SEASONAL WEATHER EFFECTS ON MASS VARIATION AND TORPOR EXPRESSION IN AN OBLIGATE HIBERNATOR

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By

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ABSTRACT

Global climate change, characterized by long term changes in temperature and precipitation patterns, can present an energetic challenge to terrestrial species. Seasonal hibernators are potentially vulnerable to projected changes because their annual energy management is tightly connected to predictable fluctuations in environmental resources. I studied the relationships between environmental factors and seasonal energy management in a wild population of Columbian ground squirrels (*Urocitellus columbianus*) between 2009 and 2020. I hypothesized that vegetation availability and torpor expression mediated the direct relationships between weather (i.e., temperature and precipitation) and ground squirrel energy budgets (measured as seasonal mass variation). I found that separate weather patterns influenced summer mass gain for each sex immediately following reproductive investment. As measured, vegetation availability did not significantly influence the total summer mass gain of either sex. Males lost more mass in cold winters (-11.49 ± 1.64 g body mass/1°C decrease in mean air temperature between Oct 2 and Mar 28), but female mass loss was more strongly influenced by entry mass and emergence phenology than winter weather.

I conducted a two-way factorial field experiment to test the influences of entry mass and hibernation microclimate on torpor expression and over-winter mass loss. I manipulated pre-hibernation entry mass by supplementally feeding a treatment group of females prior to hibernation. On average, fed females weighed 40 g more than unfed females entering hibernation (fed mean mass 567 ± 51 g, unfed mean mass 527 ± 40 g, $t_{25} = 2.33$, df = 25.09, p = 0.03) . I manipulated the hibernation microclimates of a treatment group containing fed and unfed females by installing snow fences upwind of their hibernacula. The snow fences created deep snowpack, which raised and stabilized the ambient temperature of the hibernacula compared to females hibernating without snow fences. Females in colder hibernacula increased torpor expression (e.g., expressed colder torpid skin temperatures, T_{sk}s), but females with larger pre-hibernation energy stores reduced torpor expression. Thus, warmer winters may reduce hibernation thermoregulatory costs and allow large ground squirrels to invest energy in torpor reduction. This study shows that the effects of projected climate changes on seasonal hibernator energy management will have complex and opposing effects for separate demographic classes depending on when the changes occur within the calendar year.

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LIST OF ACRONYMS

ACIS	Alberta Climate Information Service
AIC_c	Sample size corrected Akaike Information Criterion value
CI	Confidence Interval
HTI	Highly Thermally Insulating snowpack
$INDVI_{aug}$	Integrated (summed) NDVI values for the month of August
$INDVI_{jun}$	Integrated (summed) NDVI values for the month of June
NDVI	Normalized Difference Vegetation Index
Pc	‘climwin' metric calculating the probability of Type I errors (false positives)
PDO	Pacific Decadal Oscillation
pSEM	Piecewise Structural Equation Model
PUFAs	Polyunsaturated fatty acids
QMR	Quantitative Magnetic Resonance
SD	Standard Deviation
SE	Standard Error
T_a	Ambient temperature (°C)
T_{gr}	Ground temperature (°C)
T_{min}	Species-specific set point at which torpor metabolic rate is minimized
T_{sk}	Skin temperature (°C)
VIF	Variance Inflation Factor

CHAPTER 1:

GENERAL INTRODUCTION

1.1 Climate change and hibernators

Northern temperate regions are characterized by seasonally varying weather patterns, which produce long resource-poor winters and brief resource-rich summers (Parmesan and Yohe 2003; Lenoir and Svenning 2015). Many species inhabiting these environments are highly synchronized to their food sources and tend to conduct all, or most, reproduction and growth when food is abundant (Yoccoz and Ims 1999; Parmesan 2006; Mayor et al. 2017; Varpe 2017; Carrier et al. 2022; Smiley et al. 2022). When food is scarce, some species cope by migrating to more favourable climates (Alerstam and Bäckman 2018; La Sorte and Graham 2021; Weller et al. 2022). Other species adopt energy-minimizing strategies such as torpor or hibernation (i.e., multi-day torpor), which are characterized by profound reductions in metabolism, activity and (typically) body temperature (French 1988; Geiser 2004, 2013; Heldmaier et al. 2004; Kobbe et al. 2011; Tøien et al. 2011; Ruf and Geiser 2015).

Climate change is causing seasonal temperature and precipitation patterns to shift (Roland and Matter 2013; Pachauri et al. 2015). Species experiencing these changes are under increasing pressure to shift their own seasonal phenologies, life history strategies, and/or distributions to remain synchronized with essential food sources and habitable climes (Humphries et al. 2004; Parmesan 2006; Møller et al. 2008; Boutin and Lane 2014; Wells et al. 2022). Opportunistic heterotherms (i.e., species that do not need to maintain constant body temperatures) may be uniquely equipped to endure changes to seasonal weather patterns due to their flexible energy requirements (Geiser 2013). In contrast, strongly seasonal heterotherms, such as obligate hibernators, may be challenged by climate change if reproduction becomes desynchronized from food availability during their relatively brief active periods (Lane et al. 2012; Dobson et al. 2016). For example, Columbian ground squirrels (*Urocitellus columbianus*) hibernate for 8–9 months each year, and limit reproduction and regaining of fat stores to sustain themselves throughout the following hibernation season to the brief 3-4 month energetically favourable period (Dobson et al. 2016). Their active season is highly tuned to the annual peak in resources, and any shift in resource phenology may thus have negative consequences on annual reproduction. Understanding how climate change will influence hibernator fitness and phenology requires careful study of hibernator eco-physiology and seasonal mass variation.

1.2 Hibernation description

Among mammals, hibernation is a taxonomically widespread physiological and behavioural adaptation to seasonal periods with little to no resource availability that drastically reduces daily energy and water requirements (Geiser and Ruf 1995). For example, although they hibernate for nearly two-thirds of the year, golden-mantled ground squirrels (*Callospermophilus lateralis*) only spend 13–17% of their total annual energy budget during the hibernation season (Kenagy et al. 1989). During multi-day bouts of metabolic suppression (i.e., torpor), hibernators' body temperatures typically fall to within a few degrees of the ambient temperature (Figure 1.1; Buck and Barnes 2000; Geiser 2004).

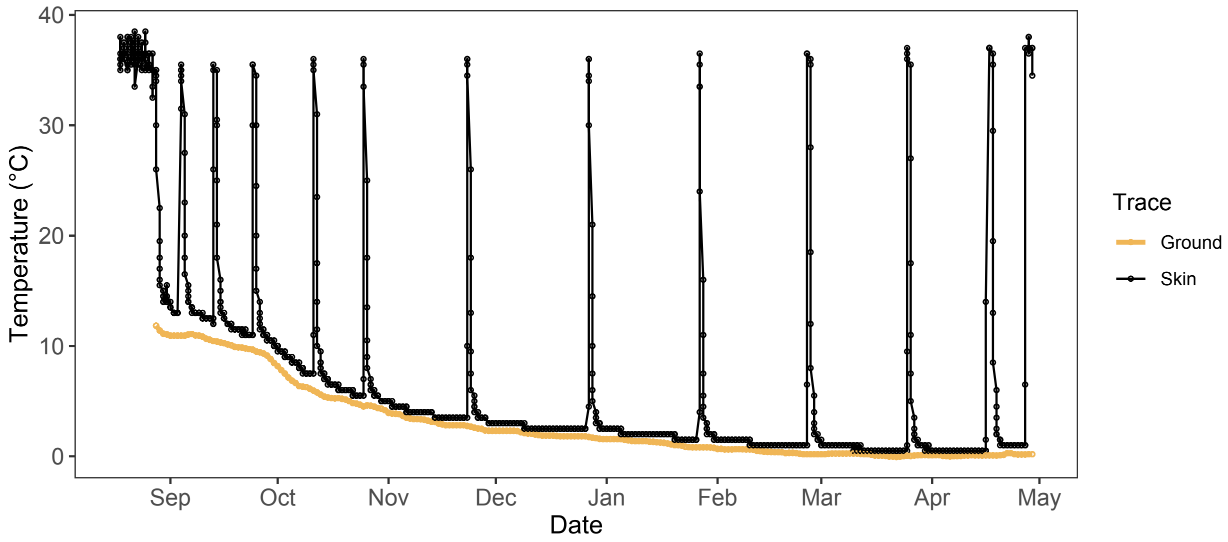

Figure 1.1: Representative ground (orange) and skin (black) temperature trace recordings (0.5°C resolution) from a female adult Columbian ground squirrel. Her first torpor bout was initiated on August 28, 2019. Her last torpor bout ended on April 27, 2020. The spikes in skin temperature are euthermic arousals. Ground and skin temperatures were recorded every four hours. Ground temperature was recorded 1 m underground.

Periodically, hibernators will rewarm to euthermic body temperatures. Euthermic arousals are energetically expensive and comprise ~70 % of the total energy spent during hibernation (Wang 1978). While their function is debated, euthermic arousals are presumed necessary for restoring physiological homeostasis and mitigating the negative physiological costs of metabolic suppression. Some of these functions include (1) restoration of depleted metabolites (Ruf et al. 2021), (2) sleep and memory consolidation (Daan et al. 1991; Popov et al. 1992; Larkin and Heller 1999), (3) restoration of renal function (Moy et al. 1972), and (4) restoration of the immune system (Prendergast et al. 2002). Maximizing body mass/resource stores at emergence, while minimizing the negative consequences of suppressed metabolism, therefore requires a careful balance of energy-saving torpor bouts and energetically expensive arousals (Young 1990b; Humphries et al. 2003b).

Torpid metabolic rate and total energy spent during hibernation are highly correlated with the ambient temperature experienced during hibernation (Buck and Barnes 2000). Torpid metabolic rate decreases as ambient temperature (T_a) decreases until a species-specific temperature (T_{min}) is reached. When T_a is equal to T_{min}, torpid metabolic rate is at its lowest, arousals occur infrequently, and the overall energetic savings are highest, due to the drastically reduced cost of thermoregulation (French 1982b; Geiser and Kenagy 1988). If T_a decreases below T_{min}, torpid metabolic rate increases to generate body heat and prevent freezing (Barnes 1989; Buck and Barnes 2000). Arousal frequency and total energetic costs also increase as ambient temperature decreases below T_{min} (Geiser and Kenagy 1988; Buck and Barnes 2000). Because of the relationships between metabolism and ambient conditions during torpor, weather has the potential to influence the amount of energy spent during hibernation. This will be explored in the following sections.

1.3 Individual variation in hibernation expression

At the individual-level, mass loss and the expression of various hibernation traits—such as arousal frequency, minimum torpid body temperature, and entry and emergence dates—are thought to be set by a circannual rhythms and modified by environmental cues (e.g., hibernacula temperature) and endogenous influences such as energy reserves (Körtner and Geiser 2000). Hibernators with larger energy reserves tend to display more energetically costly hibernation traits (e.g., maintain warmer mean torpid body temperatures or longer euthermic arousals; Boyles et al. 2007; Landry-Cuerrier et al. 2008; Fietz et al. 2020). Although it is energetically costly, reducing torpor is thought to beneficial as it mitigates the physiological costs of metabolic suppression (Boyles et al. 2020). Individuals with access to higher energy reserves also tend to enter and emerge from hibernation earlier, which may allow for predator avoidance and earlier reproduction (Michener 1978; Murie and Harris 1982; Körtner and Geiser 2000; Bieber et al. 2018).

1.4 Seasonal hibernator responses to intra-annual weather variation

The Intergovernmental Panel on Climate Change (IPCC) projects that snow depth at low mountain elevations in western North America will decrease by 25% (range 10–40%) in the near future (2031–2050; Hock et al. 2022). Without deep snowpack, soils will not be well insulated, leading to colder winter ground temperatures and potentially posing a problem for seasonal hibernators (Zhang 2005). Using modelling techniques, Boyles and McKechnie (2010) estimated that hibernating at two degrees below T_{min} increases energetic costs by up to 428%, while hibernating at two degrees above T_{min} only increases costs by up to 35% (Boyles and McKechnie 2010). These predictions are supported by empirical evidence. For example, individuals in a study of hibernating edible dormice (*Glis glis*) lost more mass in warm winters with low snowpack, presumably due to a faster rate of energy consumption due to longer euthermic arousals (Bieber and Ruf 2009; Fietz et al. 2020). Similarly, thinning snowpack was the presumed driving mechanism behind the increased mass loss of hibernating alpine marmots (*Marmota marmota*; Tafani et al. 2013). The authors suggested that a thinner layer of insulating snowpack could have allowed frost to reach deeper underground, increasing thermoregulatory costs.

While a thin snowpack provides less thermal insulation, it can also be correlated with earlier spring snowmelt—though this is complicated in areas with an increasing frequency of late season snowstorms (Neuhaus et al. 1999; Lane et al. 2012; Ernakovich et al. 2014). Early spring snowmelt signals earlier access to forage, which benefits seasonal hibernators who must fuel activities using cached or on-body resources until vegetation is available. For Columbian ground squirrels, reproduction happened earlier, and mean population fitness was higher during years of early snowmelt, potentially because they could access forage earlier compared to years with late snowmelt (Murie and Harris 1982; Neuhaus et al. 1999; Lane et al. 2012). Early snowmelt has also benefited yellow-bellied marmot (*Marmota flaviventris*) juveniles by affording them more time to gain mass prior to their first hibernation (Vuren and Armitage 1991; Ozgul et al. 2010). Further, in temperate alpine regions, early snowmelt is correlated with a slower rate of vegetation growth, which extends access to high quality new growth vegetation (Bliss 1971; Pettorelli et al. 2007; Hamel et al. 2009). This is beneficial for seasonal hibernators who depend on early summer vegetation availability and quality (i.e., digestibility and crude protein content) to recoup the mass lost during hibernation and reproduction and to accumulate body mass (i.e., fat stores) or food stores for the upcoming hibernation (Dobson and Kjelgaard 1985; Ritchie 1990; Bennett 1999; Carrier et al. 2022). In contrast, early snowmelt and early emergence could extend the active season if the subsequent hibernation entry does not also occur earlier. Therefore, early snowmelt may have negative effects on some, but not all, hibernating species.

1.5 Sex-specific responses to intra-annual weather

The timing of energetic investment in reproduction typically differs by sex, potentially creating unique windows of vulnerability to environmental conditions (Haines et al. 2022). Adult male ground squirrel's primary reproductive investment (e.g., competing for mates) occurs in early spring and can last for several weeks (Murie and Harris 1982; Michener 1992; Buck and Barnes 1999b). Because the mating season occurs at a time when there is still limited access to forage, at least a portion of the energy spent on reproduction must come from energy reserves remaining post-hibernation (Murie and Boag 1984; Michener 1998). Their dependence on remaining post-hibernation energy stores makes males vulnerable to any additional energetic challenges during the mating season. For example, male Columbian ground squirrels were disproportionately affected by a snowstorm in 1996 which extended the mating season (only 66% of males survived compared to 85% of females; Neuhaus et al. 1999). Female mammals tend to invest in reproduction over a longer period of time compared to males (e.g., Columbian ground squirrel females are gestating and lactating for 51 days out of their 3–4-month active season) but most of their energetic requirements can be met by foraging (Murie and Harris 1982; Michener 1998). Females still had much lower reproductive success in 1996 after the spring snowstorm (only 17.9 % successfully bred in 1996, compared to 76.7% in 1994, which had a warm, dry spring; Neuhaus et al. 1999). Because they have limited time to gain pre-hibernation body mass after weaning their litters, female ground squirrels may be sensitive to late-summer weather changes if they reduce their forage time or quality (Allison and Conway 2022).

1.6 Study species

Columbian ground squirrels are native to alpine and subalpine meadows of the northern Rocky Mountains (Boag and Murie 1981a; Elliott and Flinders 1991). They are medium-sized rodents (maximum pre-hibernation weight = 552–880 g; Boag and Murie 1981b) that hibernate individually for 8–9 months each year (Shaw 1925; Murie and Dobson 1987). Columbian ground squirrels at my study location are not known to create food caches (Young 1990a), which means that all metabolic activity during hibernation is fueled by on-body fat stores accumulated prior to hibernation. The general pattern of Columbian ground squirrel euthermy and torpor (of all age and sex classes) is similar to those of other small seasonally hibernating mammals: torpor bouts increase in length mid-hibernation and decrease toward the end of hibernation (Figure 1.1), while the duration of euthermic arousals decreases mid-hibernation (Shaw 1925; Young 1990b). Columbian ground squirrels are an excellent study species to understand the effects of intra-annual weather trends on intraspecific mass variation, due to their relatively long hibernation bouts, brief active seasons, and well-researched ecology.

1.7 Objective and research questions

The objective of this thesis is to test several hypotheses related to energy management across the full calendar year in a wild hibernator. I addressed three related research questions:

1) How does intra-annual variation in energetically relevant weather variables (i.e., precipitation and air temperature) influence ground squirrel body mass changes?

2) How does variation in vegetation quality and availability during the active season affect ground squirrel body mass gain?
3) How does hibernation expression interact with winter weather (e.g., snowpack depth and air temperature) to affect body mass changes?

1.8 Thesis structure

This thesis is comprised of four chapters. In **Chapter 1: General introduction**, I described the topics and emerging trends in the literature that this thesis connects. **Chapter 2: Intra-annual weather effects on sex-specific hibernator (*Urocitellus columbianus*) seasonal mass variation** tests specific hypotheses regarding ground squirrel summer mass gain and winter mass loss using long-term (11-year) biological and weather datasets. Because their energetic investments in reproduction differ temporally, I consider males and females separately. I also briefly characterize typical sex-specific ground squirrel hibernation phenotypes before investigating the influence of weather conditions on the expression of torpor and consequent mass loss. The scope of this chapter is broad and covers all three research questions. **Chapter 3: Seasonal hibernator (*Urocitellus columbianus*) energy availability determines torpor expression within microclimate limits** investigates the results of a field experiment in which I manipulated the pre-hibernation energy stores (i.e., body mass) and ambient conditions of hibernating adult female ground squirrels over the winter of 2019–2020. This chapter builds on Chapter 2 by directly evaluating the third research question through experimental manipulation of the relevant ecological variables identified in the long-term data analysis. The field experiment was limited to adult females to eliminate any confounding sex- or age class-related effects. **Chapter 4: General discussion** summarizes the key findings from both data chapters and suggests areas for future research.

CHAPTER 2:
INTRA-ANNUAL WEATHER EFFECTS ON SEX-SPECIFIC HIBERNATOR SEASONAL MASS VARIATION

2.1 INTRODUCTION

Species in northern temperate regions must adjust seasonal energy budgets to accommodate intra-annual resource variation. During the summer months, when food is abundant, these species tend to invest in growth and reproduction, while in winter, they may use behavioural and physiological adaptations such as hibernation or migration to overcome resource limitations (Careau et al. 2013; Halsey et al. 2019; Carrier et al. 2022). However, extreme weather events or disruptions to seasonal weather patterns due to climate change can threaten the fitness of these species by causing them to become desynchronized from resource availability in the environment or by increasing thermoregulatory costs (Parmesan et al. 2000; Humphries 2004).

Environmental stochasticity favours individuals that can plastically respond to or anticipate weather conditions (Bårdsen et al. 2008, 2011). Risk-sensitive energy allocation may be a critical adaptation of species living in seasonal environments to overcome periods or seasons with fewer resources (Monteith et al. 2013; Kusch et al. 2021; Smiley et al. 2022). If resources are scarce, for example during winter, theoretical and empirical evidence suggests that individuals will invest energy in somatic maintenance, even if that means having fewer resources for future reproduction (Bårdsen et al. 2008, 2011; Therrien et al. 2008; Monteith et al. 2013). The amount of energy that can be invested in somatic maintenance during the winter may depend on the amount of energy gained (i.e., fat stores) the preceding summer. Individuals or populations that have more body mass at the start of winter also tend to lose more mass during the winter so that there is less variation in body masses at the start of the summer than the start of the winter (Monteith et al. 2013; Smiley et al. 2022).

Hibernation is an energy-saving adaptation characterized by deep reductions in metabolic rate and, for most hibernating species, body temperature (French 1988; Geiser 2004, 2013; Heldmaier et al. 2004; Kobbe et al. 2011; Tøien et al. 2011; Ruf and Geiser 2015). All hibernators periodically arouse to euthermic body temperatures throughout hibernation (Humphries et al. 2003b), which is presumed necessary for restoring physiological homeostasis (e.g., urination or memory consolidation; Humphries et al. 2003b; Dobson et al. 2016; Staples 2016). Maximizing the amount of fat maintained throughout hibernation, while minimizing the negative consequences of suppressed metabolism, therefore requires a careful balance of energy-saving torpor bouts and energetically expensive arousals (Young 1990b; Humphries et al. 2003b).

Torpid metabolic rate, body temperature, and arousal patterns are highly correlated with the ambient temperature experienced during hibernation (French 1982b; Buck and Barnes 2000; Németh et al. 2009). Torpid metabolic rate decreases as ambient temperature (T_a) decreases until a species-specific temperature (T_{min}) is reached. When T_a is equal to T_{min}, torpid metabolic rate is at its lowest, arousals occur infrequently, and the overall energetic savings are highest, due to the drastically reduced cost of thermoregulation. If T_a decreases below T_{min}, torpid metabolic rate increases to generate body heat and prevent freezing (Buck and Barnes 2000). Arousal frequency and the total energetic costs of hibernation also increase as ambient temperature decreases below T_{min} (Geiser and Kenagy 1988; Buck and Barnes 2000). Thus, weather patterns that occur throughout hibernation have the potential to influence torpor expression and the amount of energy spent during hibernation.

Weather may also indirectly affect seasonal hibernators by influencing the availability or quality of forage (i.e., vegetation) during their brief active season. Seasonal hibernators that spend most of the year hibernating have relatively little time to reproduce and accumulate on- or off-body resources for the following hibernation season (Humphries et al. 2003b). Columbian ground squirrels (*Urocitellus columbianus*) are active for only 3–4 months of the year. They had higher mortality after a spring snowstorm in southern Alberta presumably delayed access to vegetation and caused the ground squirrels to deplete fat stores (Neuhaus et al. 1999). In contrast, midsummer precipitation correlated positively with adult female Columbian ground squirrel fitness (Dobson et al. 2016). One suggested, but untested, explanation was that midsummer rain encouraged plant growth, facilitating energy acquisition for both females post-lactation and juveniles (Dobson and Murie 1987; Ritchie 1990; Skibiel et al. 2013; Rubach et al. 2016). Dobson et al. (2016) also found evidence that hot, dry late summers were negatively correlated with female fitness, supporting the idea that summer forage quality and availability are important determinants of female ground squirrel fitness.

Males and females experience different seasonal energy demands, leading to the prediction that intra-annual weather variation will have sex-specific energetic consequences (Wells et al. 2022). During the active season, male Columbian ground squirrels experience peak energy expenditure during mating in the spring, while peak female energy demand occurs during midsummer lactation (Kenagy et al. 1989; Wells et al. 2022). Neuhaus et al. (1999)

reported higher male Columbian ground squirrel mortality after a late-season spring snowstorm compared to females (Neuhaus et al. 1999). Since they must emerge earlier than females to undergo gonadal recrudescence and spermatogenesis so as to be reproductively capable, male Columbian ground squirrels usually emerge earlier in the spring compared to females, potentially making them vulnerable to spring environmental conditions (Young 1990b). Female Columbian ground squirrels typically emerge after males when the weather is more likely to be mild and vegetation is more accessible (Dobson et al. 2016).

Sex-dependent time constraints and hibernation trait plasticity create unique windows of vulnerability or resiliency to weather stressors for each sex. For example, after a spring snowstorm, female Arctic ground squirrels (*Urocitellus parryii*) re-entered or extended hibernation (Williams et al. 2017). Reproductive males, however, did not re-enter hibernation, suggesting that re-entry was prevented by circulating testosterone, in anticipation of reproduction (Williams et al. 2017). The ability to plastically re-enter or extend hibernation in response to unfavorable weather conditions gave females an energy management strategy unavailable to males. During the active season, however, female Columbian ground squirrels may be more vulnerable to summer weather variation compared to males, due to their limited time to gain pre-hibernation mass (Dobson et al. 1992; Neuhaus and Pelletier 2001). Female Columbian ground squirrels have only 1-2 months for pre-hibernation fattening after weaning litters, compared to males who have 2–3 months to gain mass after spring mating. Thus, male Columbian ground squirrels may be better positioned to cope with unfavorable summer weather compared to females.

In highly seasonal or unpredictable environments, species should ensure that they have accumulated enough energy during the summer to sustain themselves through the energetically demanding winter. Thus, seasonal mass dynamics should follow predictable rhythms. It is also well established that energy stores (e.g., body fat or food stores) and environment (e.g., ambient temperature) influence the torpor expression of seasonal hibernators (Buck and Barnes 2000; Humphries et al. 2003b; Boyles et al. 2007; Landry-Cuerrier et al. 2008). However, the environmental mechanisms regulating torpor expression and seasonal mass dynamics in hibernators are not well studied—particularly in a natural setting.

In this chapter, I used long-term (11-year) weather and energetics datasets to test two hypotheses regarding the potential effects of intra-annual weather patterns on seasonal hibernator energy management. First, I hypothesized that Columbian ground squirrel mass gain can be limited by foraging time and vegetation quality or availability (the summer mass gain hypothesis). I predicted that male mass gain would not show substantial variation across years (2010–2019). Males have a relatively long period of time (2–3 months) with which to gain mass between mating and the following hibernation which should make their total summer mass gain less sensitive to weather. In contrast, I predicted that female mass gain would be more sensitive to late-summer (mid-July to late August) vegetation availability because they have a limited amount of time (< 2 months) to regain mass between weaning their litters and re-entering hibernation. Second, I hypothesized that winter mass loss is influenced by entry mass, torpor expression (e.g., frequency of arousals or mean torpor temperature), and hibernaculum ambient temperature (the winter mass loss hypothesis). I predicted that both males and females would lose more mass in cold winters with thin (i.e., poorly insulating) snowpack. I also predicted that heavier individuals would express energetically costly hibernation phenotypes, because they have the energetic resources that allow them to avoid the negative consequences of prolonged deep torpor (e.g., through a higher frequency of arousals, warmer torpor skin temperatures, more time proportionally spent euthermic), which would cause them to lose more mass than would be expected by enter mass alone.

2.2 METHODS

2.2.1 Study population

I used data collected from 2008–2020 from a continuously monitored and censused population of Columbian ground squirrels. As part of this program, researchers permanently marked individual ground squirrels with unique alphanumeric ear tags. Beginning at emergence from hibernation which typically occurred in mid to late April, researchers captured ground squirrels using live traps (Tomahawk Live Trap, Tomahawk, WI) baited with peanut butter, and weighed them to the nearest 5 g using Pesola (Prazisionswaagen AG, Schindellegi, Switzerland) spring scales (median captures per individual per year = 10, range 1–49). A subset of individuals (n = 193) was collared with temperature-sensitive data loggers (Thermocron iButton DS1921G-F5#; Maxim Integrated, San Jose, California; 0.5°C resolution) that recorded skin temperature (T_{sk}) every four hours throughout hibernation. The population occupies a mixed-species grass meadow (‘Kite Field’, ~28 ha) in Sheep River Provincial Park, Alberta (50.63° N, 114.68° W, elevation: 1480 m), which is shared in the summer with grazing cattle (*Bos taurus*) and bighorn sheep (*Ovis canadensis*).

2.2.2 Body mass

I determined summer body mass gain and winter body mass loss for each individual (Table 2.1), using the capture masses closest to hibernation entry and emergence each year. Due to variation in the number of days elapsed between when an individual emerged from, or entered, hibernation and when it was captured, it was necessary to correct for potential mass changes over these periods. I did so by calculating daily mass change after emergence or before entry using broken stick regressions and adjusting body masses by the slope of the regression for each day elapsed between capture and entry or emergence (further details are provided in Appendix A). I centred the summer mass change for each individual on emergence mass (i.e., entry mass minus emergence mass), and I centred winter mass change on entry mass (i.e., emergence mass minus entry mass). Centering mass change on seasonal starting mass accounted for the effect of body size on mass change.

Table 2.1: Sample sizes, mean mass gain, mean mass loss, and number of adult Columbian ground squirrels (*Urocitellus columbianus*) collared with temperature-logging collars prior to hibernation for each year of the study (2009–2019). Year represents the summer of mass gain and the entry year of hibernation (winter). No ground squirrels were collared in 2017, therefore entry and emergence dates could not be determined, and mass gain could not be estimated. Late-summer 2009 female mass data is sparse, and masses could not be accurately corrected for days elapsed between final capture and hibernation entry day. See Appendix A for more details on mass corrections.

Year	**Sex**	**Season**				**Adults collared (n)**
		Summer (n)	*Mass gain SE (g)*	*Winter (n)*	*Mass loss SE (g)*	
2009	Males	4	27.210	11	18.106	10
	Females	-	-	-	-	6
2010	Males	14	14.187	12	13.839	7
	Females	18	8.761	6	10.528	10
2011	Males	19	9.653	15	15.945	5
	Females	23	8.934	15	7.524	5
2012	Males	16	14.342	7	15.003	7
	Females	42	5.864	23	8.814	18
2013	Males	24	9.692	17	10.196	13
	Females	44	5.945	26	10.016	15
2014	Males	12	12.380	10	4.851	6
	Females	25	11.663	8	11.008	5
2015	Males	20	17.880	15	10.440	3
	Females	16	12.165	8	18.985	2
2016	Males	11	20.942	7	18.254	1
	Females	20	10.977	16	14.076	7
2018	Males	8	21.216	17	12.641	7
	Females	9	25.936	3	14.376	4
2019	Males	16	18.581	16	11.550	13
	Females	10	10.960	10	13.75	30

Because juveniles and yearlings do not tend to reproduce, are still growing, and male yearlings display different hibernation phenotypes compared to older individuals (e.g., later entry and emergence dates, less time spent euthermic), I restricted my analyses to adults two years old or older (Young 1990b; Dobson 1992). Females that do not successfully wean litters terminate gestation and lactation earlier than successful breeders and thus have longer to forage and fatten before hibernating (Michener 1978; Neuhaus 2000). For this reason, I excluded non-reproductive females because the mass gain hypothesis assumes that females have limited time to forage due to reproduction.

2.2.3 Hibernation trait measurement

Researchers weighed and collared a subset (n = 193) of ground squirrels prior to hibernation. Between 2009 and 2019, each collared ground squirrel was captured, weighed, and had their collar removed immediately upon capture after emergence. Some individuals were collared in multiple years (N = 145 individuals, n = 193 total temperature traces; mean = 1.35 traces/individual; range = 1-4). I calculated hibernation traits from the temperature traces, which are listed in Table 2.2. Two individuals in 2016–2017 only aroused to 28°C (potentially due to collar shift or a loose collar) therefore I considered a torpor bout initiated the first time the T_{sk} recording was $\leq$ 27°C for $\geq$ 24 hours and ended the first time the T_{sk} recording exceeded 27°C after remaining $\leq$ 27°C for $\geq$ 24 hours.

Table 2.2: Descriptions of hibernation traits measured using skin temperature recordings from a wild population of Columbian ground squirrels (*Urocitellus columbianus*) in southwest Alberta, Canada from 2009–2019 (102 adult female traces, 72 adult male traces).

Hibernation trait	**Description**
Mean torpid T_{sk} (°C)	Mean skin temperature recorded during all torpor bouts
Mean minimum torpid T_{sk}	Mean minimum skin temperature recorded during all torpor bouts
Proportion of hibernation spent euthermic	Proportion of hibernation spent euthermic (> 27 °C)
Arousal frequency (arousals/hour)	Number of arousals corrected for hibernation length
Arousal time (hours)	Total hours spent euthermic (> 27°C) between torpor bouts
Physiological emergence day	The day of year of the end of the last torpor bout (i.e., the last time T_{sk} returned to ≥ 27°C after remaining ≤ 27°C for ≥ 24 hours)
Relative physiological emergence day	Within-year mean-centred physiological emergence day
Behavioural emergence day	The day of year the individual emerges from its hibernaculum. Considered the end of hibernation.
Relative behavioural emergence day	Within-year mean-centred behavioural emergence day
Sequester time (hours)	Total hours euthermic (> 27 °C) between physiological and behavioural emergence days

2.2.4 Vegetation phenology and productivity

I estimated proxies for the quantity of vegetation available to ground squirrels using the normalized difference vegetation index (NDVI), which correlates strongly and linearly with vegetation biomass in temperate areas (Pettorelli et al. 2005). The NDVI estimates plant productivity from satellite data by calculating the difference between near-infrared and red light reflected by vegetation (Knipling et al. 1970; Michener 1977; Pettorelli et al. 2005, 2011). I chose the NDVI dataset with the highest resolution available for the years of this study. The MOD13Q1 dataset computes NDVI values from MODIS satellite images every 16 days with a spatial resolution of 250 m (Didan, 2021). Values of NDVI can range from -1 to +1, where +1 represents the maximum greenness possible. To evaluate early and late summer vegetation productivity, I calculated integrated (sum) bimonthly NDVI values, which strongly correlate with temperate vegetation biomass, for June (INDVIjun) and August (INDVIaug; Pettorelli et al. 2005, 2007). See Appendix Figure B.1 for a graphical representation of calculated NDVI metrics.

2.2.5 Weather

I used mean daily air temperature and precipitation data from an Alberta Climate Information Service (ACIS) weather station approximately 1 km from the study site (50.65, -114.62, elevation: 1525 m; https://agriculture.alberta.ca/acis). I obtained snow depth data from the Environment Canada historical weather archive (https://climate.weather.gc.ca/). The closest weather station to the study site is in Okotoks, Alberta (approximately 40 km distant; 50.73, -113.96; elevation: 1,081 m). It lies in the same East-West weather path as Kite Field, receives snow at similar times, and has been used previously to investigate the influence of snowmelt patterns on ground squirrel hibernation phenology (Lane et al. 2012). The ACIS and Environment Canada weather stations receive similar amounts of winter precipitation (i.e., January–March, 2008–2020, snow and rainfall; Wilcoxon signed rank test: $Z = -0.96$, $n = 573$, $P = 0.33$, Pearson $r = 0.05$); but the ACIS weather station near the study site receives more spring precipitation (i.e., April–June, 2008–2020, snow and rainfall, median = 2.3 cm) than the lower elevation Environment Canada station at Okotoks (median = 1.6 cm; Wilcoxon signed rank test: Z = 3.88, n = 573, P < 0.0001, Pearson r = 0.16). Okotoks snow depth data is therefore a conservative estimate of snow depth in the study area.

To effectively decouple ground and air temperatures, snowpack must be deeper than a certain threshold, which can range from 60 to 100 cm or more, depending on snow density, structure, timing, duration, and terrain characteristics (Zhang 2005; Luetschg et al. 2008; Staub and Delaloye 2017). Snowpack in Okotoks never reached 60 cm from 2008–2020 (annual maximum depth range 16–44 cm, mean 26 cm). As a proxy for highly thermally insulating snowpack (HTI), I calculated the start and end dates of long-lasting (> 1 day) snowpack that reached > 16 cm, which was the lowest recorded snowpack maximum from 2008–2020 (Zhang 2005). Because snowpack and air temperatures have opposing and unequal effects on soil temperatures depending on the time of year I also used the average January–April Pacific Decadal Oscillation (PDO) index value as a summary measure of winter conditions (Zhang 2005). I accessed data from the National Centers for Environmental Information online data archive (https://www.ncei.noaa.gov/access/monitoring/pdo/) (Mantua and Hare 2002). Negative PDO values indicate colder (Pearson r 2009–2020: P = 0.03, Pearson r = 0.63, n = 12) and snowier (Pearson r 2009–2020: P = 0.02, Pearson r = -0.67, n = 12) winters in Sheep River.

2.2.6 Ground temperature

Columbian ground squirrels in the Kite Field population hibernate at depths ranging from 80 ± 5.4 cm (adult males) to 65 ± 4.8 cm (adult females) deep (Young 1990a). Winter and spring ground temperatures that approximated the temperatures experienced by hibernating ground squirrels were measured by averaging the recordings from iButton data loggers buried approximately 50 cm deep from 2010–2016 and 2017–2020. I did not have ground temperature data from the 2016–2017 winter. One to eight data loggers were buried each year in locations with varied slopes and soil types and recorded temperature every four hours. Data loggers in 2019 and 2020 were buried consistently one metre deep. Due to inconsistencies of the buried depth of data loggers prior to 2019, some variation in measured soil temperatures existed across sample periods.

2.2.7 Statistical analyses

2.2.7.1 Sliding window analyses

All analyses were performed using R Statistical Software (v4.3.0; R Core Team 2023). I ran sliding window analyses using the R package ‘climwin’ to identify the periods during which mean summer mass gain or mean mass loss is most sensitive to the influence of weather (i.e., air temperature or precipitation) for each sex and season (Bailey and van de Pol 2016; van de Pol et al. 2016). Sliding window analyses iteratively aggregate weather patterns over all defined windows of time within a dataset and include each aggregate as a covariate in separate models. Multi-model inferencing techniques are then used to compare the strength of each model (e.g., sample size corrected measure of Akaike Information Criterion, AIC_c; Bailey and van de Pol 2016; van de Pol et al. 2016). Here, the base models used mean mass gain or loss as the response variable in weighted linear regressions. The models were weighted to account for unequal sample sizes across years. As large individuals may have lost or gained more absolute mass compared to small individuals due to their difference in size and not because of weather effects, I also included mean mass at the start of the season as a covariate. Because weather may have effects on mass gain or loss which are not captured by measuring a mean value, I also calculated weather patterns in each window as the maximum value to identify climatic thresholds (e.g., limiting foraging ability), the sum to identify cumulative effects (e.g., growing degree days), slope to capture rate of change (e.g., rate of fall cooling or spring warming), as well as the mean weather value.

To avoid the situation where a weather signal was identified in a window of time that was not biologically relevant for all years of data (e.g., winter temperature occurring after mean emergence had already occurred in another year), I calculated relative and absolute window types. Absolute windows count back from a fixed date across all years. Relative windows count back from the mean day of entry (summer) or behavioural emergence (winter) each year. Relative window signals therefore describe conditions that existed during a weather-sensitive period of the active season (summer) or hibernation (winter) rather than an annual weather window.

The risk of generating false positives is high when running so many models. To assess the reliability of signals, I therefore performed 10 randomizations and calculated the chance that the best window was a false positive using the built-in 'climwin' metric 'P_c' (Bailey and van de Pol 2016). The number of randomizations used to calculate the P_c metric was chosen based on guidance from Bailey and van de Pol (2016). A P_c value > 0.5 had a greater than 50 % chance of occurring by chance. I, therefore, considered a weather signal likely to be reliable if it had a

P_c value < 0.5. For each sex and season, I selected the best weather aggregation and window type combinations based on delta AICc and P_c values (Burnham et al. 2002).

2.2.7.2 Summer mass gain

I tested the hypothesized relationship between weather and individual relative mass gain using a linear mixed model (R package 'lme4'; Bates et al. 2015). I used linear regressions to identify relationships between summer sliding window weather signals and vegetation metrics. If a vegetation metric was related to a sliding window weather signal, I substituted the weather signal in the mixed model with the related vegetation metric as a fixed effect. The mixed model also included year and individual identity as random effects to account for repeated measures across years and individuals. I assessed model assumptions visually and tested for multicollinearity using plots and variance inflation factors.

2.2.7.3 Winter mass loss

I determined which hibernation traits (Table 2.2) varied with entry mass and weather using generalized linear mixed models. Plotted partial residuals of hibernation trait responses to entry mass variation are presented in Appendix C. Because weather could potentially have different effects on hibernation expression depending on entry mass, I also tested for interactions between entry mass and weather. I logged male mean torpor T_{sk}, mean minimum torpor T_{sk}, arousal frequency (arousals/day), arousal time (hours), physiological emergence day, behavioural emergence day, and female proportion of time spent euthermic, arousal frequency (arousals/day), and arousal time (hours) before modelling to account for moderate negative skew. Finally, I checked each model for normal distribution of residuals.

I tested the hypothesized relationships between winter weather, individual hibernation expression, and relative mass loss using piecewise structural equation modelling (pSEMs; R-package 'pSEM'; Lefcheck 2016). Structural equation models connect multiple predictor and response variables in a single network, making them ideal to visualize the direct and indirect relationships specified in my hypotheses. Arrows represent directional relationships (paths) between variables which can appear as both predictors and responses (Lefcheck 2016). I included hibernation trait variables in the pSEMs which responded to winter weather predictors in linear mixed models, as well as weather signals identified in the sliding window analysis. I removed eight males and five females from the pSEM dataset that had been caught too long after emergence to estimate accurate emergence masses to avoid fitting separate paths to different subsets of data. Consequently, three relationships between weather variables and hibernation traits that had been statistically significant in mixed models (Appendix Table B.1) became non-significant in the pSEM. Those paths are noted with asterisks in Figure 2.4.

Piecewise structural equation models are assessed using Shipley's test of directed separation, which summarizes the strength of all unspecified relationships in the hypothesized path diagram and compares this statistic (Fisher's C) to a chi-square distribution with $2k$ degrees of freedom (k = the number of tests of unspecified paths; Shipley 2000; Lefcheck 2016). If all significant relationships were connected in the input (hypothesized) path diagram, then the strength of the unconnected paths represented by C would be low and the chi-square P value non-significant (> 0.05), indicating adequate model fit. Piecewise structural equation models are less sensitive to small sample sizes compared to traditional variance-covariance structural equation models and can incorporate random effects (Lefcheck 2016). Each path included year and individual identity as random effects. I assessed path model assumptions visually and tested for multicollinearity using variance inflation factors (VIFs).

2.3 RESULTS

2.3.1 Summer

2.3.1.1 Males

The T_a and precipitation sliding window signals that most strongly influenced male summer mass gain did not significantly correlate with one another ($r = 0.34$, $p = 0.27$, $n = 12$). In the sliding window analyses, both signals positively related to mean male mass gain (Table 2.3). The precipitation slope signal was positively correlated with INDVIjun ($r = 0.64$, $p = 0.03$, $n = 12$).

Table 2.3: Top air temperature (T_a) and precipitation (Precip.) signals identified from summer sliding window analyses affecting mean male (n_{years} = 10, 2010–2019) and female (n_{years} = 9, 2011–2019) wild Columbian ground squirrel (*Urocitellus columbianus*) mass gain in southwest Alberta, Canada (2009–2019). Agg. = weather data aggregation; Date = window open – window close; ∆AICc = model improvement from baseline; P_c = probability of false positive; Std. Error = standard error; 95% CI = 95% confidence intervals

Sex	**Variable**	**Agg.**	**Window type**	**Date**	**∆AICc**	**P_c**	**Estimate**	**Std. Error**	**95% CI**
M	T_a	Mean	Absolute	May 31 – Jun 3	-9.35	0.39	8.40	1.66	4.47, 12.34
	Precip.	Slope	Absolute	Apr 18 – May 19	-18.92	0.10	204.80	23.25	149.83, 259.77
F	T_a	Max	Absolute	Aug 19-22	-11.68	0.48	9.78	1.49	6.13, 13.44
	Precip.	Mean	Absolute	Jul 16 – Aug 23	-14.54	0.23	-17.44	2.23	-22.90, -11.98

Males gained more mass in years when mean T_a from May 31 to June 3 was warmer (Table 2.4). There was a significant interaction between emergence mass and emergence day (Appendix Figure B.2) wherein large early emerging males gained less mass than small early emerging males. Late emerging males lost similar amounts of mass, regardless of relative emergence day. Although not statistically significant, population density was negatively, and $INDVI_{jun}$ was positively, related to mass gain.

Table 2.4: Male Columbian ground squirrel (*Urocitellus columbianus*) summer mass gain model summary (n = 144, conditional R^2 = 0.54). Individual identity was included as a random effect. Year was initially included as a random effect but was dropped from the final model as it explained no variance. Confidence intervals that did not cross zero are highlighted in bold. (INDVIjun = integrated June NDVI)

Fixed effects	**Estimate**	**Std. Error**	**95% CI**
Intercept	1402.00	396.00	**571.66 – 2191.65**
INDVIjun	17.10	58.14	-100.52 – 133.80
T_a	7.17	2.10	**3.20 – 11.45**
Population density	-139.00	82.84	-303.07 – 22.60
Emergence day	-8.26	2.97	**-1.42 – -2.28**
Emergence mass	-2.68	0.87	**-4.41 – -0.88**
Emergence mass: Emergence day	0.02	0.01	**0.00 – 0.03**
Individual variance	1160.00		
Residual variance	1761.00		

2.3.1.2 Females

The female T_a and precipitation sliding window signals were significantly correlated (r = -0.63, p = 0.03, n = 12), meaning mean female mass gain was higher in years with relatively warm, dry late summer weather (Table 2.3). The sliding window precipitation signal positively correlated to the number of days in the same late summer period (July 16 – Aug 23) with substantial (> 4.0 mm) rainfall (r = 0.91, p < 0.0001, n = 12) and high INDVIaug (r = 0.67, p = 0.02, n = 12). The maximum T_a signal negatively correlated to the number of rainy days (r = -0.69, p = 0.01, n = 12) but not INDVIaug (r = -0.19, p = 0.55, n = 12).

To test whether maximum T_a and mean precipitation influenced female mass gain by reducing foraging opportunities or influencing INDVIaug, I fit a linear mixed model with the number of rainy days and INDVIaug as fixed effects (Table 2.5). More rainy days from July 16 to August 23 decreased female summer mass gain. August integrated NDVI did not affect female mass gain.

Table 2.5: Female Columbian ground squirrel (*Urocitellus columbianus*) summer mass gain model summary (n = 203, conditional R^2 = 0.43). Individual identity was included as a random effect. Year explained zero variance as a random effect and was removed from the final model. Confidence intervals that did not cross zero are highlighted in bold. (INDVIaug = integrate August NDVI)

Fixed effects	**Estimate**	**Std. Error**	**95% CI**
Intercept	283.09	149.27	-6.52 – 587.72
Rainy days	-6.64	1.32	**-9.23 – -3.99**
INDVIaug	82.12	86.25	-92.98 – 245.66
Population density	3961.94	6120.05	-8291.36 – 16526.04
Emergence mass	-0.48	0.05	**-0.60 – -0.38**
Emergence day	-0.52	0.36	-1.22 – 0.18
Individual variance	140.20		
Residual variance	1358.70		

2.3.2 Winter

2.3.2.1 Correlation of weather signals

The mean T_a (Oct 2 – Mar 28) and maximum snowpack (Feb 9 – Mar 4) sliding window signals for male winter mass loss were strongly correlated ($r = -0.95$, $p < 0.0001$, $n = 10$; Figure 2.1), indicating that males tended to lose more mass in cold, deep snowpack winters (Table 2.6). Females lost more mass when the mean T_a 42–39 days before mean emergence was relatively cold and snowpack depth 119–107 days before mean emergence decreased. The female sliding window signals correlated strongly with one another ($r = 0.86$, $p = 0.003$, $n = 9$) and with winter PDO (snowpack slope: $r = 0.75$, $p = 0.02$; T_a mean: $r = 0.69$, $p = 0.04$).

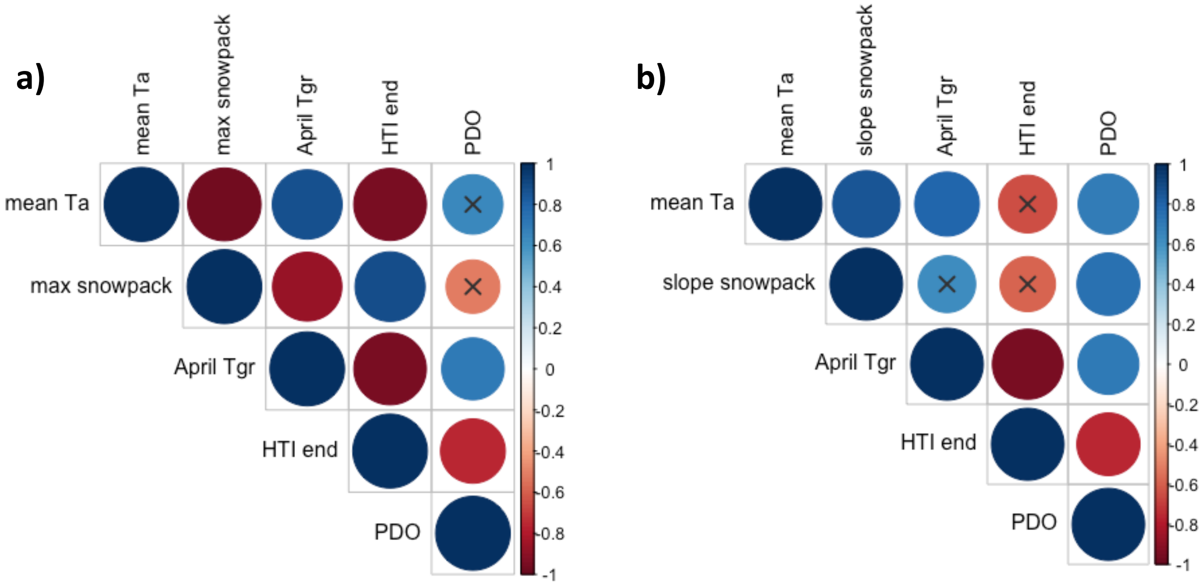

Figure 2.1: Correlation plots of sliding window signals and environmental variables affecting a) male and b) female Columbian ground squirrel winter mass loss (2010–2015, 2017–2019; n = 9). In both plots, the T_a and snowpack variables (top two rows) are sliding window signals. Mean April ground temperature (mean T_{gr}), the day of year when highly thermally insulating snowpack melted (HTI end), and the January–April averaged Pacific Decadal Oscillation (PDO) index value are the bottom three rows. Non-significant ($p \geq 0.05$) Pearson correlations are indicated by an "X." All other correlations were statistically significant ($p < 0.05$).

Table 2.6: Top air temperature (T_a) and snowpack (Snow) signals identified from winter sliding window analysis affecting mean male (n years = 10, 2010–2019) and female (n years = 9, 2011–2019) Columbian ground squirrel (*Urocitellus columbianus*) mass loss. Agg. = weather data aggregation; Date = window open – window close; ΔAICc = model improvement from baseline; P_c = probability of false positive; Std. Error = standard error; 95% CI = 95% confidence intervals

Sex	**Variable**	**Agg.**	**Window type**	**Date**	**ΔAICc**	**P_c**	**Estimate**	**Std. Error**	**95% CI**
M	T_a	Mean	Absolute	Oct 2 – Mar 28	-14.75	0.13	11.49	1.64	7.598, 15.377
	Snow	Max	Absolute	Feb 9 – Mar 4	-13.29	0.19	-1.41	0.22	-1.934, -0.893
F	T_a	Mean	Relative	42-39 days prior to mean emergence	-14.00	0.32	1.89	0.25	1.275, 2.495
	Snow	Slope	Relative	119-107 days prior to mean emergence	-17.00	0.12	6.34	0.70	4.630, 8.051

2.3.2.2 Weather effects hibernation expression and mass loss

2.3.2.2.1 Hibernation expression

Males and females both delayed physiological and behavioural emergence in cold winters with high precipitation. For example, when maximum snowpack (Feb 9–Mar 4) was lower and mean April T_{gr} was warmer, males emerged earlier ($\log_{10}$(physiological emergence day) -7.46 ± 2.03 days/1°C April T_{gr}, 95% CI [-11.63, -3.65], $\log_{10}$(behavioural emergence day) -6.62 ± 1.64 days/1°C April T_{gr}, 95% CI [-9.92, -3.46]). Females also emerged earlier when deep snowpack melted earlier and mean April T_{gr} was warmer (physiological emergence day -8.01 ± 1.49 days/1°C April T_{gr}, 95% CI [-11.06, -4.96]; behavioural emergence day -7.66 ± 1.49 days/1°C

April T_{gr}, 95% CI [-10.46, -4.37]). See Appendix Table B.1 for other weather effects on emergence phenology.

In warm winters, males and females tended to express less torpor, depending on entry mass. For example, in positive PDO years (warm, less snow), large individuals expressed warmer minimum torpor T_{sk} compared to smaller individuals (males: $7.70\text{x}10^{-4} \pm 3.41\text{x}10^{-4}$ SE °C, 95% CI [$5.19\text{x}10^{-5}$, $1.44\text{x}10^{-3}$]; females: $8.44\text{x}10^{-6} \pm 3.44\text{x}10^{-6}$ SE °C, 95% CI [$1.35\text{x}10^{-6}$, $1.61\text{x}10^{-5}$]). In negative PDO winters (cold, more snow), males and females expressed cold mean minimum torpor T_{sk}, regardless of entry mass (Figure 2.2). The female snowpack sliding window signal strongly correlated to spring PDO ($r = 0.75$, $n = 9$, $p = 0.02$) and showed the same effect on female torpor expression. When snowpack depth 119–107 days before mean female emergence became deeper, heavier females expressed warmer mean minimum torpor T_{sk}s compared to lighter females ($0.01 \pm 4.99\text{x}10^{-3}$ SE °C, 95% CI [$4.01\text{x}10^{-3}$, 0.02]; Appendix Figure B.3).

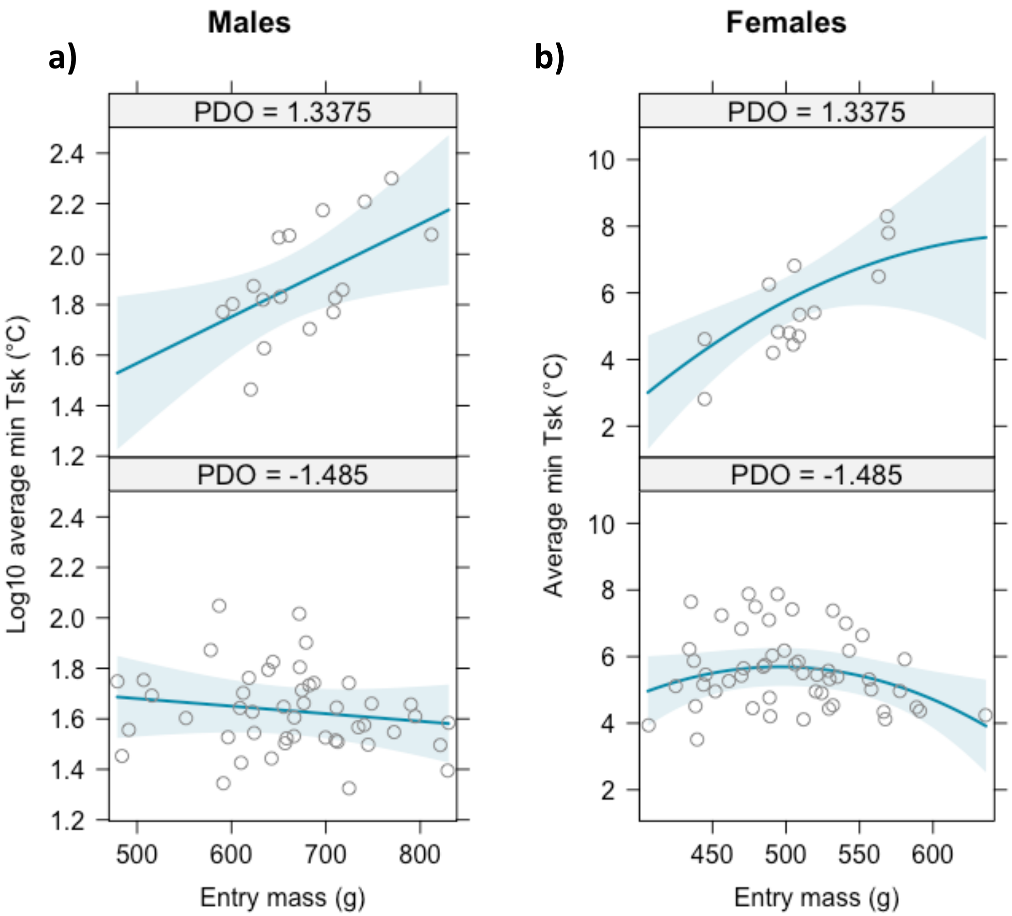

Figure 2.2: Partial residual plots of mixed models showing the effects of an interaction between January–April averaged PDO and entry mass on adult Columbian ground squirrel (*Urocitellus columbianus*) (a) male and (b) female mean minimum torpor skin temperature (T_{sk}) in southwest Alberta, Canada (n males = 52; n females = 77). Female entry mass is non-linearly related to minimum torpor T_{sk}. Plotted PDO values are the upper and lower quantiles of all years (2009–2019).

2.3.2.2.2 Mass loss

The path analysis revealed that large males tended to lose more mass than small males (-0.58 ± 0.059 SE g, 95% CI [-0.696, -0.459]) and all males lost more mass when the mean T_a from October 2 to March 28 was cold (19.70 ± 5.793 SE g mass lost for every 1°C, 95% CI [7.775, 30.855]; Figure 2.3a). The effect of entry mass had a greater effect on mass loss than the effect of mean T_a, as seen by the greater absolute value of the standardized path coefficient (-0.94 compared to 0.45). However, the non-significant paths between torpor traits and mass loss show that torpor expression does not affect the amount of mass lost beyond what is explained by winter weather and entry mass.

Relative mean T_a affected mean female relative mass loss between years (Table 2.6), but no effect was detected at the individual-level in the path analysis (Figure 2.3b). Female hibernation traits that responded to weather (arousal frequency, proportion of time spent euthermic, mean minimum T_{sk}) did not influence mass loss when accounting for entry mass. The strongest influence on female mass loss was the interaction between entry mass and relative emergence day. In this interaction, large early-emerging females tended to lose more mass than large late-emerging females (0.03 ± 0.008 SE g/day, 95% CI [0.012, 0.044], conditional R^2 0.53; Figure 2.4). Relative emergence day did not affect small female mass loss (1.25 ± 0.69 SE g/day, 95% CI [-0.100, 2.593]).

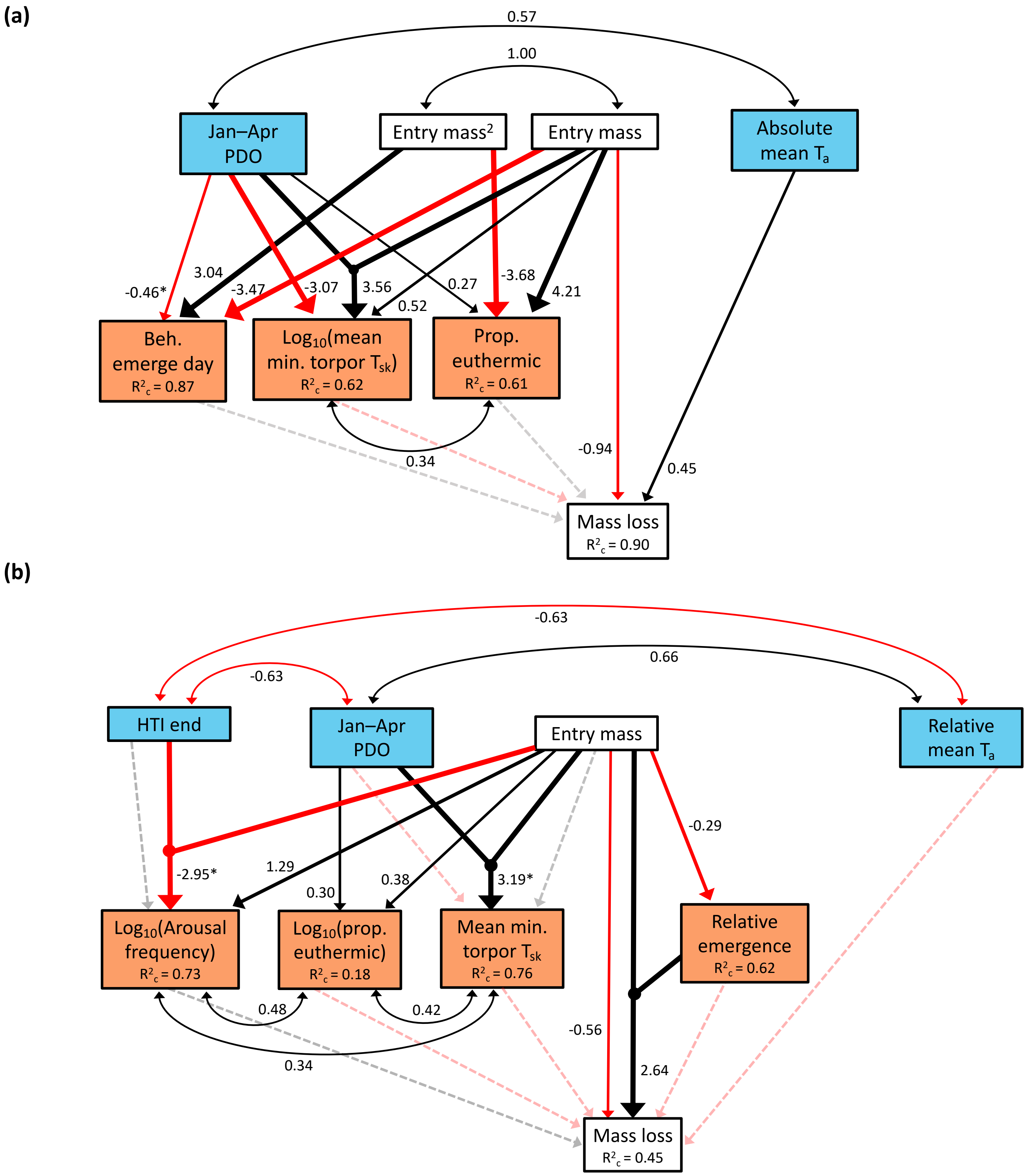
(a)
0.57
1.00
Jan–Apr PDO
Entry mass²
Entry mass
Absolute mean T_a
3.04
-0.46*
-3.47
-3.07
3.56
0.52
0.27
-3.68
4.21
Beh. emerge day R²c = 0.87
Log10(mean min. torpor T_sk) R²c = 0.62
Prop. euthermic R²c = 0.61
0.34
-0.94
0.45
Mass loss R²c = 0.90
(b)
-0.63
-0.63
0.66
HTI end
Jan–Apr PDO
Entry mass
Relative mean T_a
-0.29
-2.95*
1.29
0.30
0.38
3.19*
Log10(Arousal frequency) R²c = 0.73
Log10(prop. euthermic) R²c = 0.18
Mean min. torpor T_sk R²c = 0.76
Relative emergence R²c = 0.62
0.48
0.42
0.34
-0.56
2.64
Mass loss R²c = 0.45

Figure 2.3: Path analyses of effects of weather on individual *Urocitellus columbianus* hibernation expression and mass loss. (a) Males, n individuals = 65; (b) Females, n individuals = 61. All paths include year and individual identity as random effects. The numerical values shown are standardized model coefficients and can therefore be used to evaluate relative path strengths. Boxes (blue = weather, orange = hibernation trait, white = mass related) represent measured variables, which can appear as responses in one path or explanatory variables in another path (black = positive effect, red = negative effect). Combined arrows represent interactions. Single-headed arrows represent unidirectional relationships among variables. Curved double-headed arrows represent explanatory variables with correlated errors. Non-significant paths ($p > 0.05$) retained in the model are dashed and transparent. The thickness of significant paths is scaled by the magnitude of the associated standardized path coefficient. Conditional R^2s are presented in the boxes of response variables. T_{sk} = skin temperature, T_{gr} = ground temperature, T_a = air temperature.

[*] Relationships that were significant in individual-based mixed models (Appendix Table B.1) but became non-significant due to reduced pSEM sample size. See methods for more information.

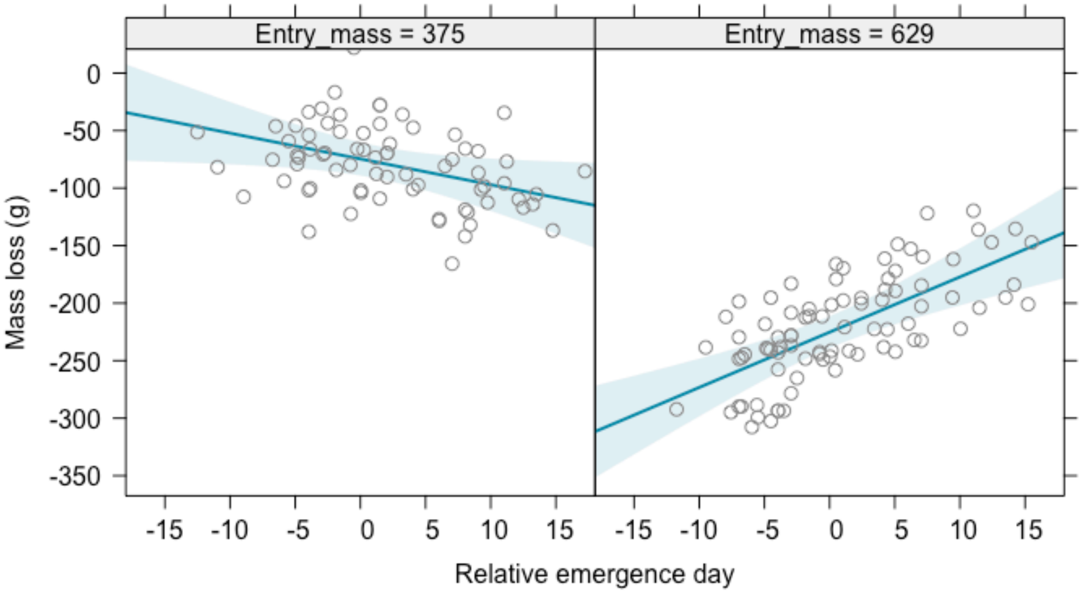

Figure 2.4: Partial residual plots showing the effect of the interaction between female entry mass (g) (upper and lower quantiles plotted; entry mass range 375–629 g, mean 504 g) and relative emergence day on winter mass loss (g). Shaded areas represent the 95% confidence intervals.

2.4 DISCUSSION

In this chapter, I tested several mechanisms through which seasonal weather may affect sex-specific ground squirrel mass changes and torpor expression. Using long-term biological, weather, and vegetation datasets, I found that summer weather directly influenced ground squirrel mass gain immediately after each sex's energetic investment in reproduction. Males gained more mass when early summer weather—immediately following the mating season—was warm. Females gained less mass when there were more rainy days in the late summer, pre-hibernation fattening period. During hibernation, male mass loss was highly affected by winter weather conditions, but individual-level female mass loss was not. Taken together, the biggest challenge to males maintaining a positive annual energy budget are cold winters and springs, while the female energy budget is influenced primarily by late-summer precipitation.

Female mass gain trends partially supported the summer mass gain hypothesis. Contrary to predictions, vegetation greenness (NDVI) did not significantly relate to female mass gain. However, late summer precipitation negatively correlated with female mass gain, suggesting that rain limited female foraging time, which did support the mass gain hypothesis. This result echoed a previous study which found that late-summer precipitation negatively affected female Columbian ground squirrel fitness (Dobson et al. 2016). Columbian ground squirrels and other semi-fossorial mammals, such as northern Idaho ground squirrels (*Urocitellus brunneus*), arctic ground squirrels, and short-tailed shrews (*Blarina brevicauda*), reduce aboveground activity on rainy days (Getz 1961; Neuhaus et al. 1999; Long et al. 2005; Williams et al. 2016; Allison and Conway 2022). Presumably, this is to avoid evaporative heat loss from wet fur (Gebremedhin and Wu 2001). The lack of direct relationship between NDVI and female mass gain may suggest that the temporal resolution of the NDVI measurement (16-days) was too coarse to detect meaningful vegetation changes.

Grazing cattle and bighorn sheep also cohabit the study area in the summer months. It is possible that late summer vegetation metrics did not influence female mass gain if forage quality consistently exceeded female's energy requirements each year. Grazing tends to extend temporal access to high quality forage, defined as high crude protein and low fibre content (Mysterud et al. 2011; Miao et al. 2015; Wu et al. 2020). Low fibre content (i.e., digestibility) in particular may be an important nutritive quality for ground squirrel mass gain. Bennett (1999) studied the juvenile growth rate of Columbian ground squirrels from Kite Field and two nearby colonies. Juveniles grew faster and had a higher probability of survival in sites that had a higher proportion of forb plant species, which occurred in colonies that also had grazing cattle present (Belovsky 1986; Bennett 1999). Forbs have higher crude protein content and are more digestible than grasses, because they have a relatively lower fibre content (Holechek 1984). Like juveniles, adult females have relatively limited time to gain mass prior to hibernation after weaning their litters. Females may not have shown any response to vegetation metrics if large herbivores extended access to or out-competed females for access to high-quality forage every year.

According to the summer mass gain hypothesis, males should not have shown much variation in total amount of mass gained from year to year due to their relatively long period to gain mass after reproduction (3–4 months, compared to the female 1.5–2 months). Surprisingly, late spring (May 31 to June 3) T_a positively related to mean male mass gain. The signal was not correlated to any NDVI metrics, implying that its effect on mass gain was not through an influence on vegetation quantity or quality. Rather, the T_a signal may have influenced the time and thermoregulatory cost males faced when foraging after the mating season ended. The mating season is the most energetically costly time of year for males, demonstrated by their low rate of mass gain during that time (Appendix A.2a), and can last two to six weeks depending on when they emerge (Neuhaus et al. 1999). During the years of this study, mean male emergence ranged from April 15 to May 7. Thus, the T_a signal reflected conditions males experienced while trying to recoup the energy spent during mating. When T_a was warmer, males may have been able to spend more time foraging aboveground. Potentially, more time foraging and less energy lost thermoregulating allowed males to gain more mass (Chappell 1980; Vispo and Bakken 1993).

The relationship between T_a and total energy spent during hibernation has generally been thought to resemble that of torpor metabolic rate (Boyles et al. 2020; Fietz et al. 2020). At T_a below a species-specific thermal setpoint (T_{min}), torpid metabolic rate and total energy spent increase sharply as T_a decreases. At T_a above T_{min}, torpid metabolic rate and total energy spent increase as T_a increases until euthermy occurs (Geiser 2004). In this chapter, I found that ground squirrel hibernation mass loss and torpor expression is influenced by a combination of extrinsic (e.g.., weather conditions) and intrinsic factors such as pre-hibernation energy stores.

For males, the path analysis results supported the winter mass loss hypothesis, showing that in cold, snowy winters males lost more mass and expressed more energetically conservative torpor patterns. Although they spent 2–17 days sequestered in their hibernacula undergoing gonadal recrudescence and maintaining energetically costly euthermic body temperatures, males were affected by winter weather over the majority of the hibernation season (Oct 2–Mar 28) rather than just the pre-emergence sequestration period. While hibernating, males expressed colder minimum torpor T_{sk}s and spent less time proportionally euthermic when the T_a weather signal was cold. In cold years, males likely expressed colder mean minimum torpor T_{sk}s and spent proportionally less time euthermic to conserve energy. However, males still lost more mass in cold winters, indicating that the energy saved by expressing energetically conservative torpor patterns did not outweigh the thermoregulatory costs incurred by cold ambient temperatures.

Contrary to the winter mass loss hypothesis, female mass loss was not influenced at the individual-level by winter weather patterns. Instead, an interaction between entry mass and relative emergence day determined how much mass females lost over winter (Figure 2.4). If large females lost more winter mass than other large females, they emerged earlier than average . Small females appear to have lost a comparable amount of mass regardless of emergence day. Thus, large females (i.e., high body fat/energy availability during hibernation; Wishart et al. 2023) may have the ability to flexibly adjust torpor expression in a risk-sensitive fashion, while small females do not. Since relative emergence day was mean-centred within each year, weather severity was not the reason why early emergers lost more mass. Potentially, large early emerging females lost more mass because they invested energy in maintaining higher mean minimum torpor T_{sk}s compared to late emerging females (Appendix Figure B.4).

Torpor expression patterns of both sexes revealed that individuals energetically prioritized physiological well-being during hibernation, potentially at the cost of future reproduction. Rather than conserve maximal energy to invest in reproduction immediately following emergence, large individuals invested energy in torpor reduction (e.g., warmer minimum torpor T_{sk}s, higher frequency of arousals, more time spent proportionally euthermic), which presumably mitigated some physiological costs of torpor. When winter weather was cold, however, large individuals also expressed certain conservative torpor traits, such as cooler mean minimum torpor T_{sk}s (Figure 2.2, Appendix Figure B.3). Winter PDO positively correlated to winter T_a and T_{gr}. Therefore, when PDO values were negative, ground squirrels likely experienced colder hibernacula and increased thermoregulatory costs (Buck and Barnes 1999a; Ortmann and Heldmaier 2000; Geiser 2004). By expressing colder mean minimum torpor T_{sk}s in cold winters, larger individuals also prioritized survival by not exhausting their energy stores before emergence (Humphries et al. 2003a). Small individuals likely did not display the same flexibility because they lacked the energetic resources to raise their body temperatures. It should be noted that collar data could not be retrieved from individuals who died before emergence. Smaller individuals are less likely to survive hibernation (Appendix Figure B.5); therefore, the mean minimum torpor T_{sk} for small individuals may be biased if a disproportionate number of small, collared individuals died during hibernation.

Conclusion and significance:

Male and female mass gain was influenced by their ability to spend time foraging immediately after their annual investment in reproduction. For females, rainy late-summer days likely decreased foraging opportunities, and therefore mass gain, despite improving vegetation quality. Cold early-summer temperatures likely increased thermoregulatory costs and reduced foraging opportunities for males when they were at their lowest annual body mass, reducing their summer mass gain. Female winter mass loss was relatively unaffected by weather conditions, but they did show some differences in torpor expression, depending on body mass and weather. While males lost more mass in cold, snowy years, the primary predictor of winter mass loss was entry mass and secondarily T_a.

Summer conditions influenced the hibernation entry mass (i.e., body fat stores) of both sexes; therefore, annual ground squirrel energy management was just as, if not more, influenced by weather conditions that occurred during the relatively brief active season compared to the relatively long hibernation season. This is consistent with studies on hibernating dormice, Eastern chipmunks (*Tamias striatus*), and hoary marmots (*Marmota caligata*) finding that summer conditions indirectly influence winter survival and mass loss by influencing pre-hibernation energy accumulation (i.e., body fat or food stores; Humphries et al. 2003a; Fietz et al. 2020; Carrier et al. 2022). Although winter weather conditions directly influence hibernators by determining the ambient temperature regime of the hibernacula, summer weather conditions had a stronger indirect effect on torpor expression and mass change by influencing entry mass. Thus, maintaining a positive annual energy budget depends on a combination of current and previous weather conditions as well as individual trade-offs between somatic and reproductive energetic investment.

In the future, male and female ground squirrels in the Kite Field population may experience unequal energy management stresses under current climate change scenarios. The early-summer T_a signal identified in the male sliding window analysis has been increasing since 2009 at a rate of 0.49 ± 0.17°C per year (Mann-Kendall test $p = 0.02$, $n = 12$). This has been accompanied by a significant increase in male summer mass gain each year (5.25 ± 1.81 g, 95% CI [1.57, 8.93]). Female mass variation and male winter mass loss did not show any significant trends over the same study period. Environment and Climate Change Canada (https://www.canada.ca/en/environment-climate-change/services/climate-change/canadian-centre-climate-services/display-download.html) projects that, under a moderate greenhouse gas emission scenario, winters in Sheep River Provincial Park are likely to become warmer and wetter (mean air temperature +3.1°C, total precipitation +13.3% by 2100) with a higher percentage of winter precipitation falling as rain, while summers become warmer and drier (mean air temperature +3.3°C, total precipitation -0.1% by 2100). The weather trends found in this study agree with these predictions. Since both sexes conserved energy and displayed more flexible torpor patterns in warmer winters, ground squirrels are unlikely to be negatively affected by warming winters. However, if the summer weather continues to become warmer and drier, late-summer vegetation quality will decline (e.g., lower water content, decreased crude protein content, decreased digestibility) and the probability of droughts will increase (Inouye et al. 2000; Parmesan 2006; Wells et al. 2022). Should warming trends continue, females may be benefit in the short-term if they have more time to forage (i.e., fewer rainy days) but be challenged in the long-term if they are unable to obtain sufficient high-quality forage to accumulate pre-hibernation mass after reproduction. In contrast, male mass gain is currently favoured by early summer warming trends.

CHAPTER 3:
ENERGY AVAILABILITY DETERMINES TORPOR EXPRESSION WITHIN MICROCLIMATE LIMITS IN A SEASONAL HIBERNATOR

3.1 INTRODUCTION

Torpor is an energy-saving tactic characterized by a reduction in metabolic rate and, for most species, body temperature (Tøien et al. 2011; Geiser 2013). Hibernators use multi-day torpor bouts to reduce resource consumption over extended periods of time—often in anticipation of seasons of reduced access to environmental sources of food and water. For certain temperate seasonal hibernators such as ground squirrels, careful management of energy budgets is critical to ensure they have enough energy to sustain themselves throughout the winter and still have energy remaining to invest in spring reproduction.

The optimal hibernation hypothesis posits that hibernation is a trade-off between maximizing the amount of energy saved during hibernation, while mitigating the potential physiological costs of torpor (Humphries et al. 2003b; Boyles et al. 2020). Potential physiological costs of the extended metabolic suppression characterizing torpor include reduced immunocompetence, dehydration, metabolite imbalance, and/or dysregulation of memory consolidation processes (Thomas and Geiser 1997; Harlow and Frank 2001; Prendergast et al. 2002; Humphries et al. 2003b; Ruf et al. 2021). These costs are thought to be at least partially reversed during energetically costly rewarming events, called arousals, when the hibernator increases their body temperature to euthermic levels before re-entering torpor (Prendergast et al. 2002; Nowack et al. 2019; Boyles et al. 2020). Increasing and maintaining a euthermic body temperature during arousals accounts for ~70% of the energy spent during hibernation (Wang 1978). Maximizing the amount of fat maintained throughout hibernation, while minimizing the negative consequences of suppressed metabolism, therefore requires careful use of energy-saving torpor bouts and energetically expensive arousals.

Total energy spent during hibernation is a function of several variables, such as torpid metabolic rate, arousal frequency, and torpid body temperature, all of which vary with ambient temperature (T_a; Buck and Barnes 2000; Németh et al. 2009; Boyles et al. 2020). As T_a decreases, torpid metabolic rate decreases linearly with body temperature until the minimal species-specific defended body temperature (T_{min}) is reached (Figure 3.1). When T_a drops below T_{min}, torpid metabolic rate increases to generate body heat, incurring high energetic costs. The amount of energy required to arouse or maintain euthermy also increases substantially at T_as below T_{min} (Geiser and Kenagy 1988; Buck and Barnes 2000; Fietz et al. 2020). As a result, hibernators can conserve the most energy during hibernation by employing long, deep torpor bouts and limiting arousals. Employing shorter, shallower torpor bouts with more frequent arousals incurs higher energetic costs, but is believed to mitigate the physiological costs of hibernation (Humphries et al. 2003b).

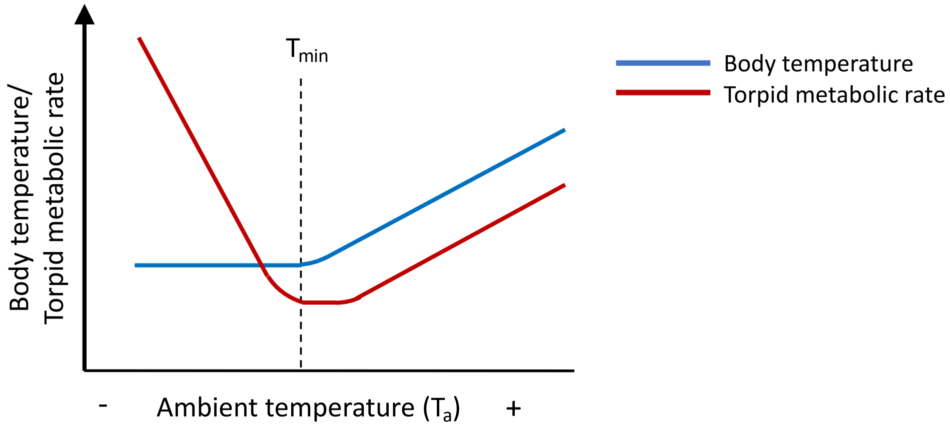

Figure 3.1: Effect of ambient temperature (T_a) on torpid metabolic rate (red) and body temperature (blue) during a torpor bout. As T_a decreases, body temperature and torpid metabolic rate decrease linearly until the species-specific minimum temperature (T_{min}) is reached. When $T_a < T_{min}$, torpid metabolic rate increases, and body temperature remains constant.

The potential range of torpor expression is broadest in cold environments, because hibernators can reach colder torpid body temperatures, but the actual expression of torpor may be regulated by access to energetic resources (Landry-Cuerrier et al. 2008). In similar T_a microclimates, hibernators with larger stores of energy, in the form of on-body fat and/or off-body food caches, often have shorter and shallower torpor bouts (i.e., reduced torpor expression; Figure 3.2) compared to individuals with smaller energy stores (French 1982b; Bieber et al. 2014; Zervanos et al. 2014; Goldberg and Conway 2021; McGuire et al. 2021). Individuals with smaller energy stores tend to have more energetically conservative torpor phenotypes presumably to ensure they have sufficient resources to survive until emergence (Murie and Boag 1984; Michener and Locklear 1990; Schorr et al. 2009). Thus, if an individual has a small store of energy, torpor expression is likely to be strongly influenced by T_a. But if the individual has large stores of energy, individual variation in torpor expression may be more strongly influenced by individual resources (Landry-Cuerrier et al. 2008).

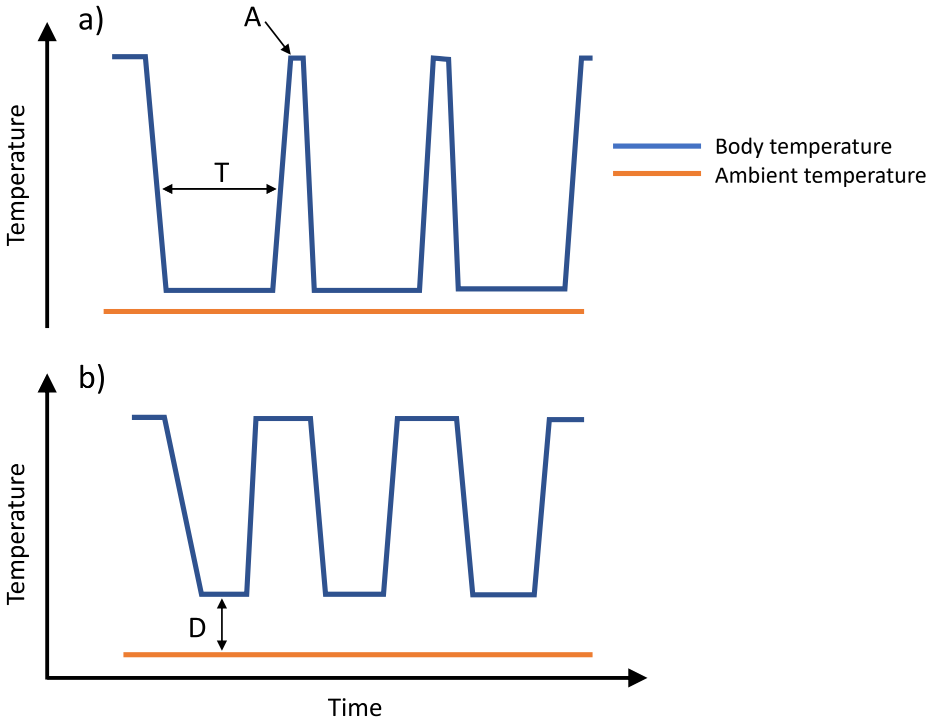

Figure 3.2: Conceptual diagrams showing partial Columbian ground squirrel (*Urocitellus columbianus*) body temperature (blue) traces of increased (a) and decreased (b) torpor expression. The orange line represents ambient temperature. In the top panel, the subject has increased torpor expression, seen by the longer torpor bouts (T), shorter euthermic arousals (A), and deeper torpor bouts (i.e., shorter distance (D) between body temperature and ambient temperature). In the bottom panel, the subject has decreased torpor expression, seen by shorter torpor bouts, longer arousals, and shallower torpor body temperatures. Torpor depth, length, and arousal length are not always correlated, but I use them here to demonstrate the concept of torpor increase versus decrease.

In this chapter, I experimentally investigate the separate and interacting effects of hibernacula T_a and individual energy stores on hibernator torpor expression and mass loss in wild female Columbian ground squirrels (*Urocitellus columbianus*). Columbian ground squirrels are seasonal hibernators, native to alpine and subalpine meadows in the northern Rocky Mountains. They are excellent subjects for studying hibernation expression and energetics. First, even subtle effects of hibernacula microclimate are potentially compounded over their remarkably long hibernation period (8–9 months each year). Second, Columbian ground squirrels sustain themselves completely during hibernation using on-body fat reserves accumulated prior to hibernation, which means torpor expression and mass loss are not influenced by food caches.

In chapter two, snowpack depth and entry mass influenced female ground squirrel mean torpor temperatures (Appendix Table B.1, Appendix Figure B.3). When snowpack depth decreased mid-hibernation, females expressed cold mean minimum torpor temperatures, regardless of entry mass. When snowpack depth increased mid-hibernation, heavy females expressed warmer mean minimum torpor temperatures compared to lighter females. In this chapter, I hypothesize that hibernacula T_a, influenced by snowpack, determines the potential depth of torpor but females regulate torpor expression depending on their energetic resources. When hibernacula T_a is cold, hibernators should be able to express colder torpor temperatures. If T_a drops below T_{min}, however, thermoregulatory costs increase drastically. At ambient temperatures < 0°C, Arctic ground squirrel (*Urocitellus parryii*) torpor metabolic rate increased proportionally with decreasing ambient temperature. Between 0 and 12°C, Arctic ground squirrel torpid metabolic rate remained constant and low (Buck and Barnes 2000). Boyles and McKechnie (2010) estimated that hibernating two degrees below T_{min} increases energetic costs by up to 428%, while hibernating at two degrees above T_{min} only increases costs by up to 35%. Thus, when T_a is below T_{min}, the energetic cost of torpid and euthermic metabolism increase, and arousals occur more frequently. But when thermoregulatory costs were not prohibitive (i.e., T_a is higher than T_{min}), hibernators with large energy stores may reduce torpor expression to mitigate the physiological costs of torpor. Together, T_a and energetic resources determine torpor expression and the amount of energy spent over hibernation.

To test this hypothesis, I manipulated the hibernacula snowpack and pre-hibernation body masses of wild female Columbian ground squirrels in a two-way factorial field experiment. Body mass was used a proxy for energy as it is positively correlated with ground squirrel fat and lean tissues, which are metabolized during hibernation (Michener and Locklear 1990; Buck and Barnes 1999b; Wells et al. 2019; Wishart et al. 2023). I predicted that females hibernating under thin snowpack would experience colder soil temperatures, express more torpor (e.g., colder mean minimum torpor T_{sk} and shorter arousals) but still lose more percent body mass compared to females hibernating under deep snowpack, due to the greatly increased energetic cost of thermoregulation if T_a fell below T_{min} (Boyles and McKechnie 2010). I also predicted that heavier females would reduce torpor expression, lose less percent body mass, and emerge from hibernation earlier compared to lighter females. Finally, heavier females under deep snowpack would express the least torpor of all treatment groups due to their warmer microclimates and large energy stores.

3.2 METHODS

3.2.1 Study population

I conducted the experiment using adult female Columbian ground squirrels from a single population occupying a mixed-species grass meadow (‘Kite Field’, ~28 ha) in Sheep River Provincial Park, Alberta (50.63° N, 114.68° W, elevation: 1480 m). Between 2008 and 2020, researchers continuously monitored and censused the population. They permanently marked each individual ground squirrel with unique alphanumeric ear tags. Beginning at emergence from hibernation which typically occurred in mid to late April, researchers regularly captured ground squirrels using live traps (Tomahawk Live Trap, Tomahawk, Wisconsin, USA) baited with peanut butter, and weighed them to the nearest 5 g using Pesola (Prazisionswaagen AG, Schindellegi, Switzerland) spring scales (median captures per individual per year = 10, range 1–49).

3.2.2 Supplemental feeding

For approximately six weeks (Jul 13–Aug 27, 2019), I supplementally fed 38 female ground squirrels *ad libitum* using RFID-activated pet feeders (Surefeed feeders Sure Petcare, Clearwater, Florida, USA). Six of the 38 females were lost before hibernation, presumably due to predation. I subcutaneously implanted each target female with a Passive Integrated Transponder (PIT) tag which allowed her to open the feeder doors and prevented non-target females from accessing the food. Target females were given access to a feeder (or multiple feeders if their territories overlapped) once they had finished weaning their litters in early to mid-July. The feeders were filled with “HI-PRO Step 3 Classic Sweet” horse feed (Trouw Nutrition, Eindhoven, Netherlands).

3.2.3 Body mass and composition measurements

To measure the effects of supplemental feeding, I used quantitative magnetic resonance scans (QMR; EchoMRI-1600; Echo Medical Systems, Houston, Texas, USA) to quantify the body compositions (i.e., amount of fat, lean mass, and free and total water) of fed and unfed females prior to hibernation. Quantitative magnetic resonance is a fast, non-invasive technique that quantifies water and body tissues (fat, lean mass, free water, and total water [g]) from live, unanesthetized animals (McGuire and Guglielmo 2010).

Prior to hibernation in 2019, I weighed and QMR-scanned each female within 15 days of her first torpor bout (range = 2–15 days; mean = 5.69). Unfortunately, logistical obstacles due to the start of the COVID-19 pandemic prevented me from obtaining emergence QMR scans of the ground squirrels in the spring of 2020. However, I did recapture and weigh each female within four days of emergence from hibernation (range = 0–4 days; mean = 0.59 days). I corrected entry and emergence body masses for days elapsed since final and initial captures using the same method described in chapter two and appendix A.

3.2.4 Hibernation monitoring

Before they entered hibernation in 2019, I fitted each supplementally fed female (n = 32) and an additional 23 unfed females (total = 55) with a collar that recorded skin temperature every four hours using a temperature-sensitive data logger (Thermocron iButtons; Maxim Integrated, San Jose, California, USA). After recapturing them in the spring of 2020, I found that 26 females had removed their collars underground before emerging. I calculated the hibernation traits described in Table 3.1 from the iButton data of the remaining 29 females.

Table 3.1: Descriptions of hibernation traits measured using skin temperature recordings from a wild population of Columbian ground squirrels (*Urocitellus columbianus*) in southwest Alberta, Canada from 2019–2020 (29 adult female traces; 4 control, 12 fed, 7 fenced, 6 fed and fenced).

Hibernation trait	**Description**
Behavioural emergence day	The day of year the individual emerges from its hibernaculum. Considered the end of hibernation.
Mean torpid T_{sk} (°C)	Mean skin temperature recorded during all torpor bouts
Mean minimum torpid T_{sk}	Mean minimum skin temperature recorded during all torpor bouts
Proportion of hibernation spent euthermic	Proportion of hibernation spent euthermic (> 27 °C)
Arousal frequency (arousals/hour)	Number of arousals corrected for hibernation length
Arousal time (hours)	Total hours spent euthermic (> 27°C) between torpor bouts

3.2.5 Snowpack manipulation

In October 2019, after all ground squirrels had begun hibernating, I used radio telemetry to locate the hibernacula of 24 females that had been fitted with radio transmitters (Holohil Systems Ltd. "PD-2C," Ontario, Canada) in addition to an iButton (13 fed, 11 unfed). I built snow fences five metres in length, 2.5 m upwind of each female's location. Four pairs of females were hibernating close enough to one another that I built one 10 m long snow fence upwind of each pair.

To measure the effect of the snowpack manipulation on ground temperature (T_{gr}) without disturbing any experimental subjects, I built eight additional snow fences in off-grid locations that would experience similar wind and precipitation patterns. Downwind (2.5 m) of each additional snow fence, and in eight unfenced control locations, I buried an iButton one metre deep and installed a post with iButtons spread 10–90 cm above ground to record T_a (i.e., snow depth, see below) and T_{gr} every three hours.

I determined snowpack depth from the iButton data by calculating daily variation (standard deviations, SD) in temperature at each iButton height. When a logger was covered by snow, it had lower daily temperature fluctuations compared to loggers that were exposed to air. I calculated maximum snowpack depth as the mean distance to the ground between the highest logger that had consistently lower variation (at least 1 SD less) than the highest logger, which recorded air temperature, for at least two weeks and the logger height above (Reusser and Zehe 2011). I defined snowmelt day as the first day that the lowest iButton's daily SD was within one SD or surpassed the highest iButton's daily SD for at least one day.

3.2.6 Statistical analyses

I performed all analyses using R Statistical Software (v4.3.0; R Core Team 2023). To quantify the effect of the snow fences on T_{gr}, I compared the mean, minimum, and variability (SD) of T_{gr}s recorded in snow fenced and control areas between November 1, 2019 (after snow fences had been constructed) and April 26, 2020, when the last snowpack melted. I used Welch's t-tests, which do not assume equal variance, to compare T_{gr} mean and minimum. To compare ground temperature variation (SD) I used a Wilcoxon test, as the data were non-normally distributed. I quantified the effect of pre-hibernation supplementary feeding by comparing the lean and fat tissues of fed and unfed treatments using Welch's t-tests. I used Cohen's *d* to compare relative effect sizes.

I calculated the hibernation mass loss and torpor traits (Table 3.1) of 29 total females (4 control, 12 fed, 7 fenced, 6 fed and fenced). To identify main effects and interactions of the feeding and fencing treatments on the mass loss and hibernation traits (Table 3.1), I used Welch's t-tests and type III ANOVAs. I used analysis of covariance (ANCOVA) to identify the interaction effects of entry mass as a continuous covariate and fencing as a categorical covariate on hibernation traits (Table 3.1). I checked all data for equal variance and normal distribution of residuals among treatments.

3.3 RESULTS

3.3.1 Snow fences

The snow fences created snowpacks that were significantly deeper (mean = 57.8 ± 10.8 cm, range = 45–77.5 cm) than control areas (mean = 19.1 ± 5.50 cm, range = 15–32.5 cm; t_{10} = -9.04, p = <0.0001; Figure 3.3). All eight fenced snow sticks, but only one control snow stick, recorded deep (> 15 cm) snowpack that persisted from late November to late April. The day when snowpack melted on controls ranged from January 19–April 23 (mean = March 8). The snowpacks behind snow fences lasted longer than control snowpacks, but warm April air temperatures (mean daily maximum T_a = 11.9 °C) caused the fenced snowpacks to melt quickly (mean April 25, range = April 22–26). On average, the soil one metre under the snow fences was 0.56°C warmer than unfenced areas (fenced mean T_{gr} 1.73 ± 0.30 °C, range = 1.29–2.29 °C; unfenced mean T_{gr} 1.17 ± 0.59 °C, range = 0.18–1.96 °C; t_{10} = -2.17, df = 10.28, p = 0.05, Cohen's d = 1.11). The fenced iButtons had minimum recorded temperatures that were 0.88°C warmer than unfenced areas (unfenced T_{gr} minimum -0.19 ± 0.75 °C, range = -1.50–1.00 °C; fenced T_{gr} minimum 0.69 ± 0.43 °C, range = 0.00–1.50 °C; t_{10} = -2.87, df = 11.15, p = 0.01, Cohen's d = 1.34). The fenced iButtons also displayed less temperature variation (i.e., greater insulation) compared to unfenced iButtons (unfenced T_{gr} variation 1.12 ± 0.12 SD, range = 0.99–1.37 SD; fenced T_{gr} variation 0.89 ± 0.10 SD, range = 0.78–1.13 SD; Wilcoxon signed rank test: Z = 2.4, p = 0.02, Cohen's d = 2.02).

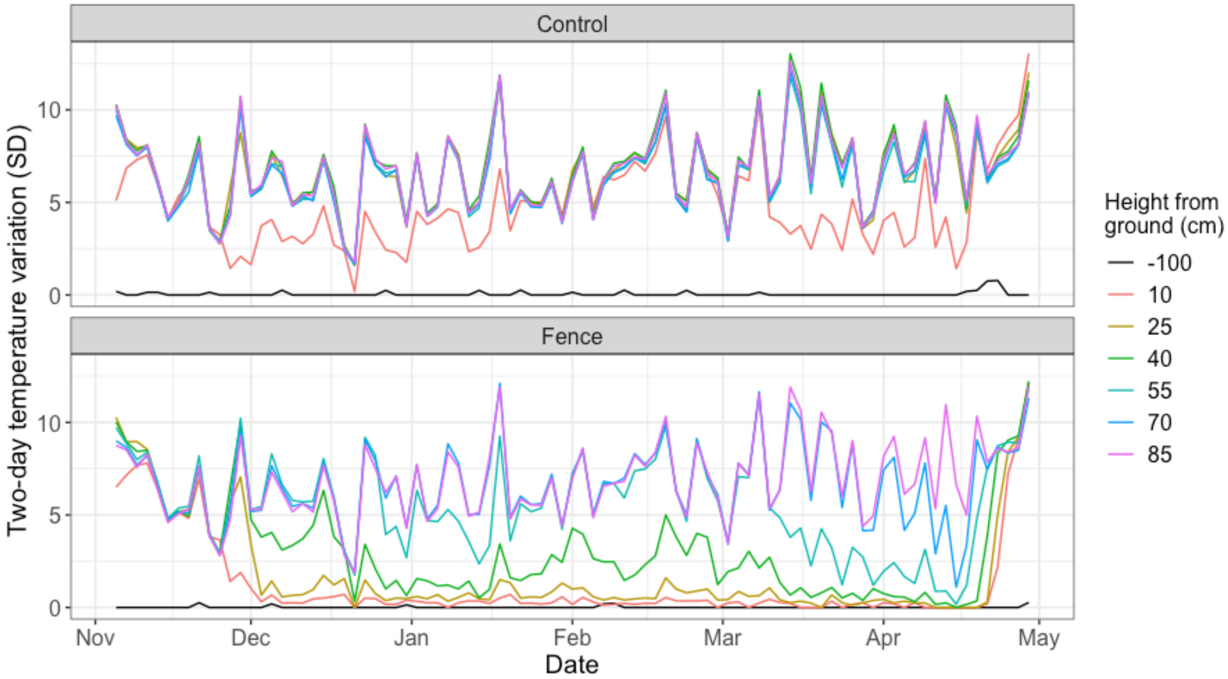

Figure 3.3: Representative time series of smoothed (two-day) temperature variations (standard deviation, SD) recorded by data loggers (iButtons) from November 2019 to April 2020. Data loggers were buried one metre belowground (-100 cm) and placed at heights 10 – 85 cm aboveground in a control area (top panel) and behind a snow fence (bottom panel). As snowpack covering an iButton increased, the magnitude of daily temperature fluctuations the iButton recorded decreased, which is represented by a lower, flatter line.

3.3.2 Supplemental feeding

At their final QMR scan in August, fed females had similar amounts of lean mass (mean 402 ± 41 g) compared to unfed females (mean 371 ± 24 g; t_9 = 1.80, p = 0.11, Cohen's d = 0.92), but significantly more body fat (fed mean 146 ± 28 g; unfed mean 103 ± 22 g; t_{11} = 3.45, p = 0.006, Cohen's d = 1.71; Appendix figure D.1). Corrected for days to entry, fed females (mean mass 567 ± 51 g) were on mean 40 g heavier than unfed females entering hibernation (mean mass 527 ± 40 g; t_{25} = 2.33, df = 25.09, p = 0.03; Table 3.2).

3.3.3 Mass loss

Fed treatments lost more percent (-35.2 ± 4.34 %) body mass than unfed treatments (-32.6 ± 5.32 %), but the difference was not statistically significant (t_{18} = -1.35, p = 0.19). Fed treatments lost more absolute body mass (-199 ± 28 g) than unfed treatments (-173 ± 38 g), and the difference was marginally non-significant (t_{17} = -1.98, p = 0.06). Unfenced treatments lost more percent (-35 ± 5.49 %) and absolute (-198 ± 34.40 g) body mass than fenced treatments (-33 ± 3.82 %, -179 ± 32.2 g), but the differences were not statistically significant (percent mass loss t_{27} = 1.00, p = 0.33; absolute mass loss t_{26} = 1.57, p = 0.12). The fed and fenced treatment lost more percent (-34.5 ± 2.61 %) and absolute body mass (-193 ± 25.9 g) than the fenced, unfed females (-32.2 ± 4.53 %, -166 ± 33.3 g) but less than the fed, unfenced females (-35.5 ± 5.07 %, -202 ± 30.1 g; Figure 3.4; Table 3.2)—although the differences were statistically non-significant (ANOVA interaction effect $F_{1, 25}$ = 0.17, p = 0.69). Emergence mass was positively correlated with entry mass (Pearson's r = 0.74, p < 0.0001).

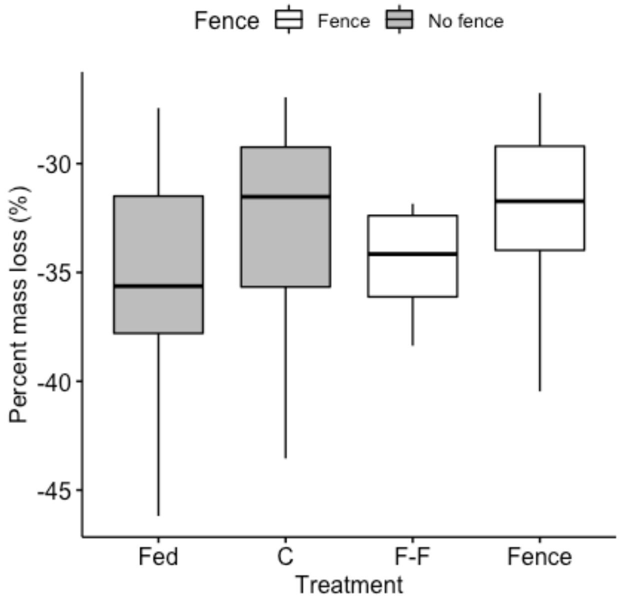

Figure 3.4: Body mass lost (as a percent of entry mass) during hibernation by supplementally fed and snow fenced female Columbian ground squirrels (*Urocitellus columbianus*, 2019–2020). On average, fed, unfenced females (“Fed”) lost the most body mass of all treatments; and fenced, unfed females (“Fence”) lost the least percent body mass. “C” = Control, “F-F” = Fed and fenced. White boxes = Fenced treatments; Grey boxes = Unfenced treatments.

Table 3.2: Grouped and ungrouped hibernation body mass changes experienced by supplementarily fed and snow fenced treatment female ground squirrels (2019–2020). Values presented are the means ± standard deviations, ranges, and sample sizes. Fenced and unfenced ground squirrel entry masses are represented by the fed and unfed categories.

Treatment (grouped)	**Entry mass (g)**	**Emergence mass (g)**	**Mass loss (g)**	**Percent mass loss (%)**
Fed (fenced and unfenced)	567 ± 51.2 (492 – 707) n = 18	372 ± 43.1 (300 – 453) n = 34	-199 ± 28.3 (-258 – -157) n = 18	-35.2 ± 4.3 (-46.2 – 27.4) n = 18
Unfed (fenced and unfenced)	527 ± 40.3 (454 – 578) n = 11	355 ± 40.4 (248 – 435) n = 43	-173 ± 38.1 (-252 – -139) n = 11	-32.6 ± 5.3 (-43.5 – 26.8) n = 11
Fenced (fed and unfed)	NA	362 ± 35.3 (300 – 435) n = 23	-179 ± 32.2 (-238 – -139) n = 13	-33.3 ± 3.8 (-40.5 – -26.8) n = 13
Unfenced (fed and unfed)	NA	362 ± 45.2 (248 – 453) n = 54	-198 ± 34.4 (-258 – -140) n = 16	-35.0 ± 5.5 (-46.2 – -27.0) n = 16
Treatment (ungrouped)				
Control	552 ± 25.7 (520 – 578) n = 4	353 ± 41.0 (248 – 418) n = 33	-186 ± 48 (-252 – -140) n = 4	-33.4 ± 7.2 (-43.5 – -27.0) n = 4
Fed	571 ± 50.1 (528 – 707) n = 12	377 ± 48.5 (300 – 453) n = 21	-202 ± 30.1 (-258 – -170) n = 12	-35.5 ± 5.07 (-46.2 – -27.4) n = 12
Fence	513 ± 41.8 (454 – 571) n = 7	360 ± 39.8 (300 – 435) n = 10	-166 ± 33.3 (-231 – -139) n = 7	-32.2 ± 4.53 (-40.5 – -26.8) n = 7
Fed + Fence	560 ± 57.3 (492 – 648) n = 6	364 ± 33.0 (310 – 410) n = 13	-193 ± 25.9 (-238 – -157) n = 6	-34.5 ± 2.61 (-38.4 – -31.8) n = 6

3.3.4 Hibernation expression

Fed females emerged earlier (123 ± 5.57 day of year) and had longer inter-bout arousals (258 ± 25.5 hours) than unfed females (127 ± 3.93 day of year; 232 ± 33.4 hours). The differences were marginally non-significant (emergence day feeding main effect F(1, 25) = [3.47], p = 0.07; arousal time feeding main effect F(1, 25) = [3.66]), p = 0.07). Type III ANOVAs of other hibernation traits did not reveal any significant main effects or interactions between treatment groups (Appendix table D.1).

Feeding and fencing both (non-significantly) increased the expression of energetically costly hibernation traits. Mean proportion of time spent euthermic (range 4.07 – 4.83), total arousal time (range 219 – 270 hours), and mean torpid T_{sk} (range 3.97 – 4.52 °C) were highest in the fed-fence treatment and lowest in the control treatment (Table 3.3).

Table 3.3: Hibernation trait expressions of four treatment groups of female ground squirrels (2019–2020). Values presented are the means ± standard deviations and ranges. Sample sizes: 4 (control), 12 (fed), 7 (fence), 6 (fed-fence).

	Treatment group			
Hibernation trait	**Unfed**	**Fed**	**Unfed-Fence**	**Fed-Fence**
Behavioural emergence (day of year)	126 ± 3.87 (120 – 129)	124 ± 6.42 (115 – 134)	127 ± 4.14 (123 – 133)	122 ± 3.31 (117 – 126)
Mean torpid T_{sk} (°C)	3.97 ± 0.40 (3.58 – 4.46)	4.23 ± 0.48 (3.55 – 5.01)	4.14 ± 0.29 (3.63 – 4.57)	4.52 ± 0.42 (3.94 – 5.03)
Mean min. torpid T_{sk} (°C)	5.13 ± 0.88 (4.15 – 6.28)	5.93 ± 0.64 (4.43 – 6.83)	5.22 ± 0.613 (4.00 – 6.82)	5.81 ± 0.86 (4.96 – 6.46)
Arousal frequency (arousals/day)	0.0521 ± 0.003 (0.049 – 0.056)	0.0565 ± 0.004 (0.050 – 0.066)	0.0542 ± 0.009 (0.043 – 0.065)	0.0562 ± 0.005 (0.052 – 0.064)
Arousal time (hours)	219 ± 31.4 (184 – 260)	252 ± 23.6 (204 – 288)	240 ± 34.4 (204 – 300)	270 ± 27.1 (228 – 304)
Prop. Time spent euthermic	4.07 ± 0.34 (3.83 – 4.56)	4.76 ± 0.47 (3.81 – 5.53)	4.60 ± 0.98 (3.65 – 6.39)	4.83 ± 0.41 (4.18 – 5.27)

In a general linear model, the interaction between continuous entry mass and fencing as a categorical covariate affected mean minimum torpid T_{sk} (interaction estimate: 0.02 +/- 0.01 °C, p = 0.01, 95% CI [0.004, 0.027]). Larger females had higher mean minimum torpor T_{sk}s when fenced (8.18×10^{-3} +/- 3.71×10^{-3} °C, p = 0.05, 95% CI [1.59×10^{-5}, 0.016], Figure 3.5a). Unfenced females had similar mean minimum torpor temperatures regardless of entry mass (-7.25×10^{-3} +/- 4.08×10^{-3} °C, p = 0.10, 95% CI [-0.016, 0.001], Figure 3.5b). Mean arousal frequency followed the same order among treatment groups as mean minimum torpid T_{sk} (highest in fed, lowest in control; Table 3.1) and was positively correlated to mean minimum torpid T_{sk} (r = 0.34, p = 0.03, n = 27).

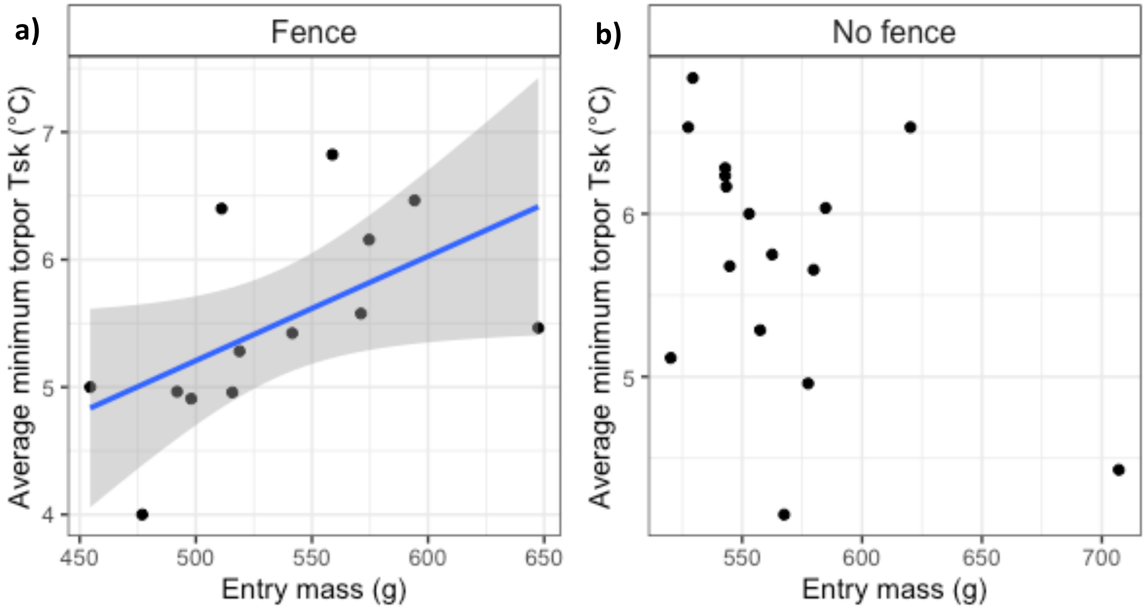

Figure 3.5: Partial regression plots showing the effect of female Columbian ground squirrel entry mass (g) on mean minimum torpor skin temperature (T_{sk} °C) of fenced (a) and unfenced (b) treatments. Larger females hibernating under snow fences had significantly warmer mean minimum T_{sk}s compared to smaller females under snow fences. Entry mass did not statistically influence the mean minimum torpor T_{sk}s of unfenced treatments. Shaded areas represent the 95% confidence intervals.

3.4 DISCUSSION

This experiment tested the separate and interacting effects of energy stores and hibernacula insulation on torpor expression and mass loss. Overall, the results supported the hypothesis that microclimate (i.e., T_a) limits maximal torpor expression and entry mass (i.e., energetic resources) regulates the expression of torpor within the bounds set by microclimate. While the data trends were in the directions predicted, in most cases they were not statistically significant. This is likely due to a lack of statistical power. Conducting this work in a wild population of ground squirrels provided a level of ecological relevance that is not achieved in a controlled laboratory, but it did present substantial challenges. These, combined with the limitations set by the COVID-19 pandemic in the spring of 2020, hindered data collection, leading to smaller-than-expected sample sizes. As expected, individuals with high entry body masses (i.e., energy stores) reduced torpor expression and lost more body mass compared to unfed treatments. The snow fences created deep snowpacks which created warmer, more thermally stable soil temperatures. Unfenced individuals reached colder T_{sk}s during torpor and lost more mass compared to fenced individuals. The total amount of mass lost during hibernation was a function of the individual's pre-hibernation energy stores and microclimate.

Thick, early established autumn snowpack traps latent summer heat in the ground, increasing mean annual ground temperature (Zhang 2005). In this experiment, the snow fences accumulated deep snowpack which persisted from late November to late April. The net effect was to increase the mean and minimum ground temperatures. In chapter two, spring ground temperatures were colder in low (negative) PDO years. The PDO value of early (January–April) 2020 was relatively low (-1.49) compared to the 1854–2021 mean (-0.30 ± 0.97). Thus, the ground regime created by snow fences mimicked warmer, high PDO years, while control areas mimicked colder, low PDO years.

In this experiment, fenced treatments experienced warmer and more stable thermal environments compared to unfenced treatments. Although the differences in mean soil temperature between fenced and unfenced treatments appear modest, the effects were accumulated over the entire 255 ± 7-day hibernation period and are likely to have substantial energetic consequences. Because torpid T_{sk} cannot be colder than the ambient temperature of the hibernacula, the potential range of torpor expression expands as ambient temperature decreases until T_{min} is reached (Landry-Cuerrier et al. 2008). Unfenced treatments, which experienced colder hibernacula, expressed more torpor (e.g., less time spent proportionally euthermic, lower arousal frequencies, colder mean and mean minimum torpor T_{sk}s) compared to fenced treatments. However, despite expressing more torpor, unfenced treatments lost a greater percentage of body mass than fenced treatments. The energetic costs of rewarming and maintaining euthermy increase as ambient temperature decreases (Humphries 2004; Karpovich et al. 2009). Therefore, unfenced treatments experienced higher energetic rewarming costs compared to fenced treatments. Unfenced individuals were also more likely to experience temperatures colder than T_{min}, which would have required them to increase their torpid metabolic rates to generate body heat and avoid freezing (Boyles and McKechnie 2010). Fenced females expressed less torpor but still conserved more energy (i.e., body mass) compared to unfenced females.

The reduction of torpor is a well-known effect of increased energy stores in both food and fat-storing hibernators (French 2000; Humphries et al. 2003b). Fed ground squirrels in this experiment entered hibernation with similar lean masses, but higher body fat stores compared to unfed females. As predicted, fed females expressed energetically costly torpor phenotypes compared to unfed females. Females that were fed and had the additional advantage of hibernating in a warm microclimate (i.e., fenced) emerged the earliest, had the warmest mean minimum torpor T_{sk}s, and had the longest arousals of all treatments. The warmer environment created by deep snowpack likely reduced the energetic cost of increasing torpor T_{sk} and maintaining euthermic body temperatures. Fed-fenced females invested their energy in further reducing torpor expression, which presumably mitigated physiological consequences of metabolic suppression, such as reduced immunocompetence, dehydration, metabolite imbalance, and/or dysregulation of memory consolidation processes (Daan et al. 1991; Thomas and Geiser 1997; Harlow and Frank 2001; Prendergast et al. 2002; Humphries et al. 2003b; Ruf et al. 2021). Fed females that were not fenced reduced torpor expression more than the control group (i.e., emerged earlier, had warmer mean and mean minimum torpor T_{sk}s, higher arousal frequency, and spent longer proportionally euthermic), but not to the same extent as fed and fenced females.

Although it was statistically non-significant, fed females emerged earlier than unfed females. Early emergence in this species is related to increased reproductive success, potentially because it gives offspring longer access to high quality vegetation and more time to grow before immergence into hibernation (Neuhaus 2000). Thermal tolerance and resource availability impose a limit on how early females may actually emerge, however (Lane et al. 2012; Allison et al. 2023). Only females which have sufficient energy stores to sustain themselves until vegetation starts growing can take advantage of early emergence and reproduction (Vuren and Armitage 1991). Early emergence may also have been beneficial for females if it reduced the physiological costs incurred by spending time in torpor (French 2000). Ground temperatures are near their annual minimum in the spring when ground squirrels emerge from hibernation. From 2010 to 2020, the annual minimum ground temperatures at the study site occurred between January and March. Reducing time spent torpid at this time therefore would have had the greatest physiological benefit. Small, unfed females likely did not have this option if they lacked the energy stores necessary to sustain euthermy until the beginning of the vegetative growing season.

I expected the deep snowpack created by snow fences to melt later than control areas, which would incline snow fenced females to emerge later than unfenced females. To varying degrees, several species of ground squirrels delay emergence during snowy springs, when melt occurs late (Michener 1977; Bronson 1980; French 1982a; Murie and Harris 1982; Lane et al. 2012; Sheriff et al. 2017; Goldberg and Conway 2021). Warm April temperatures in Sheep River caused all the snow to melt relatively quickly off the study field, however. As a result, emergence order in this experiment was much more influenced by the effect of feeding compared to fencing. Fed females emerged four days earlier on mean (marginally non-significant) than unfed females and unfenced females emerged only one day earlier on mean than fenced females.

This study assumed that the data loggers buried one metre deep accurately captured the temperature experienced by ground squirrels in their hibernacula. One metre may be deeper than the mean adult female hibernacula. Young (1990b) measured the mean adult female Columbian ground squirrel hibernacula depth at 65 ± 4.8 cm (n = 6). In the summers of 2019 and 2020, however, I dug up three radio collars that ground squirrels had removed before hibernation in burrows that were approximately one metre deep. Thus, there is some variation in hibernacula depth which may have masked the effect of the snow fences. Hibernacula at shallower depths would have lost heat faster and experienced colder spring temperatures (Goodrich 1982; Zhang 2005).

It should also be noted that the level of polyunsaturated fatty acids (PUFAs) in hibernator diets has been shown to affect torpor expression (Geiser et al. 1994; Frank et al. 2008; Frank 2012). Hibernators with moderately high PUFA diets (i.e., 33–74 mg/g) have a higher propensity to initiate torpor and have lower metabolic rates during torpor (Frank 2012). The feed used in this experiment contained only 25 mg/g (2.5%) non-hydrogenated vegetable oil, which may have prevented supplementally fed individuals from attaining a moderately high diet of PUFAs prior to hibernation. Fed individuals then had higher masses entering hibernation, which is known to reduce torpor expression, but they may have also had lower levels of dietary PUFAs which may have further predisposed them to reduce torpor expression. I did not control for individual PUFA diets in this experiment, but between-individual PUFA diets do vary considerably in natural populations (i.e., up to three-fold in populations of wild arctic ground squirrels (Frank et al. 2008). Additionally, if they had access to PUFAs in the natural vegetation, individuals may have had means to regulate their own PUFA levels by consuming more or less natural vegetation. Future studies could repeat this experiment with different concentrations of pre-hibernation PUFA diets to better understand the influence of PUFAs on Columbian ground squirrel hibernation expression under natural conditions.

Since the physiological costs of torpor increase as body temperature decreases, hibernation energy allocation represents a trade-off between investing in current somatic maintenance or future reproductive success. In this experiment, females with supplemented pre-hibernation energy stores invested energy in self-maintenance during hibernation by reducing torpor expression. Females hibernating under deep snowpacks experienced warmer soil temperatures, which presumably reduced the energetic cost of euthermy and arousals. While many of the results were statistically non-significant across treatments, the trends in torpor expression and mass loss do support the hypothesis that hibernation energy allocation is a careful balance between minimizing the physiological costs of torpor, while maximizing residual emergence mass to invest in reproduction.

To my knowledge, no study has experimentally tested the interacting effects of energy availability and thermal conditions on hibernation energy budgets in the hibernator’s natural setting. Most studies of hibernation tend to be observational or take place in laboratories, due to the difficulty of controlling environmental conditions (e.g., Geiser and Kenagy 1988; Buck and Barnes 2000; Humphries et al. 2003b; Landry-Cuerrier et al. 2008; Carrier et al. 2022). In the natural world, correlations and indirect effects amongst explanatory variables can magnify or offset the direct effects often measured in laboratory-based studies. Controlling these variables is a fundamental strength of laboratory studies because the conditions of the experiment can be reproduced, but it can also be misleading if the true effect of an explanatory variable in nature does not align with the effect measured in the laboratory (Filazzola and Cahill Jr 2021). Thus, by observing changes in the over-winter energy management trends of wild hibernators in response to manipulated energy stores and snowpack, this study lends support to the ecological significance of the optimal hibernation hypothesis.

CHAPTER 4:
GENERAL DISCUSSION

4.1 Summary

The objective of this thesis was to test several hypotheses related to energy management across the full calendar year in a population of wild Columbian ground squirrels (*Urocitellus columbianus*). Columbian ground squirrels are strongly seasonal heterotherms that hibernate for a significant portion of the year (8–9 months) and rely on a short period of favourable environmental conditions for reproduction and fat storage. As such, any alteration in resource availability resulting from climate change could have detrimental effects on their ability to accumulate sufficient fat stores prior to hibernation. Additionally, the correlation between ambient temperature and torpid metabolic rate makes winter temperature shifts or changes in snow cover particularly impactful on energy use during hibernation. To understand the effects of climate change on hibernator energy management, in-depth studies of hibernator eco-physiology and seasonal mass variation are necessary.

In chapter two I identified energetically relevant weather patterns using a sliding window approach. By using a sliding window approach to identifying climate drivers, the analysis was not constrained or influenced by *a priori* assumptions and tested a broad suite of hypotheses concurrently. I predicted that summer weather influenced ground squirrel mass gain indirectly through its effects on vegetation quality and quantity. I found that weather strongly influences ground squirrel mass gain immediately after each sex's energetic investment in reproduction during the summer. Males gained more mass when early summer weather—immediately following the mating season—was warm. Females gained less mass when there were more rainy days in the late summer (pre-hibernation fattening period). Presumably, rain limited the amount of time females could spend foraging and thus limited their summer mass gain. Vegetation quantity (NDVI) did not statistically significantly influence either male or female mass gain, which was potentially due to the coarseness of NDVI spatial and temporal measurements.

Winter weather patterns had complex effects on ground squirrel mass loss and torpor expression depending on sex and entry mass. Male mass loss was highly affected by winter temperature and snowpack patterns, but individual-level female mass loss was not. Rather, entry mass and emergence phenology determined how much mass each female lost over hibernation. Winter weather did influence the torpor expression of both sexes. When winters were cold, both males and females expressed energetically conservative torpor phenotypes. When winters were warmer and thermoregulatory requirements were lower, individuals with large energy stores reduced torpor expression, thereby mitigating the negative physiological effects of torpor.

Chapter three of this thesis investigated the trends seen in the long-term analysis of female torpor expression and mass loss. To achieve this, I manipulated pre-hibernation energy stores and hibernacula microclimate conditions of wild female ground squirrels. The results revealed that when winter soil temperatures were warmer and relatively stable, ground squirrels with larger pre-hibernation energy stores exhibited energetically costly torpor phenotypes. Conversely, in response to colder soil temperatures, the subjects tended to display energetically conservative torpor phenotypes; however, heavier females still exhibited warm torpor skin temperatures and frequent arousals compared to lighter females. These findings aligned with the optimal hibernation hypothesis, which suggests that torpor expression involves a trade-off between energy-conserving torpor bouts and energetically expensive, yet physiologically necessary, arousals (Humphries et al. 2003b). Although some of the results did not reach statistical significance, the observed biological trends in this field study corroborated the findings of chapter two and supported the notion that torpor expression is constrained by microclimate and regulated by individual energy stores (Landry-Cuerrier et al. 2008).

The analysis conducted in chapter two of this study uncovered unexpected yet influential climate drivers that impact sex-specific variation in hibernator body mass throughout the calendar year, as well as torpor expression. The field experiment conducted in chapter three validated the findings of chapter two, demonstrating that females can express colder and more energetically conservative torpor phenotypes in cold conditions, but entry mass, which reflects the mass gained the summer prior, is the stronger predictor of torpor expression. Together, both thesis chapters describe how complex interactions between intra-annual weather variation and torpor expression can influence seasonal hibernator energy management strategies.

4.2 Limitations

There were three limitations in my project which reduced its explanatory power. First, the open access NDVI dataset used for this research was somewhat coarse temporally and spatially (16-day revisit time, 250 m resolution). As such, subtle effects of vegetation quality on mass gain may have been missed. Finer resolution NDVI datasets exist but I could not use them because they either did not cover the full range of the study period (2009–2020) or they required high fees for access. Conclusions about the role of vegetation quality on ground squirrel mass gain from the current study should therefore be interpreted with caution. Second, nearly half of the subjects in the field experiment removed their collars before emergence. This substantially reduced the sample size and statistical power of all torpor trait analyses. Third, I used quantitative magnetic resonance (QMR) scans to measure the amount of fat and lean mass of each individual prior to immergence in the summer of 2019; but I was unable to make emergence QMR scans in the spring of 2020 as planned due to the COVID-19 pandemic. If I had emergence scans, I could have directly measured fat and lean mass catabolism instead of using body mass change as a proxy for hibernation energy use.

4.3 Future directions

To build upon the findings of this thesis, future studies can explore the influence of vegetation quality on ground squirrel summer mass gain and reproductive success. While the current study did not detect any effects of vegetation quality on female ground squirrel mass gain, the sampled individuals exclusively comprised those who had successfully weaned a litter. Therefore, future studies could specifically explore the potential impact of vegetation quality on female reproductive success, such as litter mass or abortion rates. Such analyses would contribute to a deeper understanding of the potential effects of future warming on ground squirrel population dynamics.

Furthermore, to extend the validity of the current experiment's results, it would be valuable to replicate the study in a different winter. The microclimate between treatments could be expanded by removing snow on top of a treatment group, as well as adding snow to another treatment group using snow fences. This would help evaluate the generalizability and robustness of the findings across varying environmental conditions and provide a more comprehensive understanding of the mechanisms involved.

Lastly, incorporating post-emergence QMR scans would offer a novel perspective on fat versus lean mass catabolism over winter in a field setting, which has not been previously explored. By conducting QMR scans after ground squirrels emerge from hibernation, researchers could assess the relative contributions of fat and lean mass to overall body composition changes. This additional data would enhance our knowledge of the physiological adaptations and energy use strategies employed by ground squirrels during hibernation.

4.4 Broader relevance

This thesis identified key windows of time and patterns of weather that have the potential to influence Columbian ground squirrel energy management should climate change cause them to shift in the future. Warming weather trends appear to be increasing hibernator reproductive success globally (Schwanz 2006; Hufnagl et al. 2011; Fietz et al. 2020; Paniw et al. 2020; Wells et al. 2022). The findings in chapter two provided some nuance to this observation by demonstrating that males and females are likely to be unequally affected by summer warming. Male Columbian ground squirrels are already benefiting from warmer springs as shown by their increase in mean body mass between 2009 and 2020. In contrast, females did not change mean body mass over the same time. If summer weather continues to become warmer and late-summer vegetation quality decreases, females may be negatively affected due to their limited time to forage and gain mass before hibernation.

Few studies of hibernation consider the effect of pre-hibernation weather conditions on the expression of torpor or winter mass loss (Sheriff et al. 2015). In this thesis, I showed that summer weather, through its effect on summer mass gain, strongly affects torpor expression in Columbian ground squirrels. Individuals that gained large energy stores prior to hibernation expressed energetically costly torpor phenotypes, which presumably reduced the potential negative physiological consequences of suppressed metabolism and allowed them to begin the following active season in better body condition (Boyles et al. 2020). For females, pre-hibernation energy stores had a stronger effect on hibernation mass loss than winter weather. Thus, the effect of pre-hibernation weather should not be overlooked when considering the effects of weather on torpor expression and mass loss in natural settings.

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APPENDIX A:

Body mass corrections

I corrected emergence body masses by isolating individuals caught and weighed on the same day as emergence and applying a broken stick regression to find the mean rate of mass change immediately following emergence (Figure A.1). I applied this slope as a correction factor to the first capture masses of each individual caught within four days of emerging (Table A.1). The number of days elapsed between emergence and first capture was determined using a combination of technician confidence, presence and amount of skin flakes and vulva condition following Lane et al., 2019 (see also Michener 1983). Females typically enter estrus within three to four days of emergence and vulva condition within this timeframe can be used as an indication of emergence date. For females, time-corrected masses at four days from emergence correlated (Pearson *r*) better to the actual emergence mass compared to uncorrected masses at four days from emergence (corrected $r = 0.92$; uncorrected $r = 0.86$). For males, corrected and uncorrected emergence masses performed equivalently (corrected $r = 0.96$; uncorrected $r = 0.96$).

Entry body masses were time-corrected in a similar manner. Since the exact day of entry into hibernation could not be determined for uncollared individuals, I adjusted body masses to the mean day of entry (i.e., disappearance) of each sex in each year. Using collared individuals whose hibernation entry dates and masses were known, I tested the entry mass correction factors in the same way as emergence masses (Figure A.2). Time-corrected male and female body masses correlated strongly with actual entry masses (males corrected r = 0.85, uncorrected r = 0.45; females corrected r = 0.84, uncorrected r = 0.59).

Table A.1: Values used to correct male and female entry and emergence body mass for days elapsed since final and initial capture pre- and post-hibernation.

Sex	**Emergence mass correction (g/day)**	**Entry mass correction (g/day)**
Males	-2.1	-2.2
Females	-11.0	-2.7

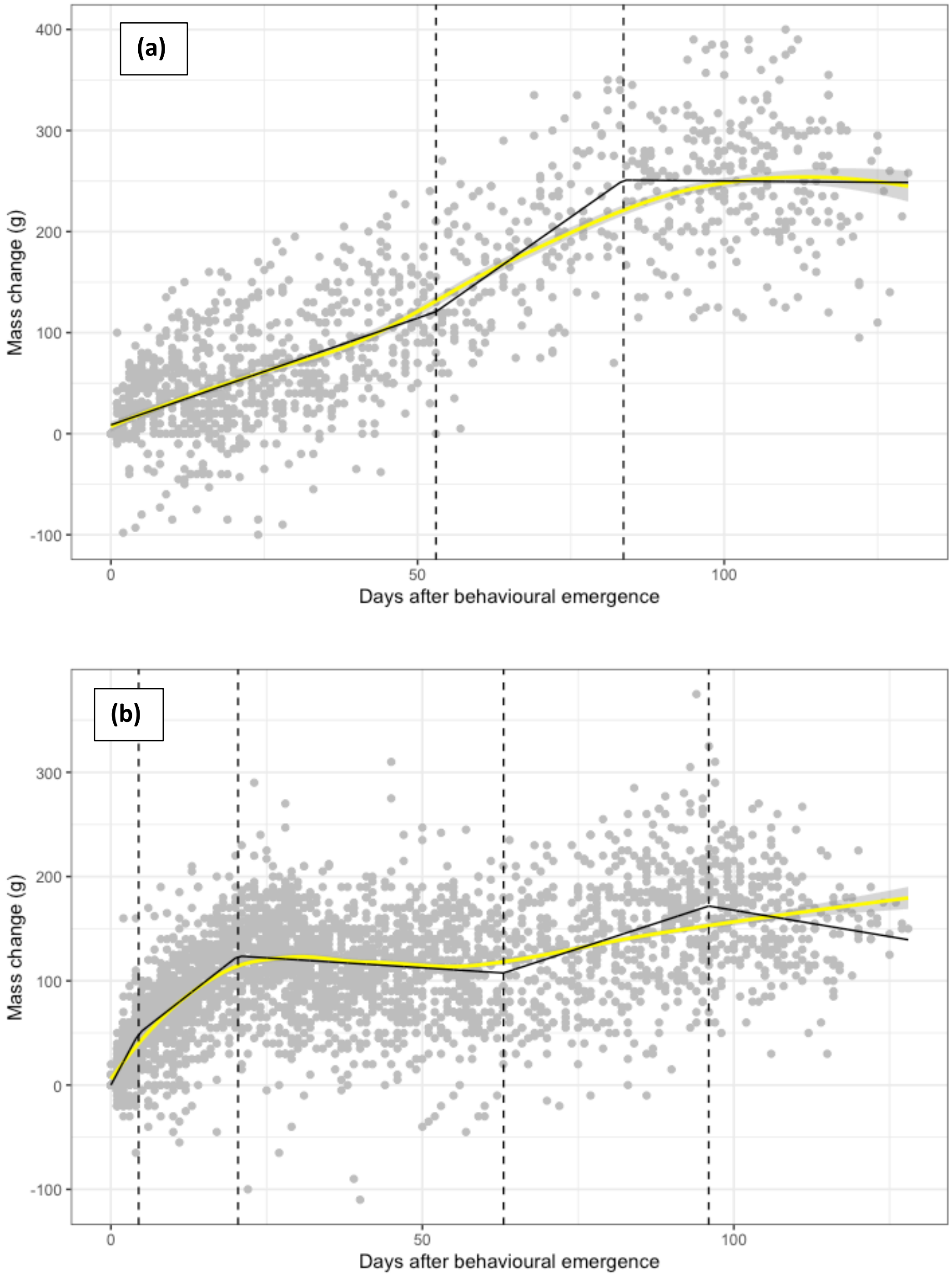

Figure A.1: Broken stick regression results showing the post-emergence summer mass changes (g) of adult (a) male and (b) female ground squirrels from 2009–2020. Breaks were detected in the male mass change plot at days 43 and 84 post behavioural emergence. The initial rate of male mass gain was 2.1 g/day. Breaks were detected in the female mass change plot at days 4, 20, 63, and 96 post-emergence. The initial rate of female mass gain was 11 g/day. The rates of change before the first break detected were used as emergence mass corrections for each sex.

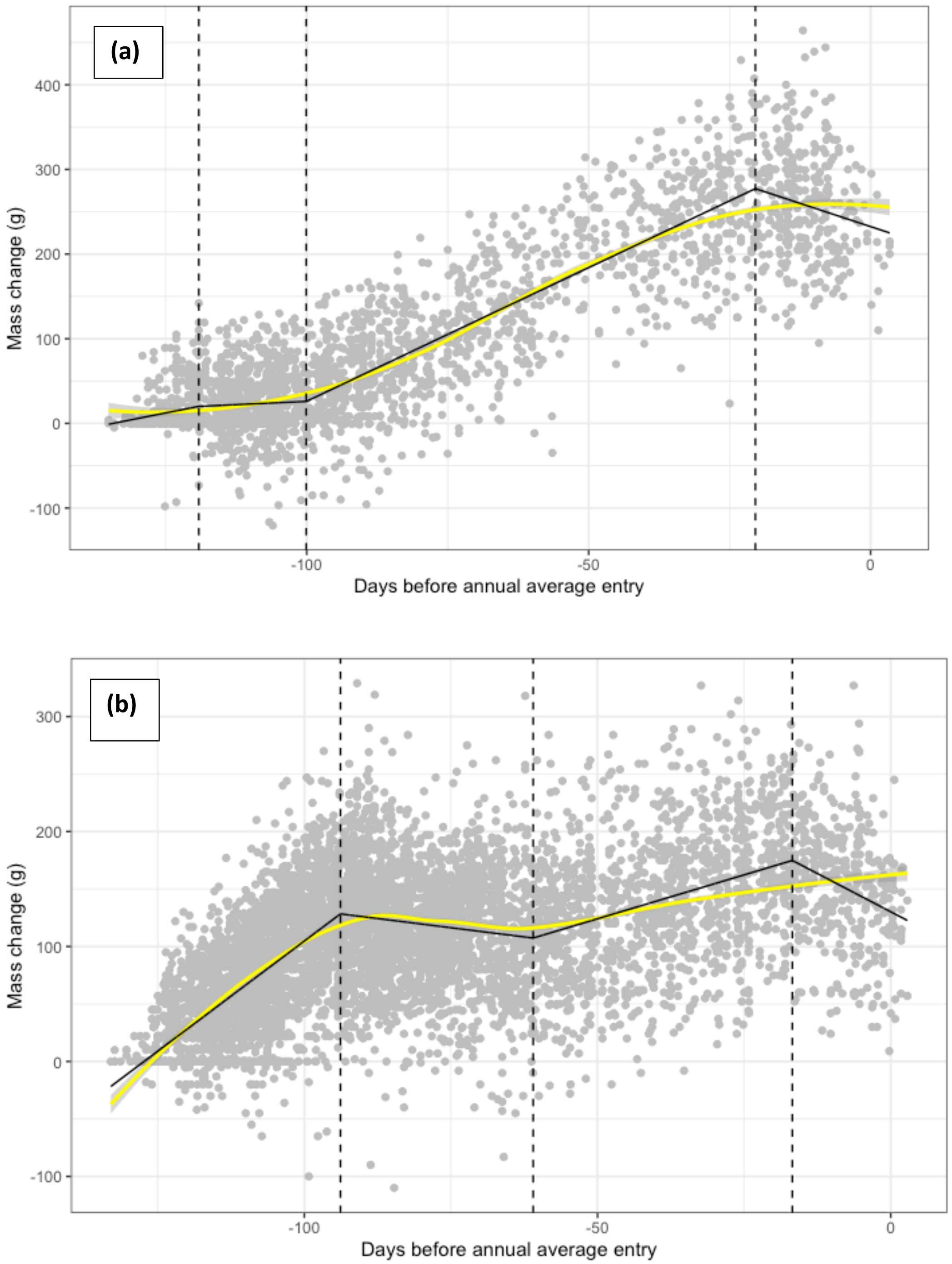

Figure A.2: Broken stick regression results showing the pre-hibernation-entry mass changes (g) of adult (a) male and (b) female ground squirrels from 2009–2019. Breaks were detected in the male mass change plot at days 119, 100, and 20 before mean entry. The final rate of male mass change before entry was -2.2 g/day. Breaks were detected in the female mass change plot at days 94, 61, and 17 before mean entry. The final rate of female mass change before mean entry was -2.7 g/day. The rates of change after the latest break detected were used as the entry mass corrections for each sex.

APPENDIX B:

Chapter 2 supplementary figures and tables

Table B.1: Linear mixed model estimates and 95% confidence intervals of various torpor trait responses to abiotic predictors of male and female Columbian ground squirrels from 2009–2019. Models account for entry mass, year, and repeated IDs. Estimates whose 95% confidence interval not cross zero are in bold. Italicized response variables had a non-linear relationship with entry mass. * Predictor significantly interacted with entry mass

Sex	Response	Predictor					
		Entry mass (g)	T$_{a}$ signal	Snow signal	PDO	HTI end (day of year)	Avg. April T$_{gr}$ (°C)
M	Mass loss (g)	**-0.62 [-0.74, -0.52]**	**16.09 [7.46, 25.15]**	**-1.92 [-2.78, -1.02]**	12.05 [-5.32, 29.93]	**-0.57 [-1.09, -0.07]**	**27.61 [10.30, 43.16]**
	Log$_{10}$ avg. torpid T$_{sk}$ (°C)	**1.06x10^{-3} [5.92, 1.53x10^{-3}]**	0.03 [-0.03, 0.10]	-2.95x10^{-3} [-9.90x10^{-3}, 4.30x10^{-3}]	**6.63x10^{-4} [4.43x10^{-5}, 1.26x10^{-3}]** *	**-2.42x10^{-5} [-4.58x10^{-5}, -2.77x10^{-6}]** *	0.07 [-0.03, 0.18]
	Log$_{10}$ avg. min. torpid T$_{sk}$ (°C)	-2.84x10^{-4} [-3.06x10^{-4}, 9.06x10^{-4}]	0.03 [-0.03, 0.08]	-2.90x10^{-3} [-8.93x10^{-3}, 0.003]	**7.70x10^{-4} [5.19x10^{-5}, 1.44x10^{-3}]** *	-1.27x10^{-3} [-3.79x10^{-3}, 1.19x10^{-3}]	0.05 [-0.04, 0.14]
	Prop. euthermic	**-6.36x10^{-5} [-1.05x10^{-4}, -2.26x10^{-5}]**	0.16 [-0.24, 0.55]	-9.07x10^{-3} [-0.04, 0.03]	**0.47 [0.06, 0.89]**	-0.01 [-0.03, 2.07x10^{-3}]	0.29 [-3.38, 1.02]
	Log$_{10}$(Arousal frequency (arousals/day))	**-5.25x10^{-6} [-7.66x10^{-6}, -2.53x10^{-6}]**	-8.98x10^{-3} [-0.03, 0.01]	1.49x10^{-3} [-4.60x10^{-4}, 3.57x10^{-3}]	0.02 [-0.01, -0.04]	1.05x10^{-4} [-8.11x10^{-4}, 1.09x10^{-3}]	-0.01 [-0.05, 0.03]
	Log$_{10}$(Arousal time (hours))	**-8.72x10^{-6} [-1.32x10^{-5}, -3.98x10^{-6}]**	-0.02 [-0.07, 0.02]	3.00x10^{-3} [-2.16x10^{-3}, 8.43x10^{-3}]	0.02 [-0.04, 0.08]	7.47x10^{-4} [-1.28x10^{-3}, 2.88x10^{-3}]	-0.03 [-0.12, 0.05]
	Sequester time (hours)	**0.60 [0.33, 0.86]**	12.51 -6.84, 31.83]	-0.91 [-3.25, 1.51]	14.48 [-12.76, 41.71]	-0.73 [-1.49, 3.86x10^{-3}]	12 [-21.99, 46.53]
	Relative behavioural emergence (day of year)	**2.57x10^{-4} [1.04x10^{-4}, 4.02x10^{-4}]**	0.04 [-1.65, 1.70]	-0.02 [-0.23, 0.17]	-1.50 [-3.55, 0.74]	0.02 [-0.06, 0.10]	0.12 [-3.35, 3.56]
	Relative physiological emergence (day of year)	**2.65x10^{-4} [8.54x10^{-5}, 4.49x10^{-4}]**	0.76 [-1.16, 2.83]	-0.15 [-0.36, 0.06]	-1.06 [-3.86, 1.87]	-0.01 [-0.10, 0.08]	1.16 [-2.72, 5.00]
	Log$_{10}$(Phys. emergence (day of year))	**2.47x10^{-6} [8.23x10^{-7}, 4.41x10^{-6}]**	**-0.03 [-0.06, -6.46x10^{-3}]**	**4.00x10^{-3} [8.71x10^{-4}, 7.16x10^{-3}]**	**-0.06 [-0.09, -0.02]**	**1.48x10^{-3} [1.84x10^{-4}, 2.79x10^{-3}]**	**-0.07 [-0.11, -0.04]**
	Log$_{10}$(Beh. emergence (day of year))	**2.17x10^{-6} [9.38x10^{-7}, 3.46x10^{-6}]**	**-0.03 [-0.05, -4.33x10^{-3}]**	**3.35x10^{-3} [8.58x10^{-4}, 5.69x10^{-3}]**	**-0.04 [-0.07, -0.02]**	1.06x10^{-3} [-3.12x10^{-6}, 2.14x10^{-3}]	**-0.06 [-0.09, -0.03]**

F	Mass loss (g)	**-0.52** **[-0.69, -0.34]**	-2.48 [-7.23, 2.53]	-1.22 [-19.93, 17.39]	-5.82 [-15.86, 4.30]	0.04 [-0.38, 0.46]	-4.91 [-17.11, 6.25]
	Avg. torpid T_{sk} (°C)	**-7.52x10^{-5}** **[-1.31x10^{-4}, -1.74x10^{-5}]**	0.03 [-0.05, 0.11]	**8.43x10^{-6}** **[2.14x10^{-6}, 1.49x10^{-5}]** *	**4.81x10^{-6}** **[3.51x10^{-7}, 9.71x10^{-6}]** *	-6.03x10^{-5} [-6.54x10^{-3}, 6.59x10^{-3}]	-0.02 [-0.24, 0.18]
	Avg. min. torpid T_{sk} (°C)	**-1.90x10^{-4}** **[-1.91x10^{-4}, -2.64x10^{-5}]**	-0.05 [-0.19, 0.08]	**1.32x10^{-5}** **[3.36x10^{-6}, 2.25x10^{-5}]** *	**8.44x10^{-6}** **[1.35x10^{-6}, 1.61x10^{-5}]** *	4.53x10^{-3} [-5.66x10^{-3}, 0.02]	-0.23 [-0.55, 0.10]
	Log$_{10}$ prop. euthermic	**1.23x10^{-6}** **[5.15x10^{-4}, 1.92x10^{-3}]**	0.02 [-5.22x10^{-3}, 0.04]	0.07 [-1.30x10^{-3}, 0.14]	**0.05** **[0.01, 0.09]**	1.48x10^{-3} [-3.31x10^{-3}, 4.67x10^{-4}]	0.04 [-0.02, 0.11]
	Log$_{10}$ arousal frequency (arousals/day)	**6.99x10^{-4}** **[2.07x10^{-4}, 1.16x10^{-3}]**	3.86x10^{-3} [-5.03x10^{-3}, 0.01]	0.02 [-7.00x10^{-3}, 0.05]	0.02 [-5.01x10^{-3}, 0.04]	**-2.70x10^{-5}** **[-5.08x10^{-5}, -5.08x10^{-6}]** *	2.01x10^{-3} [-0.03, 0.03]
	Log$_{10}$ arousal time (hours)	**1.29x10^{-3}** **[4.38x10^{-4}, 2.15x10^{-3}]**	3.58x10^{-3} [-0.03, 0.03]	0.04 [-0.08, 0.15]	0.03 [-0.03, 0.10]	5.37x10^{-5} [-2.48x10^{-3}, 2.41x10^{-3}]	-0.02 [-0.11, 0.07]
	Sequester time (days)	9.33x10^{-4} [-4.36x10^{-3}, 5.79x10^{-3}]	0.05 [-0.10, 0.17]	0.12 [-0.37, 0.55]	0.13 [-0.19, 0.41]	-6.89x10^{-3} [-0.02, 4.75x10^{-3}]	0.26 [-0.07, 0.56]
	Relative behavioural emergence (day of year)	**-0.04** **[-0.08, -7.64x10^{-3}]**	-0.51 [-1.25, 0.21]	-1.26 [-3.75, 1.20]	-0.48 [-2.25, 1.14]	0.03 [-0.03, 0.09]	-1.03 [-2.99, 0.88]
	Relative physiological emergence (day of year)	-0.03 [-0.06, 2.59x10^{-3}]	-0.23 [-0.99, 0.51]	-0.46 [-3.40, 2.22]	-0.90 [-2.60, 0.81]	0.02 [-0.04, 0.08]	-0.56 [-2.58, 1.33]
	Phys. emergence (day of year)	-0.04 [-0.08, 5.48x10^{-3}]	**-1.86** **[-3.64, -0.19]**	-4.70 [-11.71, 2.11]	-2.78 [-7.15, 1.71]	**0.21** **[0.11, 0.31]**	**-8.01** **[-11.06, -4.96]**
	Beh. emergence (day of year)	-0.04 [-0.07, 3.50x10^{-3}]	**-1.82** **[-3.49, -0.06]**	-4.64 [-11.51, 2.32]	-2.61 [-6.76, 1.53]	**0.20** **[0.10, 0.30]**	**-7.66** **[-10.46, -4.37]**

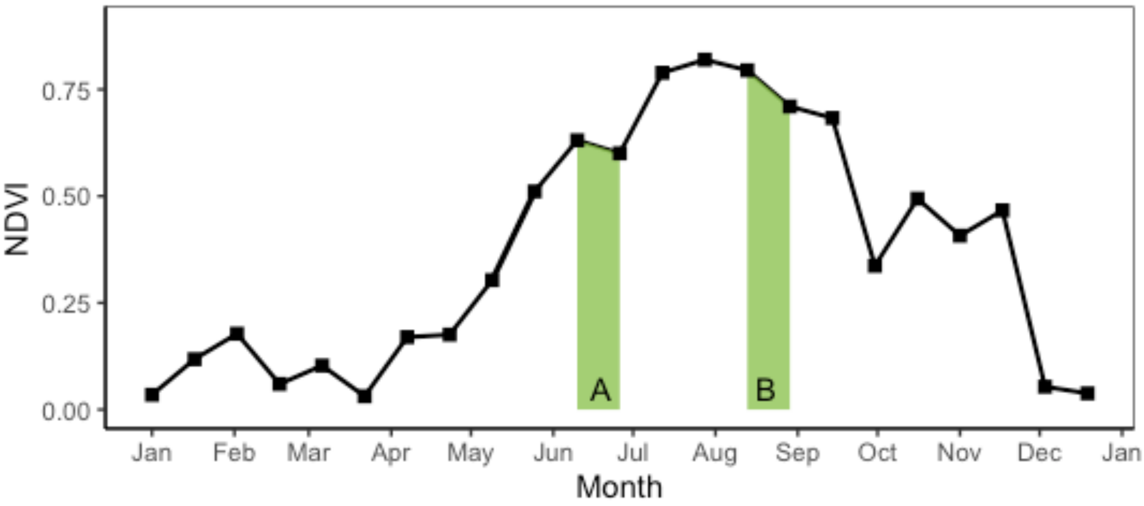

Figure B.1: Normalized Difference Vegetation Index (NDVI) values from 2009 in Kite Field, Sheep River Provincial Park, AB. Each point represents the mean "greenness" of all 10 pixels in the satellite image of Kite Field. The labels "A" and "B" represent the integrated (area under the curve) values for June (INDVIjun) and August (INDVIaug) respectively.

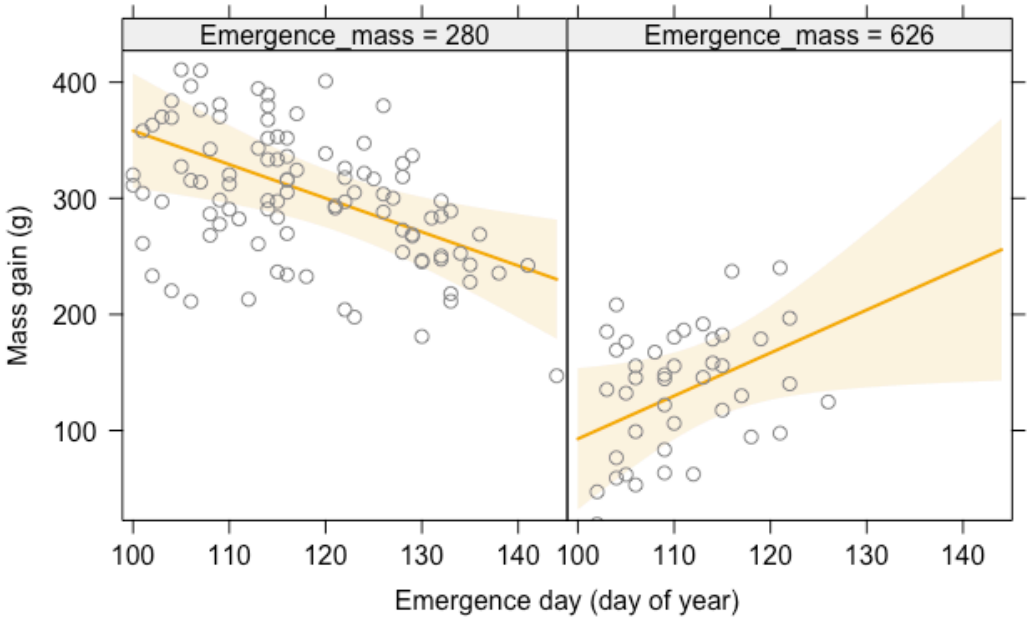

Figure B.2: Partial residual plot from the male Columbian ground squirrel mass gain model (Table 2.4) showing the interaction between emergence mass (g) and emergence day (day of year) and its effect on total mass gain (g). Early emerging males gained more mass if they were small at emergence (0.02 ± 0.01 g, 95% CI [0.00, 0.03]). Shaded areas are the 95% confidence intervals accounting for covariates. Plotted emergence masses are the upper and lower quartiles (male emergence mass range: 280–626 g, mean: 427 g).

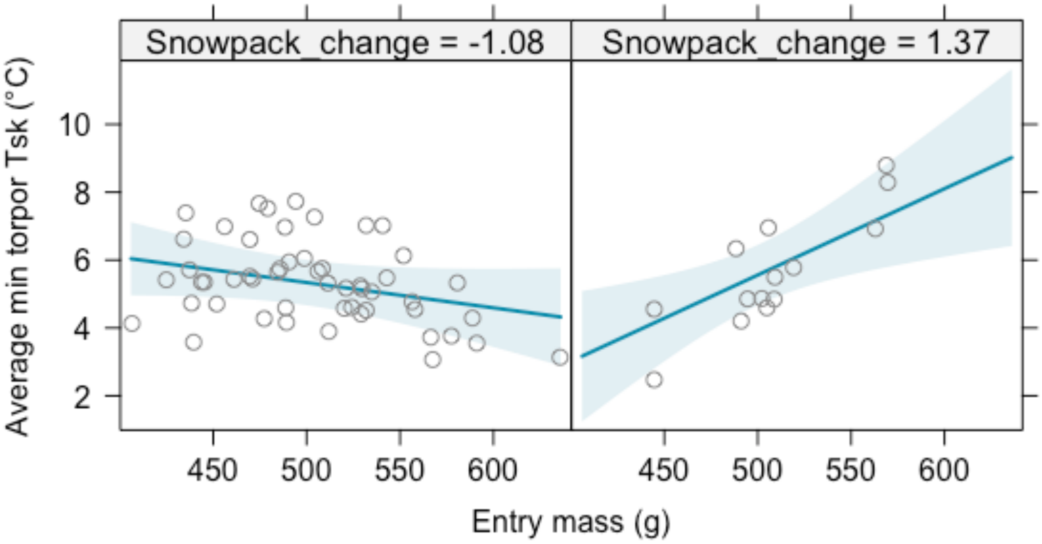

Figure B.3: Partial residual plots from a linear mixed model showing the interaction between female Columbian ground squirrel entry mass and snowpack change (upper and lower quartiles) 119–107 days before mean female emergence (2010–2019) on mean minimum torpor skin temperature (T_{sk}). When snowpack became deeper, large females expressed a warmer mean minimum torpor T_{sk} compared to smaller females ($0.01 \pm 4.99 \times 10^{-3}$ SE °C, 95% CI [4.01×10^{-3}, 0.02]). When snowpack grew shallower, all females expressed a similar mean minimum torpor T_{sk}, regardless of entry mass. Shaded areas are the 95% confidence intervals accounting for covariates.

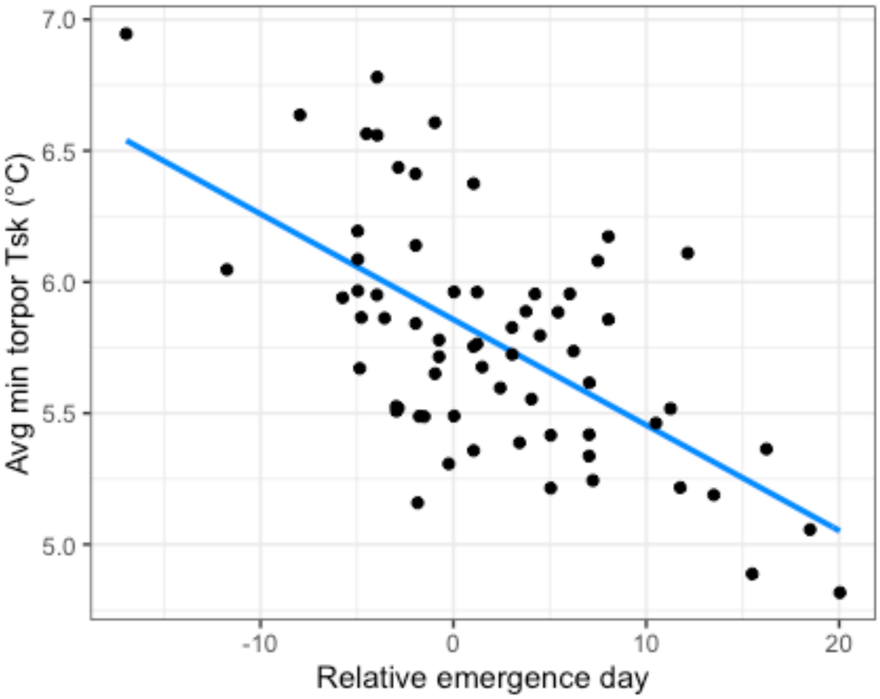

Figure B.4: Partial residual plot from a linear mixed model, showing the relationship between relative (mean-centred within year) emergence day and mean minimum torpor T_{sk}. Female ground squirrels were collared over the winters of 2010 to 2019. Late-emerging females had colder minimum torpor T_{sk}s compared to early-emerging females (-0.04 ± 0.02 SE °C, 95% CI [-0.08, 1.93x10^{-3}]).

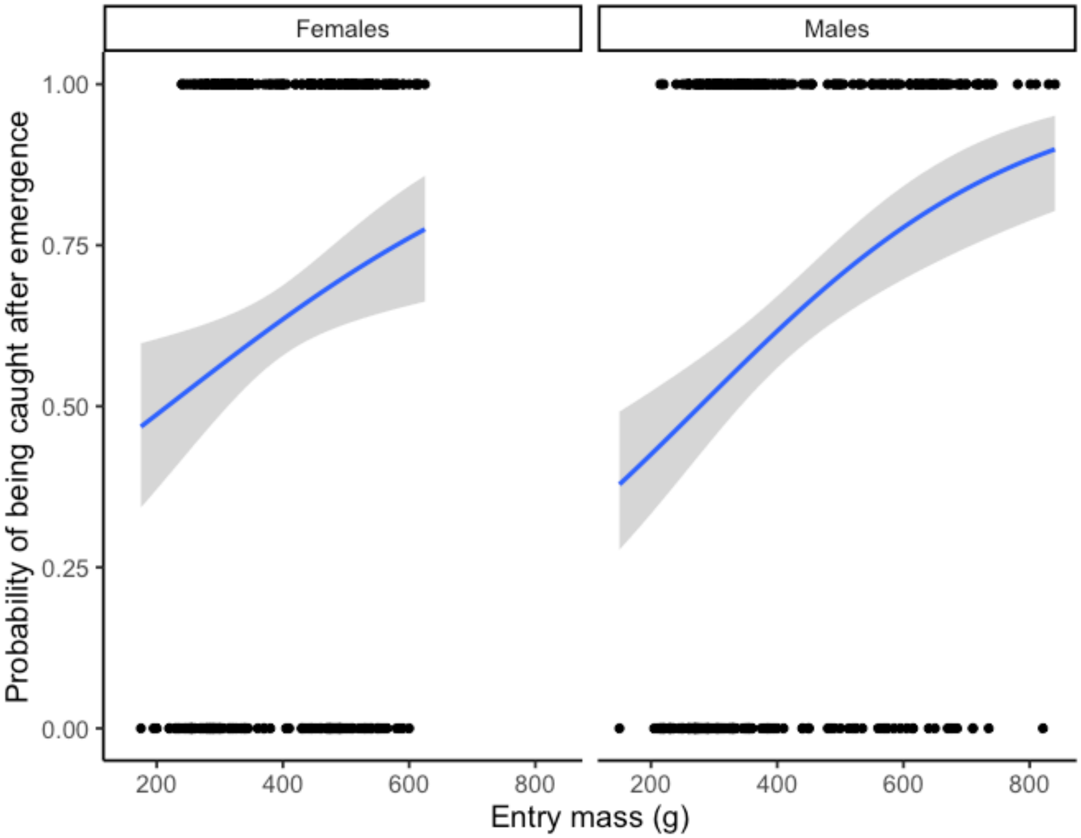

Figure B.5: Probability of Columbian ground squirrels being caught after hibernation emergence (i.e., surviving hibernation) depending on entry body mass (2009–2019). Both males and females had a higher probability of being caught the following year if they were heavier entering hibernation. In mixed effect logistic regression models (random effects: ID, year; fixed effects: entry mass, minimum age), the odds of being caught after emergence increased by 0.7 ± 0.1% (95% CI [0.4, 0.9]) for males and by 1.1 ± 0.2% (95% CI [0.7, 1.5]) for females with every one gram increase in entry mass. Entry mass was the last recorded body weight prior to autumn immergence. Only individuals caught and weighed within seven days of average immergence for each sex were included in the mixed effect logistic regressions (males n = 318, females n = 307). Mean male entry mass was 421 ± 161 g (range 150–840 g) and mean male entry age was 0.9 ± 1.5 years (range 0–9). Mean female entry mass was 400 ± 110 g (range 175–625 g) and mean female entry age was 1.9 ± 2.5 years (range 0–10).

APPENDIX C:

Entry mass effects on hibernation expression

Females:

In general, females reduced torpor expression linearly with entry mass. Proportion of time spent euthermic decreased, arousal frequency decreased, and relative emergence day became earlier as entry mass increased (Appendix Figure C.1). Females expressed a non-linear relationship between entry mass and mean minimum torpor T_{sk} (Appendix Figure C.1).

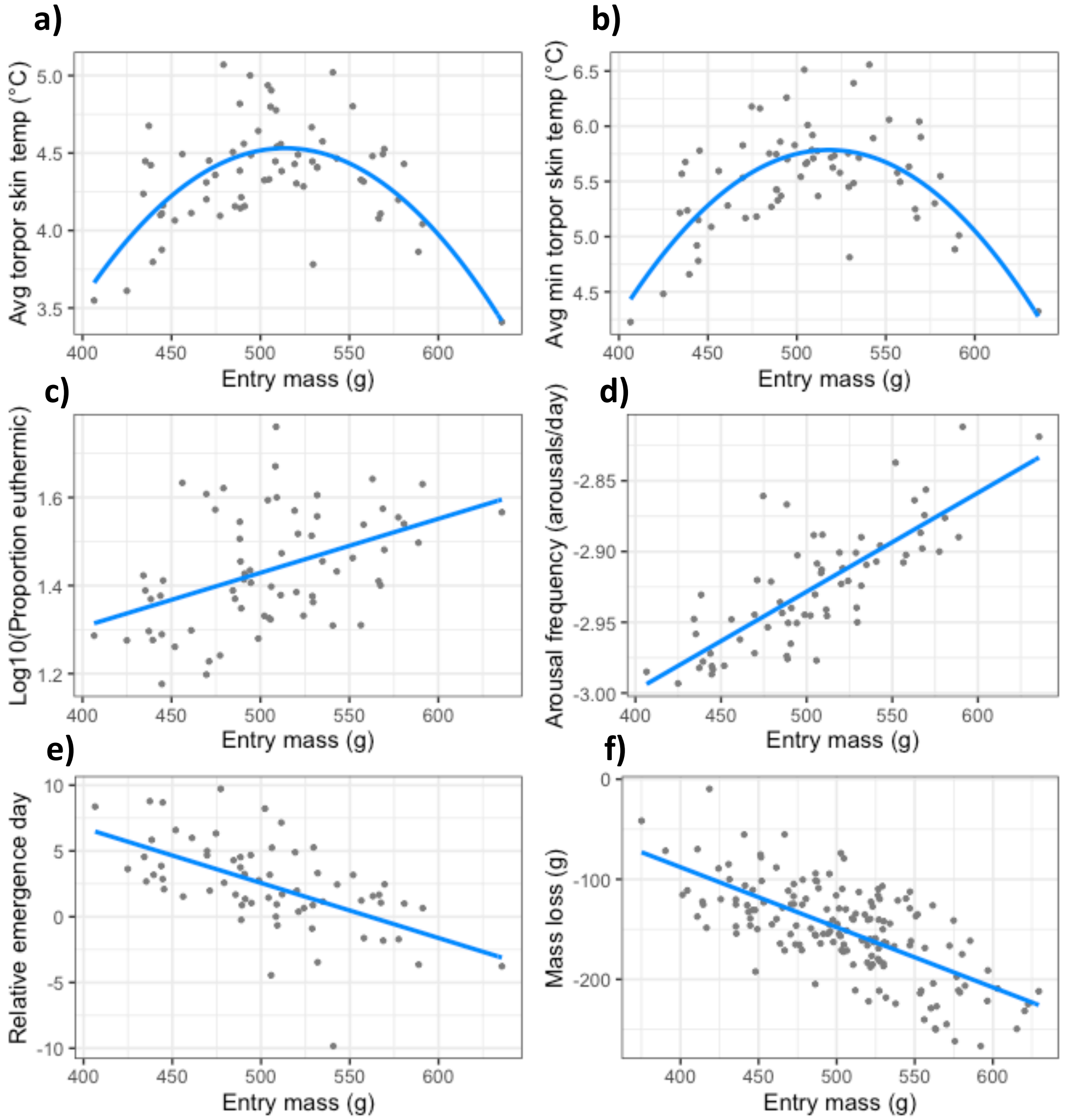

Figure C.1: Partial residual plots from linear mixed models, showing the relationship between individual female entry masses and hibernation traits. Female ground squirrels were collared over the winters of 2010 to 2019. Female (a) mean and (b) mean minimum torpor T_{sk} both peaked at medium entry masses (~525g) (mean T_{sk}: $-7.52\times10^{-5} \pm 2.79\times10^{-5}$, 95% CI [$-1.31\times10^{-4}$, -1.74×10^{-5}]; minimum T_{sk}: $-1.90\times10^{-4} \pm 4.07\times10^{-5}$, 95% CI [$-1.91\times10^{-4}$, -2.64×10^{-5}]). (c) Proportion of time spent euthermic ($1.23\times10^{-6} \pm 3.60\times10^{-4}$, 95% CI [$5.15\times10^{-4}$, 1.92×10^{-3}]) and (d) arousal frequency ($6.99\times10^{-4} \pm 2.32\times10^{-4}$, 95% CI [$2.07\times10^{-4}$, 1.16×10^{-3}]) increased with entry mass. (e) Larger females emerged earlier than smaller females (relative emergence day: -0.04 ± 0.02, 95% CI [-0.08, -7.64×10^{-3}]).

Female age-effects:

Interestingly, the relationship between female entry mass and mean minimum torpor T_{sk} was not linear. I expected mean minimum torpor T_{sk} to increase with entry mass (Humphries et al. 2003a; b), but mean T_{sk}s peaked with medium entry masses (Figure C.1a). Further investigation revealed that the largest females were relatively old (> 8 years). To test whether age influences female mean minimum torpor T_{sk}, I modelled the relationship using a linear mixed model controlling for entry mass, winter weather conditions (fixed effects), repeated IDs, and year (random effects). Young (< 4 years) and old females (> 6 years) expressed cooler mean minimum torpor T_{sk}s than middle-aged females (Figure C.2).

This relationship reflects an energetic trade-off between reproduction and maintenance (and growth) experienced by age. Young females are still investing energy in somatic growth and therefore have less energy to allocate toward reproduction (Broussard et al. 2003). In this analysis, that trend seemed to continue into hibernation with young females having less energy to increase minimum torpor T_{sk}s compared to middle-aged females, who have finished growing. Likewise, although the oldest females had high entry masses, they were also experiencing senescence and therefore had less energy to allocate somatic maintenance during hibernation, seen as low mean minimum torpor T_{sk}s (Figure C.2; Shanley and Kirkwood 2000). These findings reveal age-specific energy allocation strategies and potentially the influence of life history trade-offs and senescence on female hibernation expression.

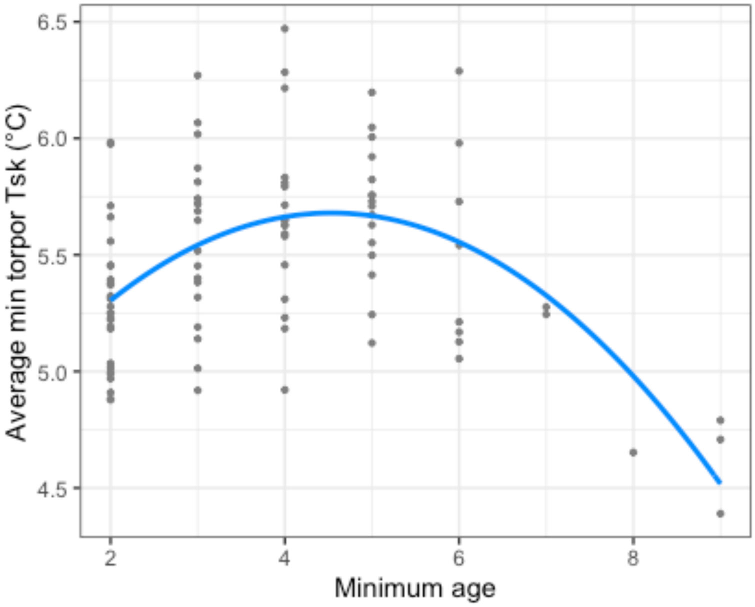

Figure C.2: Partial residual plot from a linear mixed model showing the non-linear relationship between female minimum age at entry and mean minimum torpor T_{sk}. The model controls for repeated ID, entry year, entry mass, and winter weather conditions (PDO). When the age of an individual was unknown (i.e., was not tagged as a juvenile), the minimum age at first capture was estimated using mass and reproductive status. Minimum age is therefore a conservative estimate of an individual's actual age. Mean minimum torpor T_{sk} increases with age until ~4–5 years of age then declines sharply (quadratic estimate: -0.06 ± 0.02, 95% CI [-0.11, -0.01]).

Males:

As entry mass increased, males reduced torpor expression (e.g., proportion of time spent euthermic increased, relative emergence day decreased, arousal frequency increased, mean torpor T_{sk} increased, sequester time increased; Figure C.3) and torpor related physiological costs until an entry mass threshold ~700g. Males heavier than 700g entering hibernation expressed similar degrees of torpor compared to 700g males.

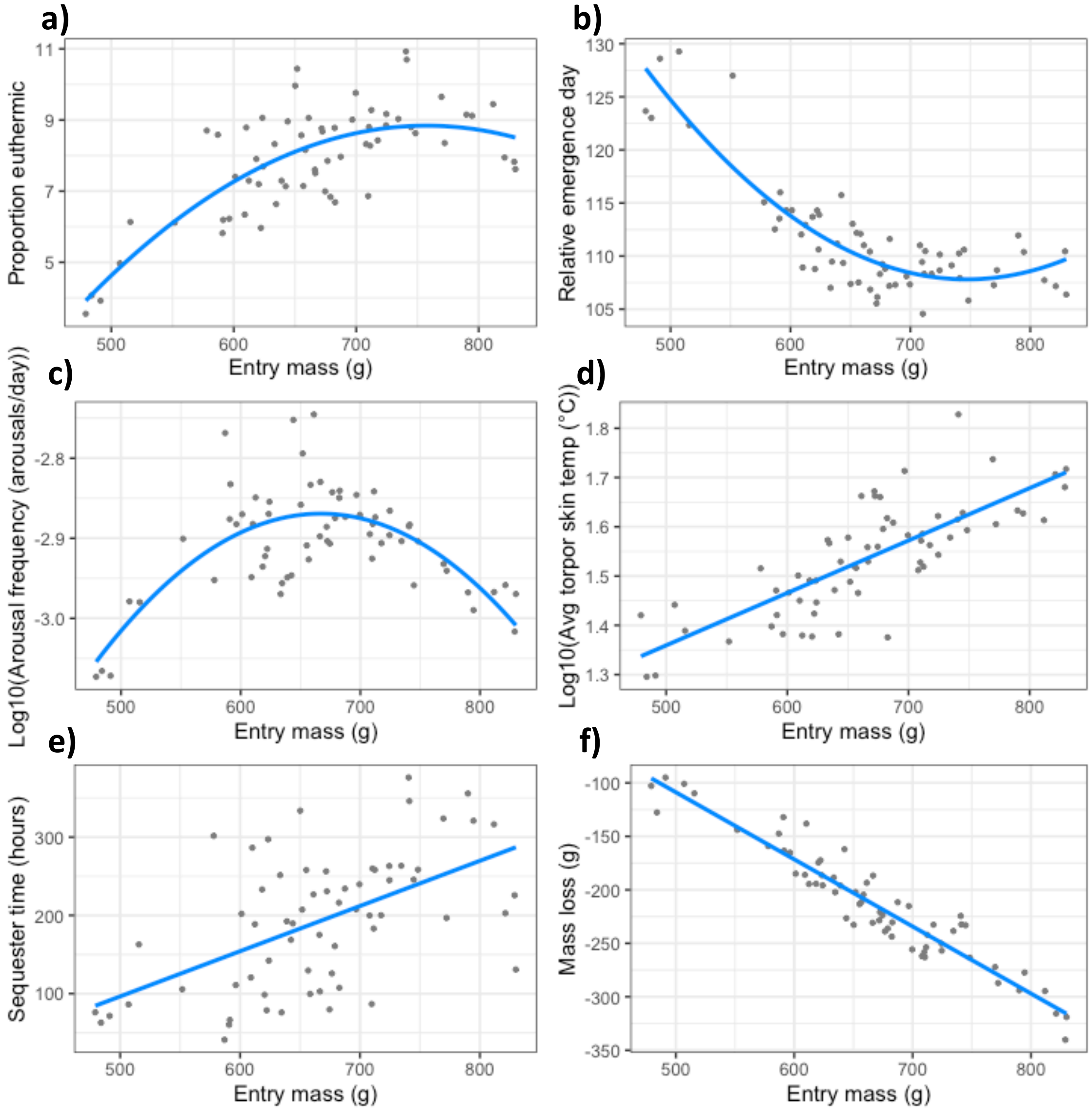

Figure C.3: Partial residual plots from linear mixed models, showing the relationship between individual male entry mass and hibernation expression. Male ground squirrels were collared over the winters of 2009 to 2019. Several male hibernation traits were non-linearly influenced by entry mass. (a) Proportional time spent euthermic increased until a point ~700g when heavier males did not spend any longer proportionally euthermic ($-6.36\text{x}10^{-5} \pm 2.08\text{x}10^{-5}$, 95% CI [$-1.05\text{x}10^{-4}$, $-2.66\text{x}10^{-5}$]). (b) Similarly, as entry mass increased, relative emergence day decreased until a point ~700g when heavier males do not emerge any earlier ($2.57\text{x}10^{-4} \pm 7.36\text{x}10^{-5}$, 95% CI [$1.04\text{x}10^{-4}$, $4.02\text{x}10^{-4}$]). (c) Arousal frequency peaked at medium entry masses (~650g) ($-5.25\text{x}10^{-6} \pm 1.25\text{x}10^{-6}$, 95% CI [$-7.66\text{x}10^{-6}$, $-2.53\text{x}10^{-6}$]). Other male hibernation traits varied linearly with entry mass. (d) Large males tended to have a warmer mean torpor T_{sk} ($1.06\text{x}10^{-3} \pm 2.43\text{x}10^{-4}$, 95% CI [5.95, $1.53\text{x}10^{-3}$]) and (e) longer sequestration times (0.60 ± 0.13, 95% CI [0.33, 0.86]).

APPENDIX D:

Chapter 3 supplementary figures and tables

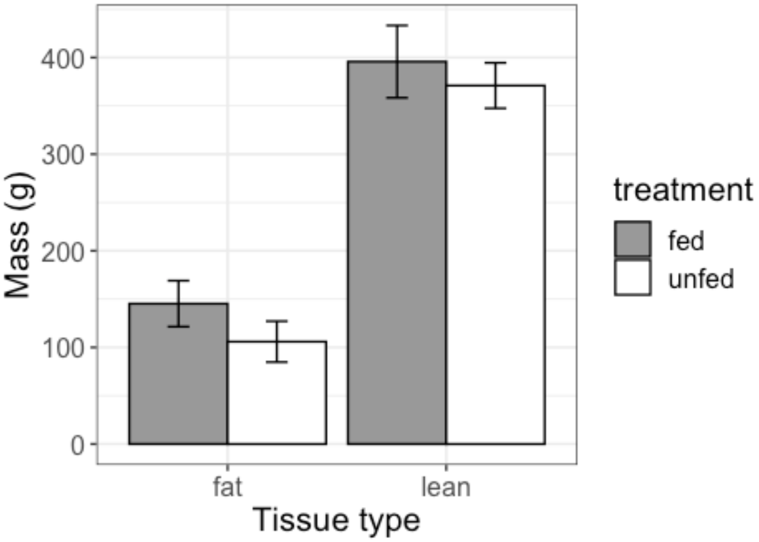

Figure D.1: Body compositions of August (pre-hibernation) body compositions of fed (n = 7) and unfed (n = 11) wild female Columbian ground squirrels in 2019. Fed females had similar amounts of lean mass (t_9 = 1.80, p = 0.11) compared to unfed females but significantly more body fat (t_{11} = 3.45, p = 0.006).

Table D.1: Two-way type III ANOVA results of supplementary feeding and snowpack manipulation on female hibernation expression. Marginally significant p-values ($0.05 < p < 0.10$) have been highlighted in bold. T_{sk} = skin temperature.

Hibernation trait	**Effect**	**F(1, 25)**	**p**
Mean T_{sk} (°C)	Fed	2.74	0.11
	Fence	1.92	0.18
	Fed:Fence	0.14	0.71
Mean min T_{sk} (°C)	Fed	2.15	0.16
	Fence	0.10	0.75
	Fed:Fence	0.13	0.72
Arousal frequency (arousals/day)	Fed	0.37	0.55
	Fence	0.01	0.92
	Fed:Fence	0.28	0.60
Arousal time (hours)	**Fed**	**3.66**	**0.07**
	Fence	1.63	0.21
	Fed:Fence	0.02	0.90
Prop. time euthermic	Fed	0.47	0.50
	Fence	0.06	0.81
	Fed:Fence	0.87	0.36
Behavioural emergence (day of year)	**Fed**	**3.47**	**0.07**
	Fence	0.71	0.41
	Fed:Fence	0.86	0.36