible that the adequate surface moisture available in this landscape allowed wood frogs to maintain a positive water balance when exposed to direct sunlight. However, similar to the negative effects of wind exposure reported in other amphibian studies, I found that for every 1 m/s increase in wind speed that wood frogs were 59% less likely to use any given location as water loss rates increased by 0.42 ug $* \min^{-1} * \mathrm{cm}^{-2}$ (Muller, Cade, & Schwarzkopf, 2018). The interactions among several environmental conditions, as well as their influence on physiological conditions, may help explain why frogs in the Subarctic avoided more exposed microhabitat locations (Stevenson, 1985). Thus, wood frogs use of locations with increased surface moisture suggests that maintaining water balance may be a more limiting physiological condition than remaining within a potentially more flexible body temperature range in this Subarctic landscape.

Although I found that environmental conditions influence body temperature of wood frogs at both the micro- and macrohabitat scale, the lack of an effect by body temperature or environmental temperature on habitat use could be attributed to spatial thermal heterogeneity and sufficient water availability. For instance, it may be possible that the optimal body temperature range of frogs in the Subarctic can be achieved in many locations due to sufficient spatial thermal heterogeneity driven by vegetation complexity (Sartorius, do Amaral, Durtsche, Deen, & Lutterschmidt, 2002). Thus, body temperature may not be a factor influencing habitat use in the Subarctic. Conversely, amphibians found in desert landscapes are often nocturnal, fossorial, or adapted to acclimate to seasonal temperature shifts to tolerate extreme temperature conditions that threaten animals and their thermal maxima (Griffis-Kyle, 2016; Sanabria, Quiroga, & Martino, 2012).

The unique amount of wetland coverage in this Subarctic landscape may also help explain why body or environmental temperature did not strongly influence microhabitat use by these wood frogs. Given they were much more likely to be found where measurable surface moisture was available, it is likely that as long as some portion of their body is in contact with liquid water, they are able to maintain a positive water balance. If wood frogs can remain within their optimal body temperature range when using microhabitats that benefit water balance, then the ability to maintain body temperature may not need to influence habitat use. *Rana temporaria* (common frog) in Poland, for example, typically used moist, vegetated locations that allowed body temperature to remain constant throughout the day (Köhler et al., 2011). Although temperature conditions were not influencing habitat use in our study, thermal physiological requirements must still be maintained by ectotherms to persist in terrestrial environments (Peterman & Semlitsch, 2014).

For wood frogs, managing body temperature during hibernation is influenced by habitat characteristics, and the ability to remain within thermal tolerances will be challenged throughout their geographic range as climate change continues (Groff, Calhoun, & Loftin, 2016). Although synthesis of cryoprotectants is crucial to overwintering success in extreme winter conditions, habitat selection for certain features (e.g. deeper snowpack) is also important for controlling body temperature minima and seasonal fluctuations (Groff, Calhoun, & Loftin, 2016; Larson et al., 2014). For example, the minimum hibernacula temperature experienced by wood frogs in northern, interior Alaska is approximately -18.1 °C, when air temperatures were as low as -36.8 °C (Larson et al., 2014). Thus, a snowpack acts as an insulating layer that buffers these frogs from extremely low temperatures. However, warmer weather and increased winter rainfall could expose wood frogs to adverse weather conditions during hibernation, which could force these frogs to experience deleterious physiological shifts due to greater temperature oscillations (Groff et al., 2016). Amphibians under these conditions are also at risk of expending more energy by having to synthesize additional cryoprotectants if the number of freeze thaw cycles increases (Harper & Semlitsch, 2007; Larson & Barnes, 2016; O'Connor & Rittenhouse, 2016). So, while the body temperatures of wood frogs may not influence Subarctic habitat use during their active period, it could become a critical limiting factor in the future should hibernacula temperatures become more erratic due to future climate warming. Research could help fill in gaps about future hibernacula habitat suitability, which is an important portion of the entire life cycle of these frogs.

Maintaining water balance may also be particularly challenging for amphibians found in extreme environments that are more vulnerable to future climate change (Blaustein et al.,

2010; Griffis-Kyle, 2016). Rapidly increasing surface temperatures and resulting habitat changes (e.g. permafrost thaw and vegetation encroachment into tundra habitat) could reduce wetland availability, as well as shorten the duration of the hydroperiod where standing water is freely available to the frogs (Johannessen et al., 2016; Lantz et al., 2013; Post et al., 2009; Walsh, 2014; Wolfe et al., 2011). Indeed, at a southern Arctic region of Nunavut, Canada, 61% of all wetlands <0.1 ha in size, along with some larger water bodies, have been decreasing in size (Carroll & Loboda, 2017). Wetland loss trends will make it more difficult for adult frogs to maintain a positive water balance and could reduce connectivity between breeding locations for frogs throughout the landscape, thereby affecting gene flow, genetic diversity, and survival of isolated populations (Furman, Scheffers, Taylor, Davis, & Paszkowski, 2016). Small populations have a greater probability of doing a random walk to extinction than larger ones, and fragmentation of habitat and subsequent isolation of frog populations into smaller groups could hasten this outcome (Allentoft & O'Brien, 2010; Furman et al., 2016). A reduction in amphibian dispersal and gene flow among populations due to habitat loss can reduce the ability of a species to tolerate environmental variation such as that caused by climate change (Cushman, 2006). Therefore, frogs in the Subarctic are likely to become more threatened as warming continues to alter wetland availability.

Globally, amphibians are susceptible to the impacts of climate change, with increased pressure from additional interacting factors that lead to declines (Hof, Araújo, Jetz, & Rahbek, 2011). As climate change continues, up to 54% of frog species and 56% of salamander species are predicted to lose habitat with suitable climate conditions by the year 2080 (Hof et al., 2011). Amphibian declines can be further influenced by interactions with other major threats including disease, land use change, and invasive species interactions (Hof

et al., 2011). Furthermore, species interactions can be altered by climate change as a result of range shifts or changes in the phenology of multiple species seasonal activities (Walther et al., 2002; Yang & Rudolf, 2010). For example, wood frogs and some Subarctic ground nesting bird species, are threatened by the range expansion of *Chen caerulescens caerulescens* (lesser snow goose), as these geese uproot and destroy important vegetational coverage used by wood frogs and other animals (Bishir et al., 2018; Peterson, Rockwell, Witte, & Koons, 2013; Davenport, personal communication). In addition, with smaller Subarctic wetlands shrinking, amphibian density within and surrounding the remaining wetlands may increase, and maintaining physiological conditions may become more difficult with increased inter- and intraspecific competition for suitable habitat and food (Carroll & Loboda, 2017; Harper & Semlitsch, 2007). However, there is still limited knowledge about how additional threats, including diseases, land use change, and altered species interactions, will impact amphibians in Subarctic landscapes. Future studies can help disentangle the influence that global threats have on amphibians in extreme, rapidly changing environments.

My study focused on a widespread amphibian species in a rapidly warming and understudied Subarctic environment. My data provide important insights into the conditions that are affecting wood frog habitat use at the macro- and microhabitat scales, as well as information that enables accurate predictions about current and future physiological conditions of frogs in this study area. With the use of physical models, environmental conditions, and habitat characteristics, predictions of terrestrial amphibian physiological conditions can be made. Our knowledge of physiological conditions can be used to enhance mechanistic modeling approaches that do not rely on current species distributions or assumptions that animals will continue to respond uniformly to different climate conditions

in the future (Araujo & Peterson, 2012; Hijmans & Graham, 2006). It is unclear how wood frogs and other ectotherms will respond to shifting environmental conditions and habitat characteristics in the future; however, with predictive mechanistic models we can begin to answer these questions. Such methodologies can be applied in all locations where climatic and habitat cover spatial data is available to predict habitat suitability across changing environmental gradients.

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Figures and Tables

Figure 1. Located in the Hudson Bay Lowlands, this picture shows the transition from boreal forest habitat with dense tree coverage (far left) to tundra habitat dominated by moss and lichen (bottom right) at a local site scale. Boreal forest patches comprise of *Picea glauca* (white spruce) and *Picea mariana* (black spruce). The ecotone habitat, or transition zone between the forest and tundra habitat, is defined by intermediate densities of Krumholtz or skirted flag trees. One of my four transect sites includes the transitional habitat on the opposite side of the forest patch seen here near Churchill, Manitoba. (Photo: Thomas Hastings)

Figure 2. Plaster models used to estimate the evaporative water loss rates, water balances, and body temperatures of wood frogs in the Subarctic landscape near Churchill, Manitoba, Canada. (Photo: Thomas Hastings)
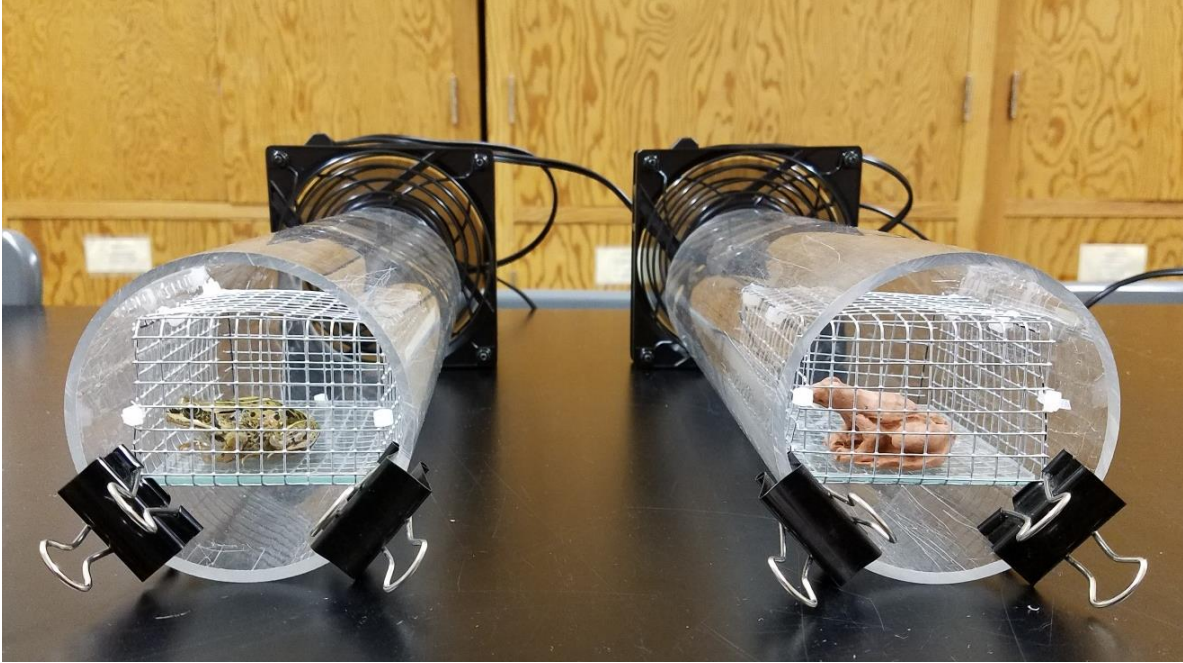
Figure 3. Wind tunnel validation trials conducted on 30 adult wood frogs and plaster models from 12 June to 4 August 2019 in Churchill, Manitoba, Canada.
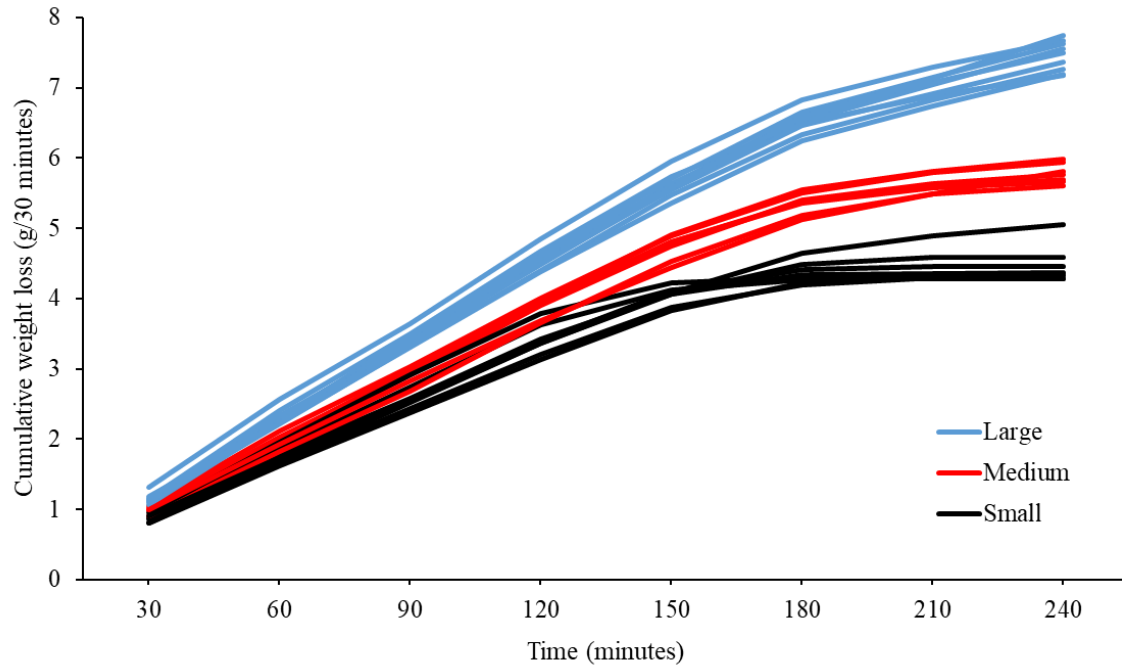
Figure 4. Outdoor water loss trials were conducted to determine how long evaporative water loss rates remain constant. Plaster models of three different size classes (distinguished by different colors) were deployed in tundra habitat with full sun, exposure to wind, and relatively humidity conditions around 31%. Models were weighed every 30 minutes and each line represents the cumulative weight loss of a plaster model over a 4-hour time period. Trials were conducted near Churchill, Manitoba on 10 June 2018.

Figure 5. Red stars indicate the location of the four sites within my study area (Churchill, Manitoba, Canada) where models were deployed along transects in each of the three macrohabitat types from June to August 2018. Blue stars indicate sites where paired plaster models were deployed at specific adult frog and paired random locations from July to August 2018. Yellow stars indicate where plaster models were deployed in 2019 at known adult frog and paired random locations from 2015 and 2016.
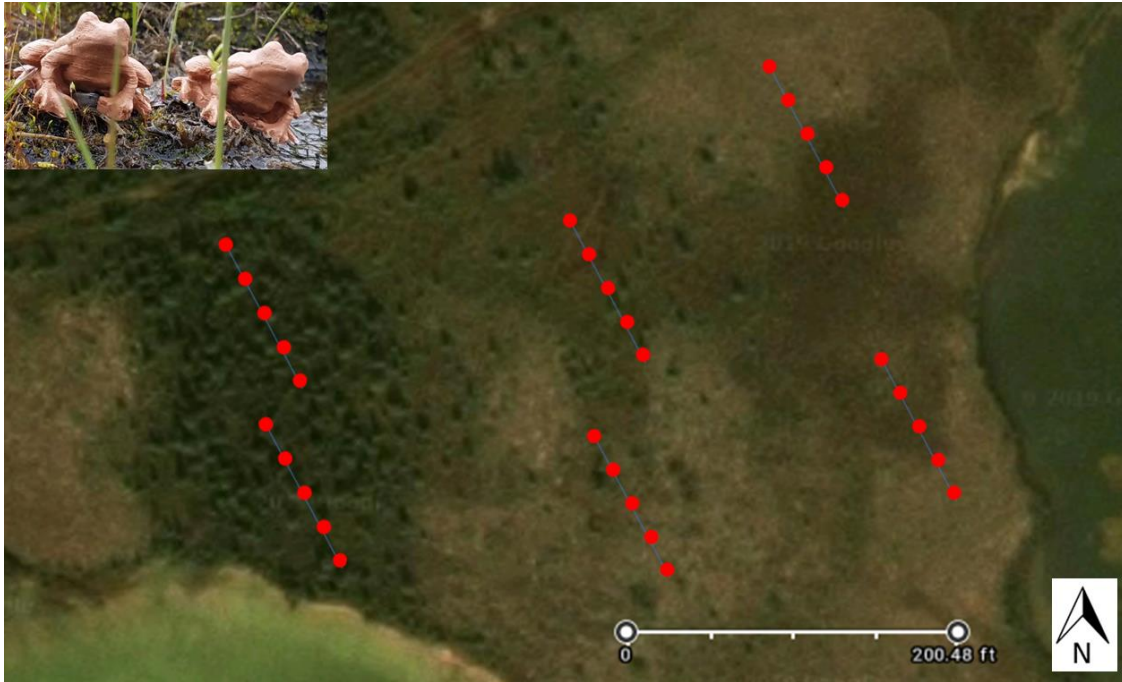
Figure 6. To compare the water balance and body temperature of wood frogs among three different Subarctic macrohabitat types, two transects were deployed in each habitat type (boreal forest patch on the left, ecotone in the middle with intermediate density of trees, and tundra on the right) with five pairs of models (top left) evenly spaced along each transect. Models were deployed along transects at my CNSC site near Churchill, Manitoba on 30 June and 25 July 2018.

The scatter plot shows the regression equation:

$$y = 0.718x - 108.64, \quad R^2 = 0.67, \quad SE = 27.24$$
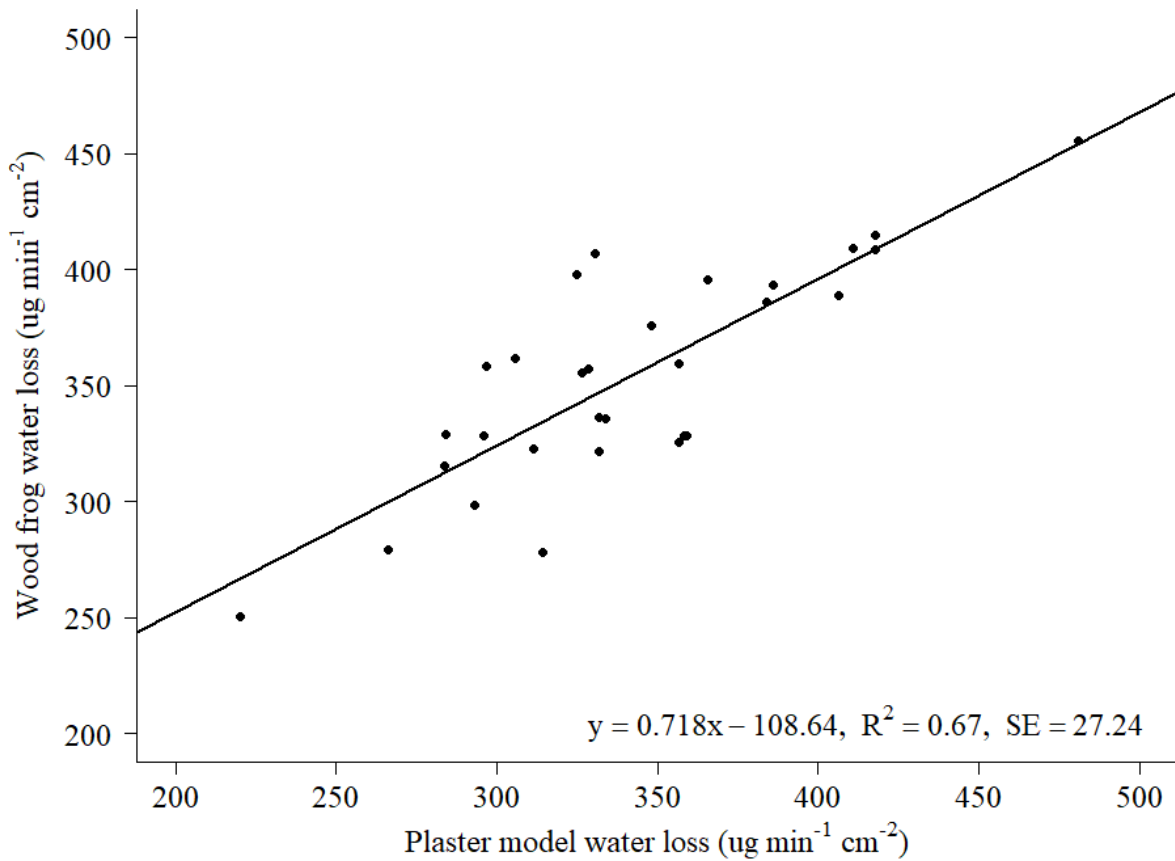
Figure 7. Linear regression with 67% of the variation in wood frog's water loss rates explained by model water loss rates ($F_{1,29} = 57.74$, $p < 0.001$, $n = 30$). Water loss data was collected from paired wood frogs and plaster models (n=30) during controlled wind tunnel trials conducted from 12 June to 4 August 2019 in Churchill, Manitoba.

$$y = 0.969x + 0.91, \ R^2 = 0.8, \ SE = 0.46$$
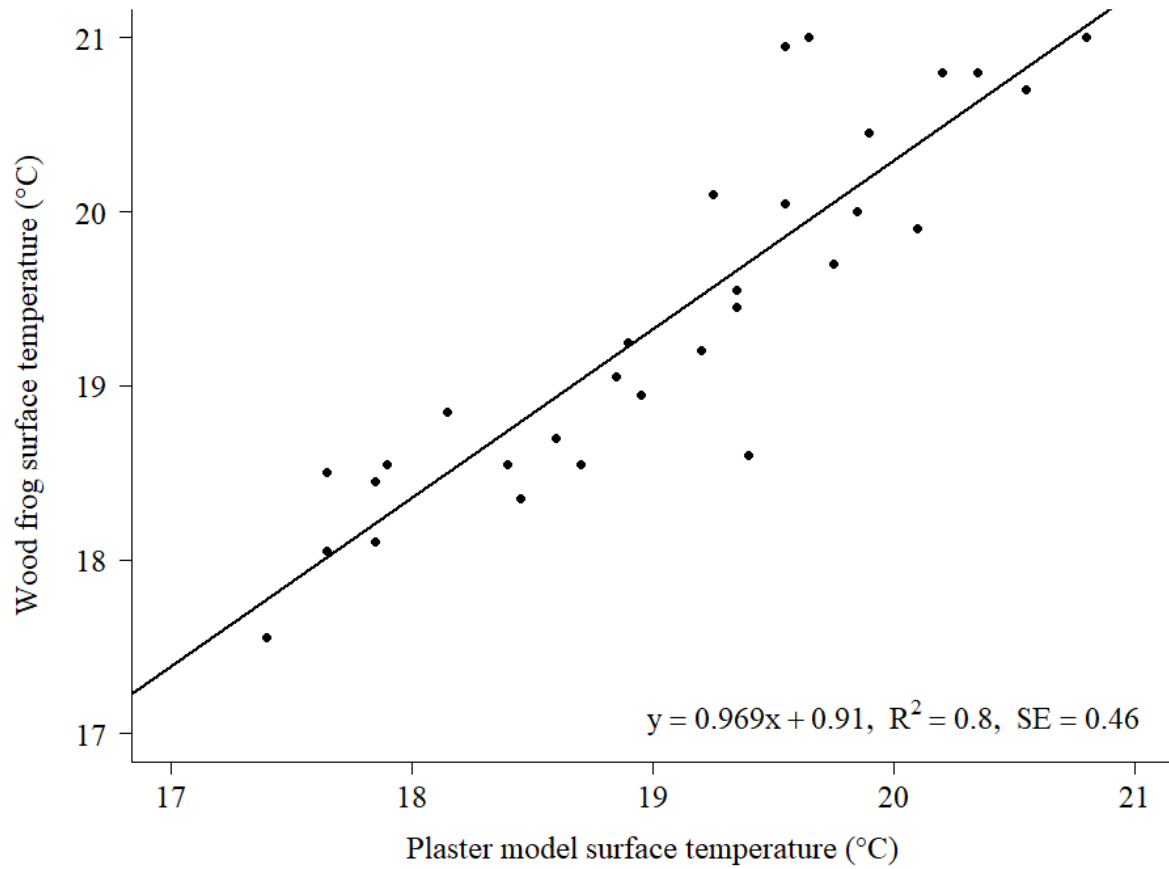
Figure 8. Linear regression with 80% of the variation in wood frog's body surface temperature explained by plaster model surface temperature ($F_{1,29} = 114.70$, $p < 0.001$, n = 30). Controlled wind tunnel trials were conducted from 12 June to 4 August 2019 in Churchill, Manitoba.

Figure 9. Linear regression with 82% of the variation in the observed body temperature of wood frogs explained by predicted body temperature when using environmental variables at the macrohabitat scale (n = 478).
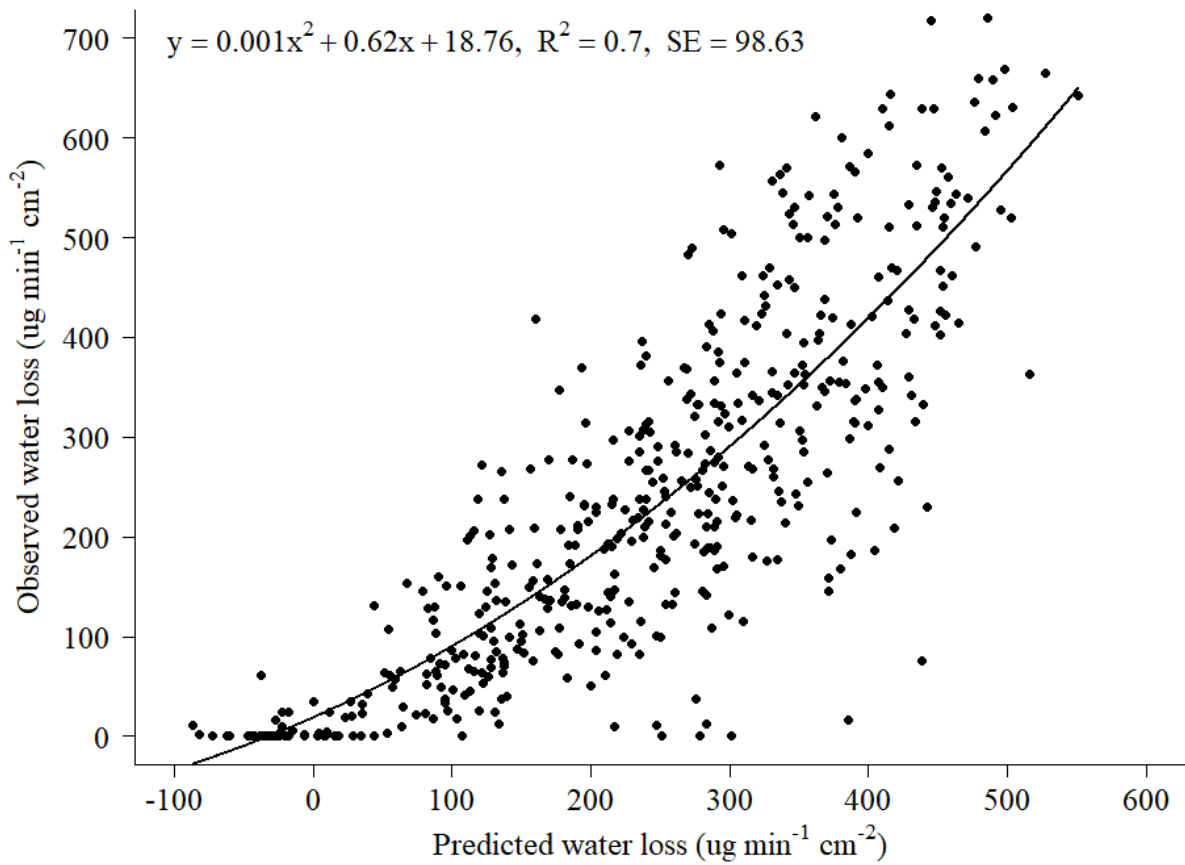
Figure 10. Quadratic regression with 70% of the variation in observed water balance of wood frogs explained by predicted water balance when using environmental variables at the macrohabitat scale (n = 478).

Figure 11. Linear regression with 60% of the variation in observed water balance of wood frogs explained by predicted water balance when using environmental variables at the microhabitat scale (n = 499).
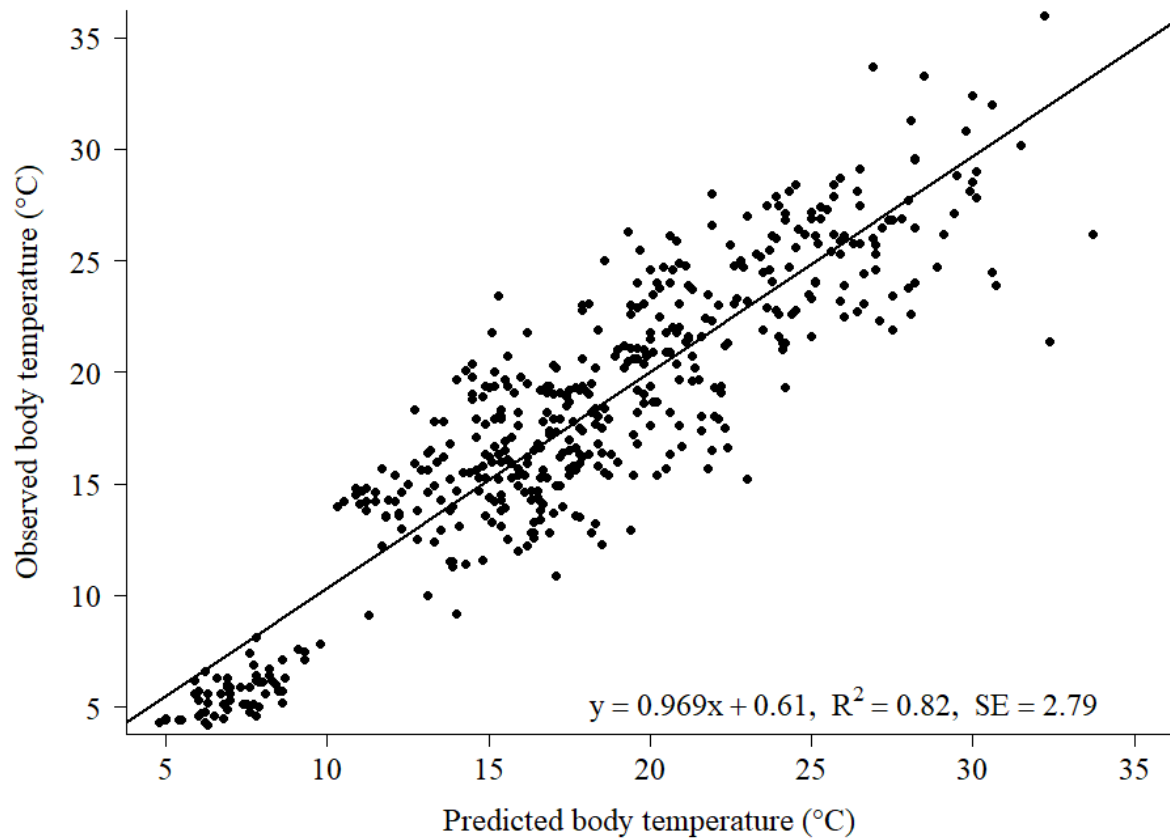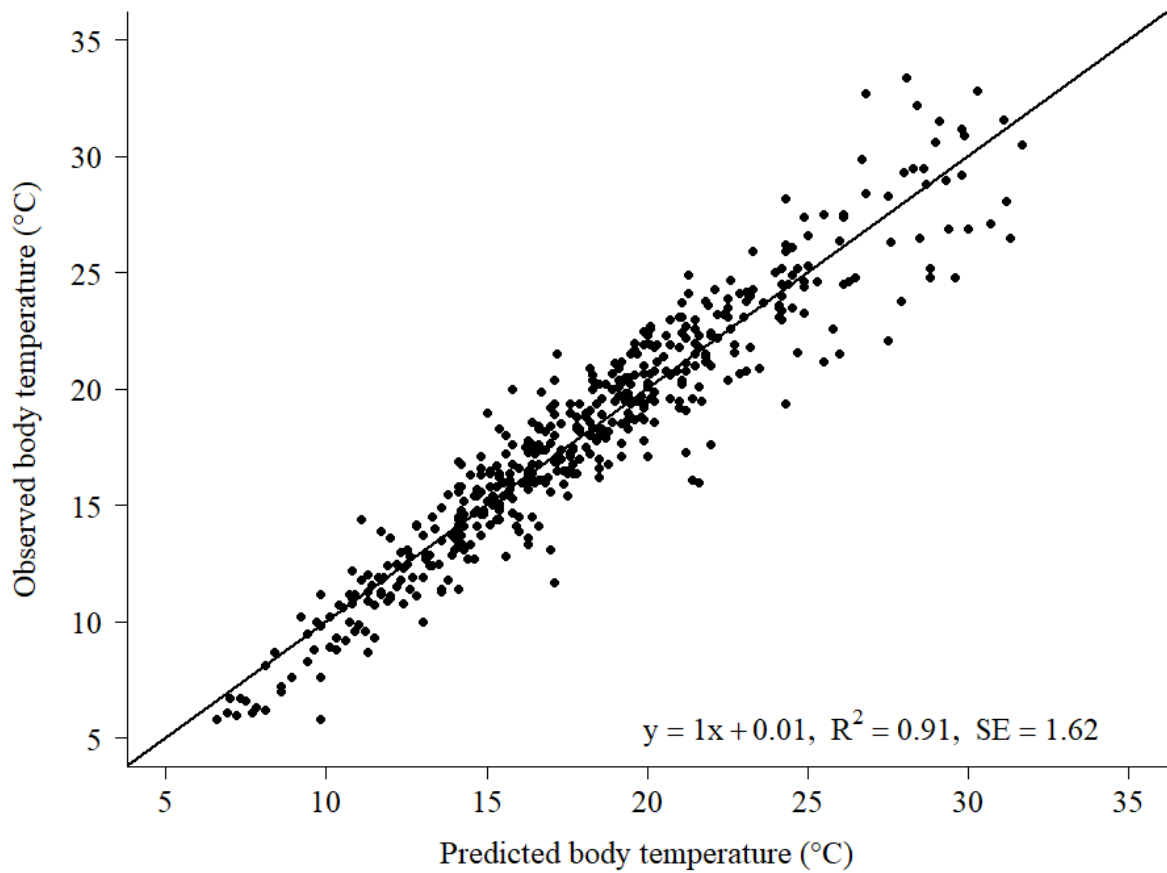
Figure 12. Linear regression with 91% of the variation in the observed body temperature of wood frogs explained by predicted body temperature when using environmental variables at the microhabitat scale (n = 498).
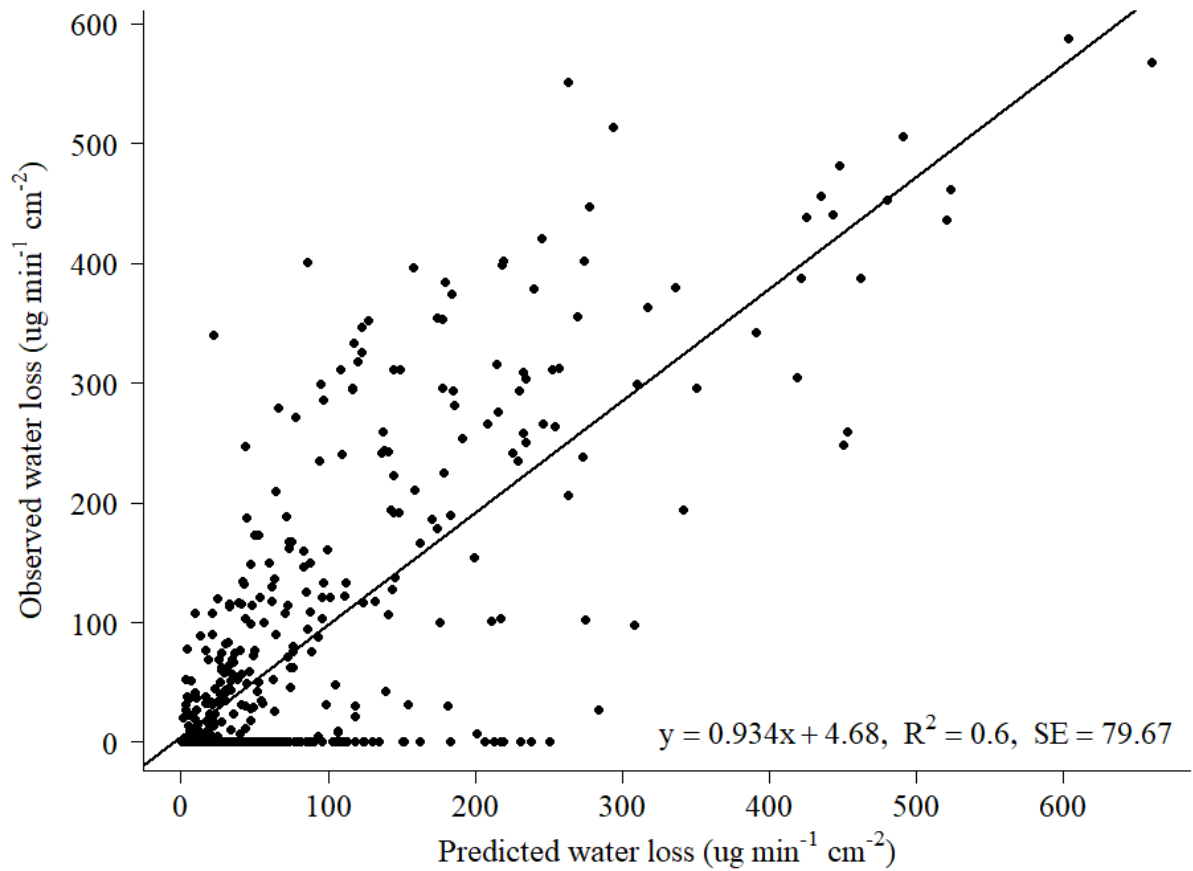
Table 1. Habitat types, environmental conditions, and habitat characteristics influencing the water balance of wood frogs at the macrohabitat scale. Water balance models were deployed at four sites near Churchill, Manitoba between 30 June and 30 July 2018. Non-important predictors, as indicated by 95% CI, are presented in bold font.

Fixed Effects	Estimate	Standard error	95% CI Upper	95% CI Lower
Intercept	182.33	46.88	92.84	270.84
Habitat: Forest	**-21.33**	**13.94**	**-47.86**	**5.64**
Habitat: Tundra	90.11	11.89	67.22	112.87
Direct sunlight	66.66	13.42	41.28	95.98
Soil (%)	-2.80	1.03	-4.75	-0.78
Moss and lichen (%)	-1.01	0.32	-1.62	-0.37
Sedge (%)	-2.56	0.64	-3.78	-1.34
Shrub (%)	-0.80	0.37	-1.51	-0.07
Humidity (%)	-2.15	0.44	-3.02	-1.31
Wind (m/s)	19.34	5.80	7.94	30.64
Ground temp. (°C)	9.32	0.79	7.73	10.82

Table 2. Environmental conditions influencing the body temperature of wood frogs at the macrohabitat scale. These environmental conditions were also used to predict the body temperature of wood frogs at the macrohabitat scale. Water balance models were deployed at four sites throughout Churchill, Manitoba between 30 June and 30 July 2018.

Fixed Effects	Estimate	Standard error	95% CI Upper	Lower
Intercept	11.36	1.04	9.37	13.36
Humidity (%)	-0.09	0.01	-0.11	-0.07
Wind (m/s)	-0.63	0.13	-0.87	-0.37
Ground temp. (°C)	0.52	0.02	0.48	0.57
Direct sunlight	1.22	0.35	0.51	1.88

Table 3. Environmental conditions, habitat characteristics, and physiological conditions that influence the odds of wood frogs using a specific microhabitat location. Habitat use of frogs was evaluated from 23 July to 25 August in 2018 and 25 June to 13 July 2019 in Churchill, Manitoba. Non-important predictors, as indicated by P-values > 0.05, are presented in bold font.

Predictor variables	Coefficient	Exp. coefficient	Odds (%)	Standard error	P-value
Water loss	-0.004	0.995	0.5	0.007	0.001
Wind (m/s)	-0.896	0.408	59.2	0.277	0.001
Direct sunlight	-0.837	0.433	56.7	0.384	0.014
Surface moisture	1.455	4.285	328.5	0.369	0.001
Sedge (%)	0.030	1.031	3.1	0.007	0.001
Shrub (%)	0.033	1.034	3.4	0.007	0.001
Soil (%)	-0.028	0.972	2.8	0.019	0.005
Body temp. (°C)	**0.041**	**1.042**	**4.2**	**0.053**	**0.283**
Air temp. (°C)	**-0.103**	**0.902**	**9.8**	**0.107**	**0.430**
Ground temp. (°C)	**0.001**	**1.001**	**0.1**	**0.050**	**0.988**

Table 4. Environmental conditions used to predict the water balance of wood frogs at four

macrohabitat sites near Churchill, Manitoba between 30 June and 30 July 2018.

Fixed Effects	Estimate	Standard error	95% CI Upper	Lower
Intercept	111.82	31.53	51.62	172.31
Direct sunlight	82.81	13.39	56.98	113.59
Wind (m/s)	43.34	5.22	30.83	54.73
Ground temp. (°C)	8.53	0.80	6.90	10.05
Humidity (%)	-2.82	0.45	-3.69	-1.94

Table 5. Environmental conditions used to predict the water balance of wood frogs at

microhabitat sites from 23 July to 25 August in 2018 and 25 June to 13 July 2019 in

Churchill, Manitoba.

Fixed Effects	Estimate	Standard error	95% CI Upper	95% CI Lower
Intercept	3.13	0.45	2.23	4.03
Humidity (%)	-0.02	0.01	-0.03	-0.01
Wind (m/s)	0.42	0.13	0.16	0.68
Ground temp. (°C)	0.11	0.02	0.07	0.14
Rain	-0.99	0.26	-1.52	-0.46
Surface moisture	-2.33	0.18	-2.69	-1.98

Table 6. Environmental conditions used to predict the body temperature of wood frogs at microhabitat sites from 23 July to 25 August in 2018 and 25 June to 13 July 2019 in Churchill, Manitoba.

Fixed Effects	Estimate	Standard error	95% CI	
			Upper	Lower
Intercept	2.48	0.28	1.94	2.97
Vapor pressure deficit (kPa)	0.58	0.12	0.34	0.78
Wind (m/s)	-0.52	0.12	-0.72	-0.26
Ground temp. (°C)	0.82	0.02	0.79	0.86
Surface moisture	0.43	0.17	0.11	0.75

Appendix A

Adult wood frogs radio-tracked from July 20, 2018 to September 11, 2018 for plaster model deployment at specific microhabitats. All frogs were measured from snout to vent (SVL).

Frog ID	Sex	Mass (g)	SVL (mm)	Site	Dates Tracked	Number of Locations	Total Days Tracked
1	F	6.51	36.63	Ramsey Road	7/20/18-7/23/18	2	4
2	M	8.02	38.32	Ramsey Road	7/20/18-8/10/18	6	22
3	M	10.03	42.88	Twin Lakes	7/20/18-8/5/18	5	17
4	F	7.55	37.27	Ramsey Road	7/21/18-8/14/18	7	25
5	M	6.92	33.30	Ramsey Road	7/21/18-7/27/18	3	7
6	M	8.56	38.98	Ramsey Road	7/21/18-7/27/18	3	7
7	M	8.68	37.79	Twin Lakes	7/21/18-8/5/18	5	16
8	M	9.37	38.98	Twin Lakes	7/21/18-7/31/18	4	11
9	M	10.97	40.97	Twin Lakes	7/21/18-8/5/18	5	16
10	-	6.67	38.10	Lindy	7/22/18-8/5/18	6	15
11	M	8.40	35.00	Lindy	7/22/18-7/24/18	2	3
12	F	9.84	36.88	Lindy	7/22/18-8/5/18	5	15
13	M	12.3	43.25	Lindy	7/22/18-9/3/18	10	44
14	-	12.27	43.44	Lindy	7/22/18-7/24/18	2	3
15	M	14.90	48.65	Lindy	7/22/18-8/20/18	7	30
16	M	7.54	33.71	Lindy	7/22/18-7/24/18	2	3
17	F	12.29	46.14	Lindy	7/24/18-9/6/18	7	45
18	F	8.70	37.31	Ramsey Road	7/25/18-8/6/18	5	13
19	M	7.70	39.46	Ramsey Road	7/31/18-8/10/18	4	11
20	F	9.96	42.65	Ramsey Road	7/31/18	1	1
21	F	11.04	40.46	Ramsey Road	7/31/18-9/5/18	9	37
22	M	9.55	39.13	Lindy_24	8/7/18-9/11/18	10	36
23	F	8.37	34.9	Lindy_24	8/7/18-9/11/18	10	36
24	F	20.43	49.11	Lindy_24	8/7/18	1	1
25	F	15.46	41.88	Lindy_24	8/7/18	1	1
26	M	9.66	39.01	Lindy_24	8/13/18-9/11/18	8	30
27	F	16.72	45.02	Lindy_24	8/13/18-9/11/18	8	30
28	F	6.79	36.65	Ramsey Road	8/14/18	1	1
29	M	9.07	40.00	Twin Lakes	8/14/18-8/18/18	2	5
30	F	12.57	42.60	Lindy	8/14/18-9/6/18	5	24
31	F	12.21	40.52	Lindy_24	8/15/18-9/11/18	7	28
32	F	12.46	45.17	Lindy_24	8/16/18-9/11/18	7	27
33	F	14.08	45.79	Lindy_24	8/16/18-9/11/18	8	27

Vita

Thomas Patrick Hastings was born in Newark, Delaware, to Philip and Susan Hastings. He is also the younger brother to Philip Hastings. He graduated from St. Mark's High School in Delaware in 2012. The following fall semester, he entered the University of Maine to study Wildlife Ecology and Conservation Biology, and in May 2016 he was awarded the Bachelor of Science degree. He remained at the University of Maine the following year to conduct research on amphibians and urban ecology. In the fall of 2017, he accepted a research assistantship in Biology at Southeast Missouri State University and transferred to Appalachian State University in the fall of 2018 to continue progress toward a Master of Science degree. The M.S. was awarded in May 2020. Following the M.S. degree, Thomas Hastings plans to work as instructor of environmental sciences. He also plans to become more involved with environmental outreach as part of his professional future.