COVARIATES OF INTRASPECIFIC VARIATION IN HIBERNATION EXPRESSION IN THE NORTHERNMOST POPULATION OF BLACK-TAILED PRAIRIE DOGS

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Abstract

Hibernation is an energy-saving strategy employed by species to survive periods of low resource abundance and inclement weather. Due to the costs and benefits associated with the use of torpor and arousal during hibernation, individuals are predicted to hibernate according to their energetic state entering hibernation and their requirements following hibernation. The timing and quantity of energetic requirements following hibernation can vary between the sexes due to differences in selective pressures. Thus, there is potential for sexual variation in hibernation expression to exist. Species and/or populations that exhibit individual variation in hibernation expression lend themselves as an ideal species to test such predictions.

Black-tailed prairie dogs (*Cynomys ludovicianus*) are a species that demonstrates tremendous variation in over-winter thermoregulatory strategies across their range. Prairie dogs within Canada comprise the northernmost population of this species and are the only population of black-tailed prairie dogs known to consistently hibernate over winter. Previous studies reveal variation in hibernation expression within this population. Yet, it is currently unknown what influences this variation, and in general, this unique aspect of their biology remains understudied. However, recent study indicates that males and females differ in their overwinter winter mass loss which may result from differences in hibernation expression.

In this thesis, I investigated the role of sex and pre-hibernation body condition on hibernation expression and over-winter energy expenditure in a single colony of prairie dogs located in Grasslands National Park, SK, CA. First, I investigated whether males and females differed in their hibernation expression and energy expenditure by assessing the use of various hibernation traits and quantifying over-winter change in body condition. I found that females had longer hibernation periods, used more bouts of torpor, and overall spent a greater amount of time in torpor compared to males. However, males spent a greater proportion of their shorter hibernation period in torpor and used longer, colder bouts of torpor. Despite differences in hibernation expression, I did not find any evidence that males and females lost similar amounts of condition over winter, though males appeared to lose condition at a faster rate. However, results regarding over-winter changes in body condition should be interpreted with caution due to discrepancies between when male and female prairie dogs hibernated relative to when I recorded body condition measurements.

I also conducted a supplemental feeding trial during a portion of the pre-hibernation fattening period of prairie dogs to expand variation in pre-hibernation body condition and combined this data with previously existing data to investigate the influence of pre-hibernation body condition on hibernation expression and over-winter change in body condition. I found

that, relative to individuals in poor condition, individuals in better pre-hibernation body condition did not shorten the duration of the hibernation period but did reduce the proportion of the hibernation period spent torpid and increased the length of arousal bouts. Prairie dogs in good pre-hibernation body condition did experience a greater decrease in body condition over winter but still emerged from hibernation in better condition compared to individuals of a poorer condition.

The work presented in this thesis increases our understanding of a relatively unknown aspect of black-tailed prairie dog biology - hibernation. Additionally, this thesis improves our understanding of the factors that influence hibernation expression and how individuals alter their use of hibernation with respect to their energetic condition. Future research investigating prairie dog body mass dynamics immediately after hibernation and the effects of sociality and predation on hibernation could be explored to further our understanding of hibernation use. Increasing our understanding of the factors that influence intraspecific variation in hibernation expression can lead to advances in understanding how hibernation evolved as well as aid us in our predictions of if and how populations will respond to changing environments.

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Chapter 1

General Introduction

1.1 Hibernation overview

At temperate latitudes, winter is typically characterized by low resource availability cooccurring with cold ambient temperatures and snowfall. As a result, individuals are energetically challenged as their daily energy expenditure is elevated while food resources are limited. To cope with this challenge, hundreds of mammalian species hibernate as an energy-saving strategy. It is important to note that while hibernation is often thought of as an adaptation to cold climates, its use is not restricted to species, periods or locations that experience cold weather (i.e., Dausmann et al., 2005; Lovegrove and Génin, 2008; Stawski et al., 2009; Geiser, 2020). Regardless, hibernation is generally characterized by consecutive, multiday periods in which metabolism and, concomitantly, body temperature (T_b) are reduced in a controlled fashion, termed "torpor bouts" (Geiser & Kenagy, 1988; Geiser & Ruf, 1995; Geiser, 2004). During hibernation, bouts of torpor are interspersed with periods of arousal, wherein an individual's metabolism and T_b return to their euthermic levels (Geiser, 2004). As metabolic rate is substantially reduced during bouts of torpor (Geiser & Kenagy, 1988; Geiser & Ruf, 1995; Geiser, 2004), an individual's energy expenditure while hibernating is drastically reduced. For example, when hibernating at 4 °C, edible dormice (*Glis glis*) can expend up to 99.6% less energy compared to if they were euthermic (Wilz & Heldmaier, 2000).

The use of hibernation can yield substantial energy savings (Geiser & Ruf, 1995); however, it does not entirely remove an individual's energetic requirements (Guppy & Withers, 1999). To meet the energy required to support metabolism during hibernation, individuals must acquire sufficient energetic resources before the onset of hibernation. Hibernators can vary in how they store their energetic resources, with energy primarily stored either endogenously as fat or exogenously as food caches (Table 1 in Humphries et al., 2003b). Food caching hibernators generally build up hordes of seeds, nuts, and/or dried vegetation within their over-winter site (hibernaculum) via an increase in foraging effort. Fat storing hibernators also increase foraging efforts in the fall, but in contrast to food caching hibernators, they

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undergo a period of hyperphagia and convert food resources to fat reserves. Indeed, before hibernation, fat-storing hibernators dramatically increase their body mass via increases in fat mass. For example, Daurian ground squirrels (*Spermophilus dauricus*) increase their body mass by 62% before hibernating (Xing et al., 2012), and Arctic grounds squirrels (*Urocitellus parryii*) can increase their fat mass by 7-8 fold; accounting for the majority of mass gain at this time (Sheriff et al., 2013).

1.2   Intraspecific variation

Generally, investigations of the evolutionary and ecological causes and consequences of hibernation have been limited to broad-scale comparisons of species that are capable of hibernation or not (Lane, 2012; Ruf & Geiser, 2015). However, given that intraspecific variation is the raw material for evolution, investigations at this level could provide important insights that have generally been overlooked (Williams, 2008). As selective pressures can differ across sex and age classes, and local environmental conditions can vary across a species' range, investigation of hibernation at the intraspecific level represents a unique opportunity to study the factors influencing hibernation expression.

Further, hibernation can account for months of a species' annual cycle (Kenagy et al., 1989; Young, 1990; Buck & Barnes, 1999). For example, Columbian ground squirrels (*Urocitellus columbianus*) spend approximately eight months of the year in hibernation (Young, 1990) while black-tailed prairie dogs (*Cynomys ludivicanus*) have been reported to hibernate for approximately four months (COSEWIC, 2011). Therefore, hibernation likely has important ecological and evolutionary relevance for such species. However, the evolutionary and ecological consequences of hibernation are currently not well understood since investigations have generally been conducted in controlled laboratory settings (Lane, 2012; Ruf & Geiser, 2015). If a species', population's, or specific cohort's hibernation expression is affected by changes in environmental conditions and, in turn, hibernation expression influences survival and reproduction, demographics may change, and/or species/population persistence may be affected (Ozgul et al., 2010). This could be particularly important in light of climate change, where precipitation and temperature patterns are changing, and extreme weather is increasingly observed (IPCC, 2013). The following section discusses the cost and benefits of hibernation use, which may be crucial for understanding and predicting how various factors influence the hibernation phenotype an individual employs over winter.

1.3 Hibernation trade-offs

The use of prolonged, deep torpor bouts during hibernation offers clear benefits of reducing energy expenditure and water use when resource abundance is low and/or local weather conditions are severe. The energy savings that accumulate throughout the hibernation period are presumed to increase survival during this time (Turbill et al., 2011). In addition to increasing survival during cold weather and low food availability, hibernation and torpor have been found to increase survival during fires (Nowack et al., 2016) and storms (Nowack et al., 2015). Hibernation may also offer benefits of increased survival via reductions in predation risk (Turbill et al., 2011). For example, the edible dormouse hibernates for longer than expected based on environmental conditions alone, which has often been attributed to the ability of hibernation to reduce the risk of mortality from predation (Bieber et al., 2014). Regardless of the mechanism (i.e., reduced energy expenditure or predation risk), a 2011 meta-analysis found evidence that during the hibernation period, individuals benefit from improved monthly survivorship relative to the active season and, overall, hibernating species had higher annual survival rates as compared to non-hibernators (Turbill et al., 2011). Further, hibernation has been associated with increased longevity (Turbill et al., 2011), which may be the result of senescence being inhibited during hibernation (Lyman et al., 1981; Turbill et al., 2013; Kirby et al., 2019) and/or life history trade-offs that result from increased survival probability (Turbill et al., 2011).

Hibernation also allows individuals to conserve additional energetic resources for future allocation. Individuals that increase torpor use (depth and/or duration) during hibernation can increase the energy savings they accumulate over winter and, thus, emerge in better condition (larger energy reserves). Sex and age-related variation in hibernation could arise depending on the benefits of having additional energetic resources following emergence. Generally, species commence reproduction following emergence from hibernation in the spring (Knopf & Balph, 1977; Morton & Sherman, 1978; Murie & Harris, 1978). Thus, individuals that increase torpor expression during hibernation have additional energy to allocate to reproduction. Females that emerge in better condition in the spring have been found to have increased reproductive success (*Urocitellus armatus*; Sauer and Slade, 1987, *U. richardsonnii*; Dobson and Michener, 1995, *U. parryii*; Karels et al., 2000). Similarly, males that emerge in better body condition may benefit from increased reproductive success (Raveh et al., 2010; Hoogland, 2001).

Despite the benefits of hibernation and the use of torpor within, hibernation and torpor use is associated with several costs. Yet, recent work has challenged whether the costs associated with torpor expression have been over-emphasized (Geiser, 2020). Nonetheless,

the existence of these costs could have implications for hibernation use (Humphries et al., 2003b). While the use of hibernation has the potential to alleviate the risk of predation, hibernators are still vulnerable to predation if those predators enter their

um while individuals are in a torpid state (Humphries et al., 2003b). Previous research has established that during torpor, individuals do not use slow-wave activity (Daan et al., 1991) and do not demonstrate REM and non-REM sleep (Blanco et al., 2016) and, as such, are thought to be sleep deprived during torpor bouts. In accordance with sleep deprivation, memory consolidation may also be impaired during bouts of torpor (Walker & Stickgold, 2004). Millesi et al. (2001) found that European ground squirrels (*Spermophilus citellus*) that hibernate have reduced retention of learned spatial and operant tasks compared to individuals who do not hibernate. In addition, torpor has been associated with immunosuppression (Burton and Reichman, 1999; Prendergast et al., 2002 but see Bouma et al., 2010 for a review). Hibernating Turkish hamsters (*Mesocricetus auratus*) have also been found to mount reduced secondary immune responses (lower levels of antibodies) when exposed to an antigen when compared to non-hibernating individuals (Burton & Reichman, 1999). Other costs associated with torpor use include an accumulation of metabolic wastes (i.e., ketones; Baumber et al., 1971, reactive oxygen species; Buzadžic et al., 1990; Carey et al., 2000; Orr et al., 2009; Brown et al., 2012; Duffy, 2020 and dehydration via evaporative water loss Thomas and Geiser, 1997). The costs that accumulate throughout a torpor bout are generally assumed to be mitigated when hibernators periodically arouse (Thomas & Geiser, 1997; Humphries et al., 2003b). However, arousals are when the majority of energy expenditure occurs during the hibernation period (Wang, 1979; Thomas et al., 1990).

1.4   Optimal hibernation expression

Consequently, an individual's hibernation expression (use of torpor and arousal) is hypothesized to reflect an optimization of the associated costs and benefits of hibernation, ultimately governed by an individual's energetic needs (Humphries et al., 2003b; "hibernation optimization hypothesis" *sensu* Boyles et al., 2007). Accordingly, an individual's hibernation expression can be affected by their energetic state before hibernation and their energetic requirement upon emergence. Specifically, individuals with sufficiently large energy reserves before hibernation are predicted to reduce torpor use and thereby its associated costs. In contrast, individuals with limited energetic resources are predicted to increase the use of torpor and incur additional physiological costs to maximize their energy savings (Humphries et al., 2003a). Support for these predictions has been found in several species, including Eastern chipmunks (*Tamias striatus*; Humphries et al., 2003a), common hamsters (*Cricetus cricetus*;

Siutz et al., 2018), edible dormice (Bieber et al., 2014), and woodchucks (*Marmota monax*; Zervanos et al., 2014). For example, fatter edible dormice reduce the depth and length of torpor bouts when compared with leaner individuals (Bieber et al., 2014).

Sexual variation in response to the size of energy stores has been found (Siutz et al., 2018), which may result from sex differences in energy requirements after hibernation is terminated. Depending on the importance of post-hibernation energy reserves on reproduction, variation in optimal hibernation strategies could exist between the sexes (Humphries et al., 2003b). If successful reproduction in one sex is more dependent on having sufficient energy stores in the spring than another, that sex may exhibit more extensive torpor use (Humphries et al., 2003b). Accordingly, if females of a species have higher energetic reproductive costs following emergence, they are hypothesized to be thriftier in their energy use over-winter and maximize torpor use during hibernation ("thrifty female hypothesis"; Jonasson and Willis, 2011). For example, in little brown bats (*Myotis lucifugus*), where males have secured mates before or during hibernation and females become pregnant shortly after emergence, females experience less over-winter loss in body condition (Jonasson & Willis, 2011) and use shorter arousal bouts (Czenze et al., 2017) compared to males.

1.5   Sex-specific hibernation

Sexual variation in hibernation expression has been found in a variety of species, including common hamsters (Siutz et al., 2016), golden-mantled grounds squirrels (*Callospermophilus lateralis*; Healy et al., 2012), Richardson's ground squirrels (Michener, 1992), and Columbian ground squirrels (Young, 1990). As a general trend, males have been found to terminate hibernation earlier than females (Young, 1990; Michener, 1992; Healy et al., 2012; Kart Gür and Gür, 2015 but see Zervanos et al., 2014 and Siutz et al., 2016), which has often been attributed to the fact that torpor use inhibits testicular development and spermatogenesis (Barnes et al., 1986). As such, earlier emergence in the spring allows males to become reproductively ready before females emerge. Earlier emergence may also provide males with more time to defend their territories (Buck & Barnes, 2003; Healy et al., 2012). In contrast, females may delay emergence in the spring relative to males until males are reproductively ready and environmental conditions are consistently favourable (Michener, 1983b). Sex differences related to the onset of hibernation have also been observed; however, these differences are species-dependent (Williams et al., 2014). For example, hibernation entrance in male Arctic ground is delayed relative to females (Sheriff et al., 2011), whereas, in Columbian ground squirrels, males enter hibernation earlier than females (Young, 1990). Male hibernators have also been found to typically hibernate for shorter periods and/or exhibit shorter bouts com-

pared to females and/or spend proportionally less time in torpor (Young, 1990; Michener, 1992; Healy et al., 2012; Kart Gür & Gür, 2015); though exceptions to this trend exist (Siutz et al., 2016).

1.6 Study species

Black-tailed prairie dogs are highly gregarious, burrowing rodents that inhabit short- and mixed-grass prairie ecosystems in North America (Hoogland, 1995). The distribution of black-tailed prairie dogs is the largest among prairie dog species, extending from northern Mexico until reaching its northern limit in southwestern Saskatchewan, Canada. Extra-limital populations are located north of the naturally occurring prairie dog range (one near Edmonton, Alberta, Canada and one near Moose Jaw, Saskatchewan, Canada; Trefry and Holroyd, 2012). However, little is known about these colonies and given that escaped captive prairie dogs established them, individuals from these colonies are not discussed further. In Canada, black-tailed prairie dogs (hereafter; 'prairie dogs') exist as a single population considered isolated from more southern populations of prairie dogs (20 km from the nearest population in Montana; (Gummer, 1999). More specifically, the Canadian population is comprised of 19 naturally occurring colonies located in and around Grasslands National Park, Saskatchewan, Canada (Stephens et al., 2018).

Across their range, prairie dogs exhibit tremendous variation in thermoregulatory strategies. Several early investigations of thermoregulation in prairie dogs concluded that they were incapable of hibernation due to behavioural observation and body temperature data of wild individuals. Indeed, in some locations, prairie dogs can be observed above ground and active throughout the winter (King, 1955; Koford, 1958; Smith, 1958; Tileston & Lechleitner, 1966). Further, Bakko et al. (1988) found that the body temperature of individuals from a colony in Fort Collins, Colorado, USA, did not drop below 31 °C. Despite observations of prairie dogs remaining belowground for several days either late in winter (Hoogland, 1995) or during periods of inclement weather (Tileston & Lechleitner, 1966; Hoogland, 1995), the short timeframe of these instances led to the belief that prairie dogs were incapable of hibernation (Hoogland, 1995). This species' high degree of sociality has often been attributed to their non-hibernating nature (Tileston & Lechleitner, 1966; Bakko et al., 1988), with year-round interaction thought necessary to maintain the social structure (Michener, 1983a, 1984 but see Kusch and Lane, 2021b).

Laboratory investigations of prairie dogs, however, have demonstrated their ability to enter bouts of torpor during low ambient temperatures (Anthony, 1955; Thomas & Riedesel, 1975; Hamilton & Pfeiffer, 1977; Harlow, 1997) and following removal of food and water

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(Harlow & Menkens Jr., 1986; Harlow & Frank, 2001). Investigations of prairie dogs in the wild have demonstrated their ability to enter minor and major bouts of torpor sporadically over-winter (Lehmer et al., 2001; Lehmer & Van Horne, 2001; Lehmer et al., 2003; Lehmer & Biggins, 2005). Despite the documented cases of torpor use in both laboratory and field settings, none of the studies mentioned above documented consistent, repetitive bouts of deep, prolonged torpor that characterize hibernation, and as a result, prairie dogs have been generally considered to only use torpor facultatively (Harlow & Menkens Jr., 1986; Harlow, 1995, 1997; Harlow & Frank, 2001; Lehmer et al., 2001; Lehmer et al., 2003; Lehmer & Biggins, 2005).

More recent investigations of prairie dogs in Colorado, USA, and Saskatchewan, Canada, have documented body temperature patterns indicative of hibernation (Gummer, 2005; Lehmer et al., 2006; Hawkshaw et al., 2021 in prep). Lehmer et al. (2006) found that five prairie dogs that were residents of a single colony in Pawnee National Grassland in Colorado, USA entered sequentially bouts of torpor that lasted on average 96.37 h with $T_{\rm b}$ dropping to an average minimum of 21.98 °C. However, individuals that resided in nearby colonies had thermoregulatory patterns similar to those previously described in prairie dogs (Lehmer et al., 2006). Comparable to the five prairie dogs in Pawnee National Park, Gummer (2005) found that prairie dogs studied from two colonies across two different years within the Canadian population regularly entered hibernation during winter, exhibiting consecutive bouts of torpor that were interspersed with periods of arousal.

Canadian black-tailed prairie dogs typically hibernate for approximately four months from November to February (Gummer, 2005). However, the Canadian population exhibits substantial individual variation in hibernation strategies (Figure 1.1, Hawkshaw et al., 2021 in prep). Despite the Canadian population's seemingly unique ability to employ hibernation regularly (Gummer, 2005; Hawkshaw et al., 2021 in prep), this aspect of their biology has received little attention. Furthermore, investigations into factors that may be driving their use of hibernation and the observed intraspecific variation in hibernation expression remain limited (Gummer, 2005; Hawkshaw et al., 2021 in prep). A recent study found that, within the Canadian population, female prairie dogs do not lose as much mass compared males (Kusch et al., 2021), which suggests that female reproduction may be dependent on spring body condition. Therefore, females may use hibernation expressions that yield larger energy savings than males to ensure sufficient energy stores are available for reproduction. Previously, Gummer (2005) found that males and females did not differ in their expression of hibernation in terms of mean minimum $T_{\rm b}$ reached during torpor and mean torpor bout duration. However, this study was limited to 11 individual prairie dogs (six males and five females); therefore, population-level conclusions may be premature. In addition, body con-

dition has been found to influence hibernation expression in other hibernators (Humphries et al., 2003a; Siutz et al., 2018; Bieber et al., 2014; Zervanos et al., 2014). As such, it may be an important factor in driving the observed between-individual variation in prairie hibernation expression.

The Canadian population of prairie dogs is currently considered threatened (COSEWIC, 2011; Species at Risk Act, 2018), and a large decline in population size has been documented (see COSEWIC, 2011). In addition, prairie dog population size has undergone large fluctuations between years (Stephens, 2012). Recently, body condition before hibernation and torpor use have been suggested as influential factors in determining prairie dog over-winter survival and reproductive success (Stephens et al., 2018). Therefore, investigating the potential effects of pre-hibernation body condition and sex on hibernation expression represent key areas of study for this species.

Moreover, given that black-tailed prairie dogs are social, facultative, fat-storing hibernators, they represent a unique opportunity to test the hibernation optimization hypothesis (Humphries et al., 2003b) and related thrifty female hypothesis (Jonasson & Willis, 2011). As facultative hibernators typically express hibernation when cold-stressed and food-limited, prairie dog hibernation expression may be more sensitive to the quantity of energetic resources they have before hibernation. Alternatively, it has been suggested that compared to food caching hibernators, those which store fat may be more limited in their ability to adjust torpor since the size of their energetic reserves is more limited, and therefore the energetic necessity of torpor is greater (Siutz et al., 2017; Humphries et al., 2003b). In addition, since this population of prairie dogs is located at the northern limit of their species range, where resources availability is likely to be more variable, prairie dogs may be more sensitive to the effect of pre-hibernation body condition. Prairie dogs are also a species where males invest earlier in reproduction relative to females but appear to lose more body mass over winter than females (Kusch et al., 2021), which suggests that females may be thriftier in their expression of hibernation. Thus, black-tailed prairie dogs are a unique species to investigate the influence of energy availability and sex on hibernation expression.

1.7 Objectives and thesis overview

In this thesis, I investigate the influences of sex and pre-hibernation body condition on intraspecific variation in hibernation expression of black-tailed prairie dogs at the northern edge of their range. My research objectives were two-fold: to determine (1) if prairie dogs demonstrate sexual variation in hibernation expression and over-winter energy expenditure, and (2) to investigate if prairie dog hibernation expression and overwinter change in body

condition correlate with pre-hibernation body condition and if these responses were sex-specific.

In Chapter 2, I address objective (1) using male and female prairie dogs across three hibernation years (2017, 2019 and 2020) to assess if female prairie dogs use torpor more extensively during hibernation and expend less energy over winter than males. In Chapter 3, I build on Chapter 2 and address objective (2) by expanding variation in pre-hibernation body condition via food supplementation of a subset of prairie dogs in the autumn of one hibernation year. While Chapter 2 includes unfed individuals across three hibernation years, Chapter 3 incorporates fed prairie dogs from the food supplementation year with unfed individuals across the three hibernation years. This allowed for a larger sample size and range of pre-hibernation body conditions to be used to address objective (2). Specifically, in Chapter 3 I experimentally tested whether prairie dogs in better pre-hibernation body condition exhibit reduced torpor use compared to prairie dogs in poorer condition, and I assessed if males and females respond differently to pre-hibernation body condition. Finally, in Chapter 4, I offer general conclusions and highlight broad implications of the results described herein.
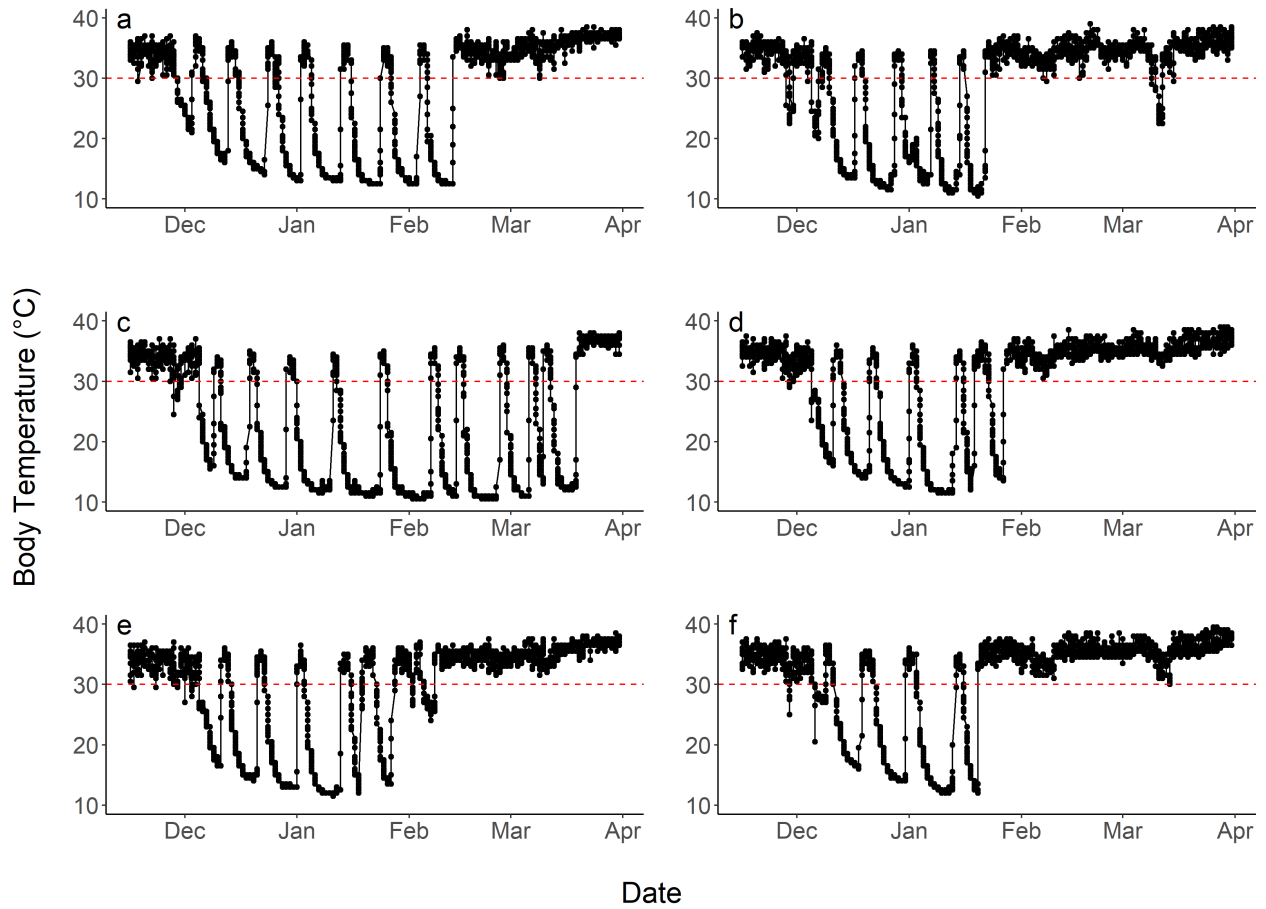
Figure 1.1: Hibernation expression in 6 black-tailed prairie dogs from 2016-2017. Panels a, c and e represent body temperature traces from female prairie dogs, and panels b, d, and f represent body temperature traces from male prairie dogs. Horizontal, dashed red line indicates 30 ℃.

Chapter 2

Sexual variation in black-tailed prairie dog hibernation expression and over-winter energy expenditure

2.1 Introduction

To survive periods of inclement weather and resource limitation in the environment, hundreds of species hibernate. Hibernation is an effective energy-saving strategy, with the metabolic rate of most hibernators reduced to less than 10% of their euthermic metabolic rate (Geiser & Ruf, 1995). During hibernation, accompanying the drastic decrease in metabolic rate, body temperature (T_b) is decreased for periods lasting multiple days in what is termed a torpor bout (Geiser and Ruf, 1995; Geiser, 2004 but see Tøien et al., 2011). However, metabolism and T_b cannot be maintained at this "torpid" level for the entire hibernation period. While bouts of torpor conserve energy, physiological costs, including memory impairment (Millesi et al., 2001), a buildup of reactive oxygen species (Buzadžic et al., 1990), and a reduced ability to respond to immune challenges (Burton & Reichman, 1999) are also associated with torpor use. Bouts of multiday torpor are interspersed with periods of arousal, when metabolic rate and T_b return to euthermic levels (Geiser, 2004), which are believed to partially offset the physiological costs of torpor (Humphries et al., 2003b; Thomas & Geiser, 1997).

By expressing torpor to a greater extent (i.e., lower metabolism and lower T_b, increased length and greater number of individual bouts), individuals can maximize their energy savings, but risk incurring more severe physiological costs that are associated with torpor use (Humphries et al., 2003b). On the other hand, if individuals maintain higher body temperatures during torpor, spend proportionally more time aroused, or increase their numbers of arousals, they increase their energy expenditure but reduce the physiological damage accrued. As such, Humphries et al. (2003b) hypothesized that individuals optimize their use of torpor and arousal (hibernation expression) based on their energetic needs ("hibernation

optimization hypothesis"; *sensu* Boyles et al., 2007).

Prior to entering hibernation, individuals need to acquire sufficient energy stores to support energy expenditure during hibernation. Species typically accumulate the required energy by storing food in caches or converting food to fat deposits. The energy accumulated prior to hibernation not only supports metabolism during hibernation but also energy requirements upon emergence (if resource abundance is limited). For example, male Arctic ground squirrels (*Urocitellus parryii*) cache food prior to hibernation and consume this food cache to support gonadal growth before emerging from their hibernaculum the following spring (Buck & Barnes, 1999; Gillis et al., 2005). Therefore, individuals can ensure they emerge with sufficient energy stores by both acquiring sufficient energy prior to hibernation and maximizing energy savings.

For several hibernating species, reproduction commences shortly after emergence in the spring (Knopf & Balph, 1977; Morton & Sherman, 1978; Murie & Harris, 1978). Since reproduction is energetically expensive (Gittlemen & Thompson, 1988), having sufficient energy stores is critical. Accordingly, one of the central predictions of the hibernation optimization hypothesis is that the sex with larger reproductive requirements upon emergence will have larger autumn energy reserves (better body condition) and express torpor to a greater extent (Humphries et al., 2003b). Furthermore, within a sex, variation in the size of energy reserves upon emergence can lead to variation in reproductive success. Individuals in several species that emerge in better body condition have been found to have improved reproductive success compared to those in poorer condition (Sauer & Slade, 1987; King et al., 1991; Dobson & Michener, 1995). For example, female Uinta ground squirrels (*Urocitellus armatus*) that have heavier body mass in the spring have larger litters than lighter females (Sauer & Slade, 1987). Additionally, black-tailed prairie dogs (*Cynomys ludovicianus*) appear to forgo reproduction altogether when body condition upon emergence is poor (Kusch et al., 2021).

Jonasson and Willis (2011) further developed the hibernation optimization hypothesis (Humphries et al., 2003b) and proposed the thrifty female hypothesis. The thrifty female hypothesis was formulated for hibernating little brown bats (*Myotis lucifugus*) and predicts that females will be more conservative ("thrifty") with their energy use over winter than males due to their higher reproductive demands. However, it is important to note that in little brown bats, mating occurs prior to and/or during hibernation, and females become pregnant shortly after emergence. Thus, in little brown bats, males have already invested energy in reproduction prior to emergence, while females will allocate post-emergent energetic resources across the breeding season. Accordingly, female little brown bats exhibit a more energetically conservative hibernation with shorter arousal periods than males (Czenze et al., 2017) and experience less change in body condition over winter (Jonasson & Willis, 2011).

In species for which there is evidence that females have higher reproductive demands in the spring, we expect females to behave similarly to little brown bats. Overall, predictions of sex-specific hibernation expressions must consider the overall energetic costs of reproduction for each sex, the timing of reproductive events, and how each may influence reproductive success in each sex. For example, even if females of a particular species overall invest more energetic resources to reproduction compared to males, but males invest energetic resources earlier in the reproductive season when resources are limited, males may employ more "thrifty" hibernation expressions and/or other energetic strategies to enhance their reproductive success. Alternatively, especially in income breeding species (*sensu* Drent and Daan, 1980) emergence and reproductive investment for females may be timed to when resources are more readily available in the environment. Therefore, females may not be as reliant on emergent energy reserves for successful reproduction.

The specific timing of hibernation emergence can also influence reproductive success. For example, if an individual emerges earlier, reproductive opportunities and the time available for successful rearing of young prior to the next hibernation season may be increased. Female European ground squirrels (*Spermophilus citellus*) that emerge earlier have earlier oestrus dates and thereby, larger litters that emerge from nest burrows earlier (Millesi et al., 1999). However, females may have to balance their emergences dates in order to time the most energetically expensive stage of reproduction (primarily lactation) when environmental resource abundance is high (Michener, 1983b). Earlier emergence dates may allow for males to increase their mating opportunities (Michener, 1983b) and, thereby their reproductive success. In several sciurid hibernators, protandry occurs, with males generally observed terminating hibernation and emerging from their hibernaculum earlier than females (Michener, 1983b; Young, 1990; Sheriff et al., 2011; Kart Gür & Gür, 2015). Hibernation has been found to inhibit spermatogenesis and gonadal development (Barnes et al., 1986). As such, males that terminate hibernation earlier increase their time to become reproductively ready prior to female emergence. Earlier emergence from hibernation may provide males with additional time to secure and defend territories (Michener, 1983b; Buck & Barnes, 2003). However, it should be noted that earlier emergence for either sex can come at the cost of increased exposure to inclement weather and predators (Bieber et al., 2018; Willis, 2017).

Black-tailed prairie dogs are highly social, burrowing, small mammals that inhabit short and mixed-grass prairie ecosystems. In southwestern Saskatchewan, Canada, at the northern edge of their range, prairie dogs have been found to regularly hibernate over winter and exhibit consecutive bouts of deep multiday torpor (Gummer, 2005; Hawkshaw et al., 2021 in prep). To date, this aspect of prairie dog biology has been understudied, resulting in a poor understanding of the factors that influence prairie dog hibernation expression. Given that

reproductive costs in the spring can have important consequences for hibernation expression in other hibernating species, sex may influence prairie dog hibernation expression and energy expenditure. Kusch et al. (2021) found evidence that female black-tailed prairie dogs retain more mass over winter than males, suggesting that females may have higher spring reproductive costs and employ a more energetically conservative pattern of hibernation (i.e. spent more time in torpor, use longer and colder bouts of torpor) as predicted by the thrifty female hypothesis. However, in contrast to little brown bats, both male and female prairie dogs initiate reproduction following hibernation, with the largest reproductive energy investment of males (during gonadal maturation and mating) occurring earlier in the spring compared to females (during lactation). The timing of male reproductive investment post-hibernation may lead to male reproduction being more reliant on energy conserved during hibernation and thereby male hibernation expressions that are more energetically conservative than females. The one previous study of hibernation expression in black-tailed prairie dogs did not find evidence of sexual variation (Gummer, 2005). However, the sample size in this study was moderate, and comparisons were limited to the average depth and duration of torpor bouts. To my knowledge, no study has completed an in-depth investigation of hibernation expression in male and female black-tailed prairie dogs where traits such as hibernation phenology, duration, total time spent torpid and aroused, duration and temperature of arousal bouts, the proportion of time spent torpid and overall use of heterothermy are compared.

2.1.1 Objectives and Hypotheses

The purpose of this chapter was to investigate whether sexual variation in hibernation expression and change in body condition (use of energy reserves) exists in the northernmost population of black-tailed prairie dogs. I tested two interrelated hypotheses, namely the hibernation optimization hypothesis and the thrifty female hypothesis. I hypothesized that if spring energy requirements are higher for females and spring energy requirements influence hibernation expression, females would employ more energetically conservative hibernation and expend less energy over winter compared to males. I predicted that hibernation expression would vary between the sexes such that females would have a longer hibernation period comprised of longer and deeper (colder) torpor bouts compared to males. As a result of sex differences in hibernation expression, I also predicted that females would expend less energy overwinter as measured by a decreased over-winter change in condition as compared to males. Additionally, males in several hibernating species of ground squirrels have been found to emerge earlier in the spring as compared to females (Michener, 1983b; Young, 1990; Sheriff et al., 2011; Kart Gür & Gür, 2015), therefore, I predicted that male prairie dogs would also emerge earlier compared to females.

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2.2 Methods

2.2.1 Study site

I studied prairie dogs in a single colony ('Walker') located within Grasslands National Park, southwestern Saskatchewan, Canada (49° 3' 46.8"N, 107° 21' 28.8"W). This colony occurs within the mixed-grass prairie ecosystem (Coupland, 1992) with vegetation primarily comprised of needle and thread grass (*Hesperostipa comata*), blue gramma grass (*Bouteloua gracilis*) and western wheatgrass (*Agropyron smithii*; Stephens et al., 2018). The climate in the area is classified as semi-arid (McGinn, 2010); according to the Environment and Climate Change Canada 1981 to 2010 Climate Normals, a nearby weather station (Val Marie Weather Station, approx. 49 km from my study site) recorded 352.5 mm of mean annual precipitation, of which 77.3 cm fell as snow (Environment and Climate Change Canada, 2021). Additionally, from 1981 to 2010, ambient temperatures ranged from -49.4 to 41.1 °C (Environment and Climate Change Canada, 2021).

2.2.2 Data collection

2.2.2.1 Live-trapping and individual identification

All fieldwork was conducted under research permits from Parks Canada (GRA-2014-16101 and SAR-GRA-2014-16101) and the Ministry of Environment (20AR028W). In addition, all procedures described below follow the Canadian Council on Animal Care guidelines and received approval from the University of Saskatchewan Animal Research Ethics Board (AUP-20140042). Prairie dogs that I used in my study were residents of a colony that was part of a seven-year monitoring program (2014-2020). As such, I used previously established live-trapping and handling protocols. Briefly, I live-trapped individuals using Tomahawk live-traps (Tomahawk Live Trap Company, Tomahawk, WI, USA) baited with a mixture of peanut butter and rolled oats. I checked traps at a minimum of once every 2 h but increased the frequency of trap checks as ambient temperature increased above 25 °C. I did not trap prairie dogs above 30 °C or during periods of rain.

As a part of the long-term study, at first capture, prairie dogs received unique alphanumeric ear-tags (National Band Tag Company, Newport, Kentucky, US) and a unique symbol for identification. I followed the same practice for any new individual captured during my study. The symbol for each prairie dog was painted on their dorsal pelage using non-toxic Nyanzol-D (Greenville Colorants, Clifton, New Jersey, USA) to allow for ease of identification in the field. By marking prairie dogs with a distinct symbol on their pelage, I could locate

specific prairie dogs at a distance. This allowed me to target trapping efforts on specific areas within the colony and reduce the number of times prairie dogs were handled (i.e., if a prairie dog was recently captured, I could release the prairie dog from the trap without handling). Since Nyanzol-D can fade in the sun, and prairie dogs moult twice a year (Hoogland, 1995), I repainted a prairie dogs' symbol when necessary. In addition, during captures, I assessed a prairie dogs' sex, age (juvenile, yearling and adult) and reproductive status. To determine sex, I observed the distance between a prairie dog's external genitalia and anus (Hoogland, 1995). To determine the reproductive status of females, I assessed the condition of their vulva and nipples (vulva open or closed; nipples lactating or not) as well as palpated their abdomen (to determine the stage of pregnancy) (Hoogland, 1995; Kusch et al., 2021). To determine the reproductive status of males, I assessed the condition of a males testes (descended (scotral) testes or not) (Hoogland, 1995). I assessed the age of a prairie dog using previous trapping records if they were previously captured as a part of the long-term monitoring program. Following the convention of Kusch et al. (2021), I considered any individuals as adults (>2 years old) if they had not been previously captured as juveniles.

I restricted my study to adult prairie dogs (two years or older) that were relatively trappable. All but one of the hibernation traces previously recorded from the Canadian population are from adult prairie dogs (one trace was from a yearling), therefore the observed variation is likely not driven by differences between subadults and adults. In addition, differences between subadults and adults have been observed in other hibernating species (French, 1990; Kart Gür & Gür, 2015; Siutz et al., 2016). Thus, expanding my study to include subadults could minimize the statistical power to detect sex differences in hibernation expression. I only used individuals that were relatively trappable to maximize the retrieval of temperature-sensitive data loggers (described below). This likely did not influence any subsequent results since traps were baited with a minimal amount of peanut butter and oats (<1 tsp).

2.2.2.2 Hibernation expression

To evaluate hibernation expression, I surgically implanted temperature-sensitive data loggers (Model no. DS1921GF50 Thermochron iButtons, Maxim Integrated, California, USA) into the abdominal cavity of prairie dogs. Implanting data loggers allowed me to monitor core body temperature (T_b) overwinter and determine an individual's use of torpor and arousal during the hibernation period.

2.2.2.2.1   Surgical procedure

Prior to implantation, I programmed each data logger to record T_b once every three hours, coated them with a physiologically compatible protective wax (2 M-Coat W-1 protective coating; Vishay Precision Group, Malvern, Pennsylvania, USA) and sterilized them via ethylene oxide sterilization. All surgeries were performed or supervised by a licensed veterinarian.

For implantation surgeries, I captured prairie dogs in late autumn (end of October in 2018 and 2019). After recording their capture location, I transported them to a nearby Parks Canada facility (approx. 5 km away from the colony). Once at the Parks Canada facility, I measured the body mass of prairie dogs with a Pesola spring scale (Prazisionswaagen AG, Schindellegi, Switzerland)). We induced prairie dogs under general anesthesia (Isoflurane; Abott Laboratories, Saint-Laurent, Quebec) using an isoflurane vaporizer system and non-rebreath circuit. Specifically, we administered 0.5 to 5% Isoflurane with a 1 L/min flow of oxygen to anesthetize prairie dogs. Once induced, we monitored anesthetic depth (heart rate, respiratory rate, reflex response) throughout the duration of the surgical procedure. We also subcutaneously injected prairie dogs with buprenorphine (0.02 mg kg^{-1}) and ketoprofen (2 mg kg^{-1}) to provide analgesia and lactated Ringer's solution to account for fluid loss.

To prepare the surgical field, we placed prairie dogs in a dorsal recumbent position and shaved a 2 cm area around where the skin was to be incised. We scrubbed the shaved area around the incision site three times with a betadine solution. We rinsed the first two betadine scrubs with water and the final scrub with a 70% alcohol solution. We then applied a dilute betadine solution and draped the incision site. We wore sterile gloves and a hairnet throughout the surgery and only touched the necessary sterile surgical field and equipment.

Once the surgical field was prepped, we made a 2 cm incision along the ventral midline and dissected the subcutaneous tissue to expose the linea alba. We then made an along the linea alba to open the abdominal cavity. We extended the incision site and inserted the prepared data logger into the abdominal cavity. We closed the linea alba with an interrupted suture pattern using an absorbable monofilament suture and closed the subcutaneous tissue and skin with either a continuous or interrupted pattern with a PDSII suture, and a small amount of surgical glue After the incision site was closed and prairie dogs were recovering from anesthesia, we administered a second dose of lactated Ringer's solution.

Following recovery from anesthesia, we moved the prairie dogs to small animal cages and offered apple and lettuce ad libitum. Post-surgery, we checked the small animal cages once every 3 h over a 24 h period to monitor prairie dog condition. After the 24 h monitoring period, we administered a second dose of buprenorphine (0.02 mg kg^{-1}) and ketoprofen (2 mg kg^{-1}) to provide additional analgesia and visually assessed the incision site for healing. I released prairie dogs at their specific capture site. Post-release, I continued to assess the

healing of the incision site by attempting to capture prairie dogs 2, 4, 8 and 14 d after their surgical date.

Details on the data loggers implanted are presented in Table 2.1. Briefly, I implanted 19 in 2018 and 48 data loggers in 2019. However, 25 of the 48 individuals implanted in 2019 were from animals that received a supplemental diet in autumn 2019, these individuals are discussed further in Chapter 3 but were not included in the analyses presented in Chapter 2 due to their experimental manipulation. In addition to the temperature loggers implanted in 2018-2019 and 2019-2020, 16 data loggers were implanted in 2017 as apart of the long-term monitoring program (Table 2.1). Data loggers in 2017 were implanted using the same protocol as described above but were programmed to record body temperature once every 2 h and were implanted in November. Despite the difference in sampling intervals across hibernation years, this should not substantially affect the calculated values for each hibernation trait. If the sampling interval difference did have an influence on the hibernation traits I quantified, this effect should have been captured as year effect, where 2017 was consistently different from 2019 and 2020, which did not occur. Additionally, the differences in sampling interval should not influence the detection of sex differences in hibernation expression, which was my primary interest.

The following year, I used the same surgical protocol described above for retrieval of data loggers, except that the data logger was massaged out of the incision upon opening an individual's abdominal cavity. Retrieval of data logger took place after the reproductive period (late June in 2019 and late July – early August in 2020) to ensure that the surgical procedures did not disrupt reproduction.

2.2.2.2.2   Defining hibernation

To delineate torpor from arousal, I adopted a definition of torpor bouts being any period where body temperature was <30 ℃ (Figure 2.1). Previous studies investigating prairie dog thermoregulation have used a 33 ℃ threshold (i.e., Gummer, 2005; Lehmer et al., 2006, , however, 30 ℃ is commonly used as the temperature threshold across various hibernating species (Young, 1990; Michener, 1992; Healy et al., 2012; Kart Gür & Gür, 2015). In addition, I defined a short, daily torpor bout as a period where body temperature was <30 ℃ for ≤24 h and a multiday torpor bout as a period where body temperature was <30 ℃ for >24 h (Geiser & Ruf, 1995). I defined the onset of a torpor bout as the date and time that body temperature first decreased to <30 ℃, and termination of a torpor bout as the date and time that body temperature first returned to ≥30 ℃ after having been <30 ℃ (See inset panel in Figure 2.1)

Hibernation is frequently defined as the period from the date of entry into the hibernac-

ulum to the date of emergence from the hibernaculum, while the heterothermic period is defined as the date of the first to last torpor bout (Young, 1990; Michener, 1992; Healy et al., 2012). However, I lacked the observational data necessary to determine when prairie dogs entered and exited their hibernaculum. Following the convention of Kart Gür and Gür (2015), I defined hibernation as the date and time of the start of the first multiday torpor bout to the date and time of the end of the last multiday torpor bout (Figure 2.1), with the exception of one individual who was captured prior to their last multiday torpor bout during a prolonged period of arousal (see details in statistical analyses section). It is important to note that most but not all of the prairie dogs in my study expressed both multiday and short torpor bouts ($\leq$ 24 h, Range: 0 to 14 short torpor bouts) during hibernation. I combined an individuals use of short torpor bouts and multiday torpor bouts when assessing an individual's hibernation expression to determine their overall use of torpor during hibernation.

To assess how an individual expressed hibernation in a given year, I quantified several aspects of hibernation (hereafter, 'hibernation traits'). Specifically, I determined the duration of the hibernation period, the total time spent torpid and aroused, the proportion of the hibernation period spent torpid, the number of torpor bouts used, the mean duration of torpor and arousal bouts, the mean minimum temperature reached during a bout of torpor, the mean arousal bout temperature, the minimum body temperature reached during hibernation and lastly, the onset and termination of the hibernation period. See Table 2.2. for a list of all hibernation traits and their respective definitions. Correlations between hibernation traits have been found in prairie dogs and other hibernating species (i.e., deeper torpor bouts are correlated with longer torpor bout durations, longer hibernation periods are correlated with more torpor bouts; Geiser and Kenagy, 1988; Lehmer et al., 2003; Levesque and Tattersall, 2010; Kisser and Goodwin, 2012 and data not shown here). Investigating each hibernation trait separately allowed me to quantify how males and females are expressing each trait (fully characterize their use of hibernation) and the extent to which the expressions of males and females differed. Additionally, if the strength of correlations between hibernation traits vary between the sexes or are influenced by other factors (i.e., energetic state), the extent of differences in hibernation expression could be under- or over-appreciated if only a few traits are considered.

In addition to characterizing these individual aspects of hibernation expression, I also summarized the hibernation expression of each prairie dog with a single metric using the Heterothermy Index (HI, Boyles et al., 2011). Boyles et al. (2011) developed HI as a method to assess how an individual's T_b varies over a given period with the intent that it could be used to compare thermoregulation between different groups (i.e., species, sexes, age cohorts). The HI is mathematically equivalent to a standard deviation and is specifically a

measure of how far and long an individual's body temperature deviates from their optimal T_b over a specified period. The HI can be calculated with the following formula:

$$HI = \sqrt{\frac{\sum (T_{b-mod} - T_{b-i})^2}{n-1}} \tag{2.1}$$

Where T_{b-mod} is the most common T_b an individual experienced (a proxy for optimal performance T_b) while active, T_{b-i} is an individual's T_b at a given time, i, and n is the number of times T_b was sampled in a given period.

I determined HI for each prairie dog over a common hibernation period and when accounting for individual variation in the duration of hibernation. I defined the common hibernation period as the time from the earliest date a prairie dog began hibernating (11 November) to the latest date a prairie dog stopped hibernating (19 March). Since 2020 was a leap year, I measured HI from 11 November to 18 March in 2020, so comparisons between years would be based on the same number of days.

2.2.2.3 Quantifying over-winter change in body condition

To quantify the change in body condition, and thereby estimate the amount of energy reserves individuals used over-winter, I took morphometric measurements, namely body mass and zygomatic arch breadth, in autumn during the implantation of data loggers (October of each study year) and at first capture in the spring of each hibernation year (March in each study year). I measured body mass with either a 1000 g or 3000 g Pesola spring scale (Note: The 1000 g Pesola measured mass in increments of 10g and measurements between increments were taken to the nearest 5 g and the 3000 g Pesola measured mass in increments of 20 g and measurements between increments were taken to the nearest 10 g) and zygomatic arch breadth with an analog calliper (Mastercraft). During both the data logger implantation and the post-hibernation captures, I took three measurements of zygomatic arch breadth. I then took the average of all measurements taken during both captures (six measurements total) for each individual. This allowed me to increase the number of measurements I used to determine an individual's zygomatic arch breadth and account for any variation that may be attributed to different handlers. In most vertebrates, growth stops once adulthood is reached (Gould, 1966), and in prairie dogs, size at maturity is reached at around 15 months (King, 1955). Therefore, since all individuals in my study were adults and no longer experiencing large changes in skeletal size, it is unlikely that differences in skeletal size between the two morphometric measurement periods would be due to growth.

I then used the body mass and zygomatic arch measurements to construct a zygomatic arch-derived index of body condition, which has been shown to positively correlate with body

fat levels in prairie dogs (Wishart et al., 2021 in prep). I constructed sex-specific indices, which allowed me to account for the sexual dimorphism observed in prairie dogs (Hoogland, 1995, 2003). For each sex, I standardized body mass and zygomatic arch breadth (z-score; mean=0, standard deviation (SD)=1), after which I regressed the standardized body mass on standardized zygomatic arch breadth. The residuals from the regression served as my index for body condition, where individuals with high index values are considered to be in better condition (large quantities of fat) relative to individuals with low index values. When constructing the body condition index (BCI), I included pre-and post-hibernation measurements, which allowed for pre-and post-hibernation conditions to be captured within the same index and for comparisons to be made between the two. To assess change in body condition, I subtracted an individual's pre-hibernation body condition from their post-hibernation body condition. I also determined the rate of condition lost over winter by dividing an individual's change in condition by the duration of their hibernation period.

The date I took the morphometric measurements was more than two weeks prior to the onset of hibernation or emergence from hibernation for most individuals (Pre-hibernation range: 15 to 45 d, post-hibernation range: 3 to 62 d). Given the range in the number of days I measured an individual prior to and after hibernation, the differences in body mass I measured may reflect differences in when measurements were taken instead of individual variation nor be truly representative of an individual's body mass directly before or after hibernation. To account for this, I performed linear regression analyses to estimate population rates of body mass change before the onset of hibernation and after the termination of hibernation. Using the estimates from the pre- and post-hibernation linear regression models, I applied a correction to the body mass measurements I took so that a given individual's body mass was more representative of their "true" pre- or post-hibernation body mass. For each individual, I corrected hibernation body mass to reflect their body mass to what would have been 15 d prior to hibernation and 3 d following hibernation so that I did not extrapolate past what my linear models could predict and comparisons between individuals would not be influenced measurement date. Through estimating rates of body mass change from a linear regression, this follows the assumption that body mass changes immediately before and after hibernation are linear which may not truly reflect how body mass changes during these times. Details on this analysis and the resulting correction I applied are in Appendix A. Non-rounded corrected pre- and post-hibernation body mass values were subsequently used to calculated the body condition index described above.

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2.2.3 Statistical analyses

Across the 2017, 2019 and 2020 hibernation years, a total of 56 data loggers were retrieved. However, in 2019, 6 data loggers failed to record data, and in 2020, 2 of the temperature loggers failed to record data (Table 2.1). Additionally, in 2020, 9 of the data loggers with useable data were from individuals that did not receive the supplemental diet. In 2019, one retrieved data logger was from a prairie dog located within a feeding zone implemented by Parks Canada and the Calgary Zoo (Parks Canada, *unpublished data*). As such, across all three hibernation years, 28 data loggers with usable data were retrieved from unfed individuals. Two of these 28 data loggers were retrieved from the same individual (same individual implanted in two different years). As such, I excluded the second recorded of data for these individuals in subsequent analyses. As such, I had a total of 27 hibernation traces from unique individuals.

One individual in 2017 was captured and then re-entered a short torpor bout (4 h) 5 d later, followed by a multiday torpor bout approx. 1.4 d after the short torpor bout. Prior to being captured, this individual had been aroused for approx. 41 d since the end of the previous multiday torpor bout (there was a single 2 h torpor bout that occurred approx. 24 d prior to capture; Figure 1.1 panel b). No other individuals in the dataset demonstrated euthermic periods between torpor bouts of this duration like this. As such, I analyzed their body temperature trace from the start of their first multiday torpor bout to the end of their second last multiday torpor bout. One male in 2019 demonstrated a long arousal bout (552 h or 23 d) before re-entering one final multiday torpor bout. Though this period of arousal was extensive, I lacked observational/capture records to determine whether this individual had been above ground and active and re-entered for a spontaneous torpor bout similar to the individual in 2017, or if this extensive period of arousal was a prolonged arousal bout used during hibernation. Given this, I analyzed this individual's body temperature trace to the end of their last multiday torpor bout.

I performed all analyses within the R statistical environment (R Core Team, 2021, version 4.1.1). To assess whether males and females differed in their change in body condition over winter and hibernation expression (HI and hibernation traits outlined in Table 2.2) I used Type II Analyses of Variance (ANOVA). For each dependent variable, except for the number of torpor bouts, I constructed linear models with sex and hibernation year as independent variables. I included hibernation year as an independent variable as variation in weather conditions across hibernation years could influence hibernation expression. For number of torpor bouts, I used a generalized linear model with a Poisson distribution as this was count data and assessed this model for over-dispersion. Type II ANOVAs were appropriate as my

sample sizes were unequal across hibernation years, and Type II ANOVAs have increased statistical power relative to Type III ANOVAs when no interaction is present (Langsrud, 2003; Smith & Cribbie, 2021). I was unable to account for an interaction between sex and hibernation year since my sample sizes in 2019 for females was $n=2$, and in 2020 $n=2$ was for males. I assessed model assumptions for each model via plots of residuals (residuals vs. fitted values, normal Q-Q plot, and conditional residual boxplots against each independent variable) and calculated the ratio of the largest error variance to the smallest error variance for each independent variable. If this ratio was ≤ 4, I continued using the linear model; however, if the ratio was >4 I followed the recommendation of Zuur et al. (2009) and used a generalized least squares (gls) model. I used a cut-off value of 4, as Fox (2008) demonstrated that linear regressions are relatively robust to violations of homoscedasticity below this threshold. In generalized least squares models, a variance structure can be specified, which allows for differences in variance among levels of a factor to be accounted for without transforming the response variable. If applicable, I compared the linear model to the gls model using a likelihood ratio test, and if the gls model was better ($p<0.05$) I assessed that model assumptions were improved. If the gls model was not better ($p\geq 0.05$), I used the linear model, as the error variance ratio of 4 proposed by Fox (2008) is considered conservative. I adjusted p-values to control for the false discovery rate to account for multiple testing of hibernation traits (Benjamini and Hochberg, 1995; Table A.5). All results were considered significant at $p<0.05$.

2.3   Results

2.3.1   Sexual variation in hibernation expression

Across all three hibernation years, 27 data loggers from unique individuals were retrieved, of which 13 were from male prairie dogs, and 14 were from female prairie dogs. As such, I quantified and compared hibernation expression in 13 males and 14 females. Summary statistics for hibernation traits in each sex are shown in Table 2.3.

2.3.1.1   Hibernation phenology

The mean onset of hibernation was 3 December in 2016-2017, 2 December in 2018-2019 and 21 November 2019-2020. The onset of hibernation did not significantly differ between the sexes (Type II ANOVA, $F_{(1, 23)}=1.17$, $p=0.39$), though males tended to enter hibernation slightly later than their female counterparts (Figure 2.2). hibernation onset was not significantly different across hibernation years (Type II ANOVA $F_{(2, 23)}=3.95$, $p=0.10$).

Mean emergence was 3 February in 2016-2017, 5 February in 2018-2019 and 15 February in 2019-2020. The date of emergence from hibernation was significantly different between the sexes (Type II ANOVA, $F_{(1, 23)}$=20.87, p<0.001), with males emerging from hibernation on average 24 d earlier than females (Figure 2.2). The date of emergence from hibernation did not significantly differ across hibernation years (Type II ANOVA, $F_{(2, 23)}$=0.17, p=0.85).

2.3.1.2 Hibernation duration

Males spent significantly less time hibernating than females (Type II ANOVA, $F_{(1, 23)}$=19.90, p<0.001). Males spent 51.4 ± 4.6 d in hibernation while females spent 86.9 ± 4.7 (Figure 2.3a). Hibernation year did not significantly affect the duration of hibernation in prairie dogs (Type II ANOVA, $F_{(2, 23)}$=0.70, p=0.67).

2.3.1.3 Torpor use

Male prairie dogs spent significantly less time in torpor than females (Type II ANOVA, $F_{(1, 23)}$=15.49, p=0.002). Males spent an average of 37.8 ± 2.8 d in torpor while females spent 54.9 ± 3.0 d in torpor (Figure 2.3b). Hibernation year did not significantly affect the total time a prairie dog spent in torpor (Type II ANOVA, $F_{(2, 23)}$=0.29, p=0.82). Despite spending less time in torpor overall, male prairie dogs spent a greater proportion of their hibernation period torpid (Type II ANOVA, $F_{(1, 23)}$=5.67, p=0.04). In general, males spent 74.6 ± 1.4% of hibernation in torpor while females only spent 64.2 ± 2.6% (Figure 2.3c). Hibernation year did not significantly affect the proportion of time a prairie dog spent torpid (Type II ANOVA, $F_{(2, 23)}$=1.60, p=0.35). The number of torpor bouts a prairie dog used during hibernation also significantly differed between the sexes (Type II ANOVA, $\chi^2_{(1)}$=33.76, p<0.001) but not across hibernation years (Type II ANOVA, $\chi^2_{(2)}$=7.34, p=0.10). On average, female prairie dogs used more than double the amount of torpor bouts that males did (Figure 2.4a). However, female prairie dogs also exhibit a greater range in the number of torpor bouts they used (Figure 2.4a). The number of torpor bouts a male used ranged from 5-10 bouts during the hibernation period, while for females, it ranged from 8-24 bouts.

Mean torpor bout duration significantly differed between the sexes (Type II ANOVA, $F_{(1, 23)}$=5.68, p=0.04) with females using bouts of torpor that were on average shorter in duration compared to males (Figure 2.4b). Females used torpor bouts that were on average 90.3 ± 9.3 h long while males used torpor bouts that were 126.4 ± 7.6 h. The mean duration of torpor bouts used by an individual did not differ across hibernation years (Type II ANOVA, $F_{(2, 23)}$=3.61, p=0.10). Across all bouts of torpor the mean torpor bout depth for males was significantly colder than females (Type II ANOVA, $F_{(1, 23)}$=6.78, p=0.03). The mean torpor

bout depth for males was 15.2 ± 0.5 °C while for females was 18.4 ± 0.9 °C Figure 2.4c). Hibernation year did not significantly affect a prairie dog's the mean depth of torpor bouts (Type II ANOVA, $F_{(2, 23)}$=1.54, p=0.35). Despite differences in mean torpor bout depth, males and females did not differ in the absolute minimum T_b reached during hibernation (Type II ANOVA, $F_{(1, 23)}$=0.002, p=0.96). The minimum body temperature a prairie dog reached was on average 11.2 ± 0.2 °C (Range: 9.5 to 12.5 °C). Hibernation year did not significantly affect minimum body temperature (Type II ANOVA, $F_{(2, 23)}$=4.05, p=0.10).

2.3.1.4 Arousal use

The total time a prairie dog spent in arousal bouts was influenced by sex (Type II ANOVA, $F_{(1, 23)}$=27.85, p<0.001), with males spending less overall time in arousal compared to females. Males spent on average 13.6 ± 2.0 d in arousal during hibernation while females spent on average 32.0 ± 3.4 d in arousal. Total time spent in arousal did not vary significantly across hibernation years (Type II ANOVA, $F_{(2, 23)}$=3.83, p=0.10).

Mean arousal bout duration was on average 51.2 ± 2.9 h (Range: 28.3 to 91.7 h). Mean arousal bout duration did not differ between the sexes (Figure 2.5a; Type II ANOVA, $F_{(1, 23)}$=0.13, p=0.86) or across hibernation years (Type II ANOVA, $F_{(2, 23)}$=0.59, p=0.67). Similarly, mean arousal bout temperature did not differ between the sexes (Figure 2.5b, Type II ANOVA, $F_{(1, 23)}$=0.02, p=0.96) or across hibernation years (Type II ANOVA, $F_{(2, 23)}$=1.71, p=0.35). On average, mean T_b during arousal bouts was 33.6 ± 0.1 °C (Range: 33.0 to 34.4 °C).

2.3.1.5 Heterothermy Index

HI over the common hibernation period was not influenced by hibernation year (Type II ANOVA, $F_{(2, 23)}$=0.92, p=0.41) but was influenced by sex (Type II ANOVA, $F_{(1, 23)}$=9.40 p=0.005). Females, on average, had higher HI than males, with the HI of females being 13.9 ± 0.6 and HI of males being 11.2 ± 0.5. HI over an individual's specific hibernation period was not influenced by sex (Type II ANOVA, $F_{(1, 23)}$=0.06, p=0.80) but was influenced by hibernation year (Type II ANOVA, $F_{(2, 23)}$=92.7, p<0.001).

2.3.2 Over-winter change in body condition

I measured change in body mass over winter in 24 prairie dogs (n=10 (male=5, female=5) in 2017, n=5 (male=3, female=2) in 2019 and n=9 (male=2, female=7) in 2020. Male prairie dogs tended to experience a greater change in body condition (Figure 2.6), however this difference was not significantly different (Type II ANOVA, $F_{(1, 20)}$=0.88, p=0.36). Change

in body condition was not significantly different across hibernation years (Type II ANOVA, $F_{(2, 20)}$=1.36, p=0.28). The rate of body condition lost during hibernation was significantly different between the sexes (Type II ANOVA, $F_{(1, 20)}$=18.6, p<0.001), with males losing more body condition per day of their hibernation period than females. Rates of body condition lost during the hibernation period did not differ across hibernation years (Type II ANOVA, $F_{(2, 20)}$=0.28, p=0.76).

2.4   Discussion

In this chapter, I present evidence that black-tailed prairie dogs demonstrate sexual variation in their hibernation expression. Across all years of study, male and female prairie dogs differed with respect to some but not all hibernation traits. The sex differences in the expression of hibernation traits I report are consistent with several of my predictions on sexual variation in hibernation expression but not all. Male prairie dogs emerged from hibernation earlier, had shorter hibernation periods, and used fewer bouts of torpor relative to females; a trend that has been observed in other hibernating species of ground squirrel (Michener, 1983b; Young, 1990; Sheriff et al., 2011; Kart Gür & Gür, 2015). However, contrary to the predictions from the thrifty female hypothesis, males were the sex that spent a greater proportion of time in torpor and used bouts of torpor that were, on average, deeper (colder) and longer in duration. This indicates that males use a more energy-conservative expression of hibernation relative to females. I did find that males and females lose similar amounts of body condition during hibernation and that males lose body condition at a faster rate. However, the change in body condition I report likely captured changes in body condition that occurred outside of hibernation.

Male and female prairie dogs demonstrated similar hibernation onset dates and, on average, entered hibernation in late November/early December. Previous studies reporting hibernation onset in other hibernators found sex-specific differences; however, which sex enters hibernation first varies across species (Williams et al., 2014) and is likely driven by a combination of factors (i.e., territory defence, minimizing exposure to predators, acquiring sufficient energy stores). The similarity in hibernation onset observed in prairie dogs may result from their complex social structure, which has previously been used to explain the lack of hibernation in prairie dogs (Tileston & Lechleitner, 1966; Bakko et al., 1988; Michener, 1983a, 1984). Male and female prairie dogs may seek to maximize the time spent above ground, maintaining their social bonds by beginning hibernation on similar dates. However, to my knowledge, no studies have investigated how sociality influences hibernation onset. Prairie dogs demonstrate sex differences in the timing of emergence from hibernation sim-

ilar to those reported in other sciurid hibernators (Michener, 1983b; Young, 1990; Sheriff et al., 2011; Kart Gür & Gür, 2015), with males emerging, on average, 24 days earlier than females. The similarity in hibernation onset in males and females but earlier emergence of males likely contributes to the prolonged hibernation duration observed in females. Earlier emergence and shortened hibernation period in male prairie dogs are most likely the result of the need for males to undergo spermatogenesis (Barnes et al., 1986; Barnes, 1996) and possibly defend territories/secure access to females prior to female emergence (Michener, 1983b; Buck & Barnes, 2003).

The hibernation onset and emergence dates I report in this chapter reflect when prairie dogs first started and stopped using multiday torpor, respectively, rather than when prairie dogs entered and exited their hibernaculum. Thus, while I observed similar hibernation onset between the sexes and earlier hibernation emergence dates in males, it is possible that prairie dogs do not demonstrate the same pattern when they enter and emerge from their hibernaculum. As such, while male prairie dogs terminate the use of torpor at earlier dates, they may exit the hibernaculum at similar times as females and have an extended period of post-hibernation euthermia while sequestered in their hibernaculum. Prairie dogs reside in coteries (family group) generally composed of one breeding male, several breeding females, and juveniles (Hoogland, 1995). In harem breeding species, it is thought that since breeding males have preferential access to breeding females within the harem, they may not benefit from emerging from their hibernaculum earlier than females (Michener, 1983b). Male prairie dogs may terminate torpor earlier to undergo gonadal recrudescence, but then time the resumption of above-ground activity with females to maintain their access to them (i.e. defend from invading males, guard females). Investigating when prairie dogs enter and emerge from their hibernaculum relative to when they use torpor would shed light on whether they demonstrate sexual variation in the resumption of above-ground activity. Such studies will also elucidate if male prairie dogs exit hibernaculum earlier to defend territories from neighbouring males and guard females as they emerge and if they undergo gonadal recrudescence while sequestered in their burrows prior to emergence.

In support of my predictions, female prairie dogs had a more extended hibernation period, used more bouts of torpor, and overall spent more time in torpor than males. However, contrary to my predictions, males used torpor bouts that were on average colder and longer in duration and spent a larger proportion of their hibernation in torpor. Additionally, neither the duration nor the temperature of arousal bouts varied between the sexes. Therefore, male prairie dogs appear to use a more energy-conservative hibernation expression compared to females. Interestingly, female prairie dogs are physiologically capable of reaching body temperatures as cold as males, as evidenced by similar minimum T_b's during hibernation.

However, on average, females use warmer torpor bouts that are shorter in duration. Though not presented here, females used more bouts of daily torpor during their hibernation season relative to males (females: 5.57 ± 1.07; males: 0.77 ± 0.23), which may in part explain why their torpor bouts were on average, warmer and shorter relative to males. As previously mentioned, male prairie dogs are presumably under selection to terminate hibernation earlier to undergo gonadal recrudescence and secure access to females, which shortens the length of the male hibernation season (Barnes et al., 1986; Barnes, 1996). However, as males need to support energy requirements early in the spring (i.e., gonadal recrudescence, securing mates), they likely heavily rely on energy conserved during hibernation. Males may use torpor to a greater extent to maximize their energy savings during hibernation to support their spring energy requirements.

Additionally, given that males emerge earlier, they may be more likely to face inclement spring weather and decreased foraging opportunities and as a result, may attempt to maximize their energy savings during hibernation. Females may benefit from delaying emergence until males are reproductively ready (Michener, 1983b) and maximize the energy available for reproduction by hibernating for a prolonged period while simultaneously reducing the physiological damage they accrue by decreasing the depth and duration of torpor bouts (Humphries et al., 2003b). The hibernation optimization hypothesis predicts that individuals with larger fat stores will reduce torpor use (Humphries et al., 2003b). As such, relative to males, female prairie dogs may enter hibernation with larger energy stores prior to hibernation and, therefore, may not need to be as "thrifty" with their energy use. Alternatively, if black-tailed prairie dogs hibernate communally, it is possible that as males terminate hibernation earlier to undergo gonadal recrudescence while females continue to hibernate, they may passively increase the torpor temperatures females use. It is unclear if prairie dogs in Canada hibernate communally, though limited evidence presented by Gummer (2005) suggests they may. Future work investigating whether prairie dogs hibernate communally and when females are using warmer bouts of torpor during hibernation will help elucidate whether the earlier termination of hibernation in male prairie dogs influences torpor use in females.

Similar to prairie dogs, in other species of hibernating ground squirrels, males emerge earlier and use torpor an overall shorter amount of time; however, sex differences in the specifics of torpor use during hibernation vary across species. For example, while hibernating male Arctic ground squirrels use heterothermy for a reduced amount of time but the length of their torpor bouts and the overall proportion of time spent torpid is similar to females (Buck et al., 2008). Male Anatolian ground squirrels (*Spermophilus xanthoprymnus*) hibernate for a reduced period but use shorter bouts of torpor and spend proportionally less time in torpor than females (Kart Gür & Gür, 2015). While male Richardson's ground squirrels

(*Urocitellus richardsonii*) have been found to use similar number of torpor bouts but have shorter maximum torpor bout lengths and, on average, have longer arousals compared to their female counterparts (Michener, 1992). In contrast, in prairie dogs, I found that while males hibernate for a shortened period, they, on average, used torpor bouts that are longer and colder compared to females and spent a greater proportion of their hibernation period in torpor. This suggests that male prairie dogs use a different strategy to optimize energy savings and reproductive success than males in other species. In Anatolian ground squirrels, it has been suggested that males may have greater fasting endurance by having large fat stores than females (Kart Gür & Gür, 2015). As such, male Anatolian ground squirrels may not need to rely as heavily on torpor compared to females. Male Arctic and Richardson grounds squirrels build up food caches before entering hibernation (Gillis et al., 2005; Buck & Barnes, 1999; Michener, 1993), which are presumably consumed post-hibernation to support gonadal recrudescence. As males in these species support spring energy requirements with cached food resources, they likely do not need to use torpor to a greater extent relative to females. Conversely, if male prairie dogs do not enter hibernation with increased energy stores relative to females or do not cache food prior to hibernation, they may rely more heavily on torpor use during hibernation to conserve energy to support the energetic costs of being euthermic while undergoing gonadal recrudescence, in addition to supporting breeding efforts. Regardless, to my knowledge, the finding that male prairie dogs use longer and colder bouts of torpor and spend a greater proportion of hibernation relative to females is novel to sciurid hibernators and likely reflects a "thriftier" strategy to maximize energy savings during their shortened hibernation period so that they can emerge early in the spring with sufficient energy stores to support reproduction.

In addition to specific hibernation traits, I also compared Heterothermy Index (HI) as a means to compare hibernation expression and the use of heterothermy in a single metric. I found that HI differed between males and females over the common hibernation period but not over an individual's specific hibernation period. The discrepancy between these findings is likely the result of females hibernating on average for a longer amount of time and emerging later in the spring. As a result of these difference in hibernation duration and emergence dates, male prairie dogs had already ceased hibernation and maintained euthermic body temperatures for a portion of the common hibernation period. When comparing the HI over an individual's specific hibernation period, the HI of males and females did not significantly differ. This indicates that the T_bs of males and females are similar deviations away from their optimal temperatures when hibernating. The finding that males and females have similar HI during hibernation is surprising given that males spent proportionally more time in torpor and used longer and deeper bouts of torpor. However, variation in the use of heterothermy

across years may have decreased the statistical power of the test to detect sex differences in HI. Larger sample sizes and investigation of HI within a single year could help tease apart the relationship between HI and sex. The effect of hibernation year on HI calculated over an individual's specific hibernation period may in part reflect differences in weather severity during the hibernation period across the different years. Torpor (depth and length) in a more southern population of prairie dogs is influenced by precipitation and ambient temperature (Lehmer et al., 2003)), but studies investigating the influence of weather variables on HI, ideally with repeated measures from individuals, is needed to understand the influence winter weather has on HI.

Despite finding that males used torpor bouts that, on average, were deeper and more prolonged and spent a greater proportion of hibernation in torpor, I found that males and females did not differ in their over-winter change in body condition. Furthermore, when considering the length of their hibernation season, males appeared to lose body condition faster than females. However, as mentioned previously, these body condition data should be interpreted with caution. Due to logistical reasons, I was unable to capture prairie dogs immediately prior to and after hibernation or determine the exact date prairie dogs resume above-ground activity. This resulted in the body mass I recorded generally occurring two or more weeks before and after hibernation (see Tables A.2 and A.5 for a summary of the number of days I corrected for). For post-hibernation measures, when I measured body mass was quite delayed relative to the emergence, and due to the earlier emergence of males, I measured males and females at different times relative to their emergence. On average, I measured males 47 d post hibernation with only one male's measure within 31 d of emergence. In contrast, on average, I measured females 22 d post-hibernation, with 5 of 14 females measured within two weeks of emergence. As such, the body mass correction I applied, particularly for males, likely does not capture body mass changes that occur immediately after emergence. Male prairie dogs in winter-active populations demonstrate substantial declines in body mass during the breeding season (Hoogland, 2003). Therefore, the body conditions I reported may include the energy expenditure of males that occurs during the breeding season (including gonadal recrudescence that may occur while they are still in their hibernaculum) and any changes that may occur for females. Additionally, the correction I applied assumes a linear change in body mass before and after hibernation which may not accurately capture how body mass is changing during these periods. If the body condition I reported does capture the energy males expend during the breeding season, then males are likely saving more energy relative to females during their hibernation period, given that they are spending a greater proportion of time in torpor. However, male prairie dogs likely have a greater post-hibernation energy expenditure than female prairie dogs as males become

euthermic to undergo gonadal recrudescence and potentially defend territories while females continue to hibernate and, during the breeding season, as males mate and ward off invading males.

Alternatively, males may not accrue as much energy savings relative to females during hibernation as would be expected by their hibernation expression for several reasons. First, males and females may occupy burrows with differing microclimates that may alter the energy savings accrued from a particular expression of hibernation (Boyles et al., 2007). Second, Studier (1981) found that the energy savings from initial decreases in T_b are more substantial than any subsequent decreases in T_b. As such, even though male prairie dogs on average use colder bouts of torpor relative to females, they may not accrue proportionally more energy savings by doing so. Third, it is possible that male prairie dogs are more active during arousal bouts and therefore use more energy than their female counterparts during this time. For example, during arousals male prairie dogs may move in their hibernaculum to a greater extent or even briefly check above ground conditions. However, observations of male and female activity within the hibernaculum and above ground activity appearance during the winter would be needed to confirm this. Finally, it is unknown whether black-tailed prairie dogs cache food prior to hibernation; however, if they do, differential reliance on food caches during hibernation could influence body condition patterns across the sexes, as observed in Arctic ground squirrels (Buck & Barnes, 1999). Future research investigating body and fat mass dynamics in Canadian prairie dogs following hibernation requirements is warranted. This would allow for a deeper understanding of sexual variation in the timing and energetic costs incurred in the spring. Further, body mass measurements taken immediately prior to and following emergence from hibernation will be required to determine which sex is truly "thriftier" with their energy use.

Hibernation expression in black-tailed prairie dogs is influenced by differences in reproductive energy requirements in the spring. Like other sciurid hibernators, male prairie dogs terminate hibernation earlier than females to prepare for the breeding season while females continue to hibernate. However, in contrast to other sciurid hibernators, male prairie dogs express torpor to a greater extent which likely supports the energy expenditure associated with being active while undergoing gonadal recrudescence and mating. Thus, there is a diversity of over-winter thermoregulatory strategies that species (and sexes) employ to support their spring energy requirements. One shortcoming of this study was the inability to determine when prairie dogs enter and emerge from their hibernaculum and measures body condition at these times. Future work that investigates this aspect of prairie dog hibernation will be required to fully appreciate the over-winter strategies of prairie dogs and the energy savings they yield. Given that prairie dogs have a vast geographical distribution with varying

degrees of torpor use (Hoogland, 1995; Lehmer et al., 2001; Lehmer et al., 2006; Gummer, 2005; Hawkshaw et al., 2021 in prep), investigations of sexual variation in thermoregulatory strategies across their range could provide further insight as to how climatic and ecological conditions influence how males and females use torpor to survive over winter and support reproduction in the spring.

2.5 Conclusion

In this chapter, I investigated sexual variation in black-tailed prairie dog hibernation expression and over-winter change in body condition. Here, I present unequivocal evidence of sex differences in hibernation expression, which likely result from differences in the timing of reproductive requirements following emergence. Sex-specific patterns of emergence and overall duration of hibernation were consistent with other species of hibernating ground squirrel, with males emerging earlier and spending less time in hibernation. Male prairie dogs used hibernation expressions that are considered more energy-conservative; during hibernation, they used longer and deeper bouts of torpor and spent proportionally more time in torpor relative to females. However, females did have an extended hibernation period with more bouts of torpor. This suggests that male prairie dogs use a more energy-conservative hibernation expression to maximize energy savings during their shortened hibernation period to ensure they have sufficient energy stores to support gonadal recrudescence and breeding. In combination with studies investigating hibernation expression, the findings of my study demonstrate the diversity of over-winter strategies that species and black-tailed prairie dogs, in general, can employ to overcome ecological challenges. Further, my findings demonstrate sex-specific patterns of torpor use during hibernation are not consistent across species where males have earlier reproductive investment, which may be due to species-specific trade-offs and differential usage of other energy-saving strategies.
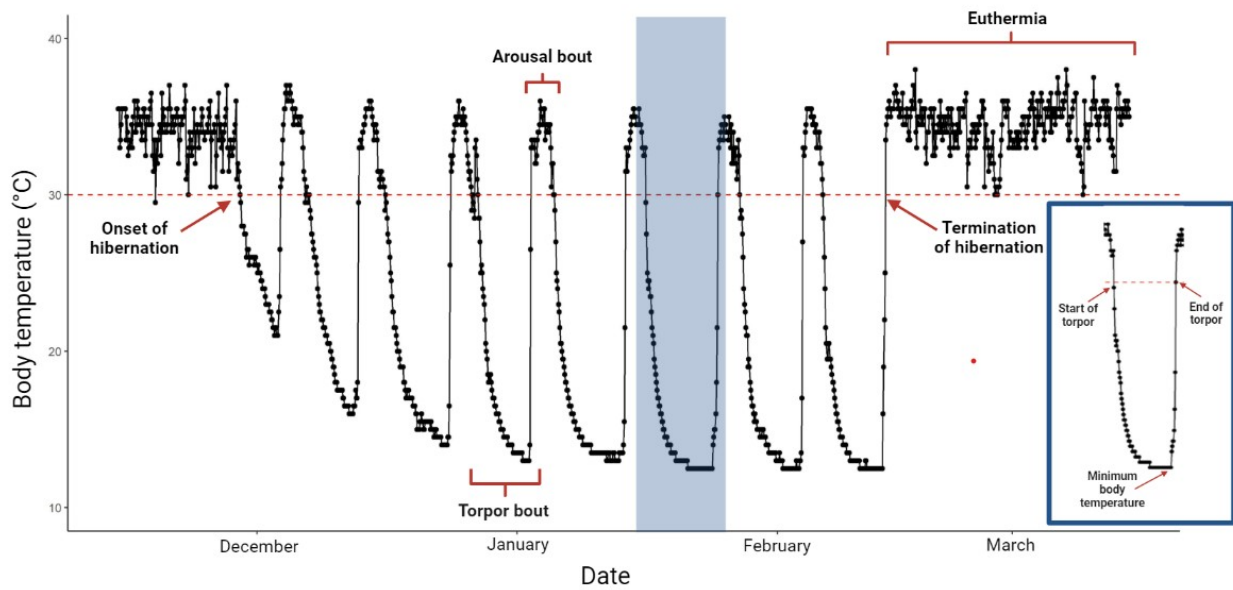
Figure 2.1: Example of hibernation expression in a Canadian black-tailed prairie dog. The red, horizontal dashed line indicates the 30 °C temperature cut-off used to delineate torpor use. Red arrows indicate the onset of and emergence from hibernation. Red brackets indicate an example torpor bout, arousal bout, and period of post-hibernation euthermia. The blue box indicates the torpor bout that is shown in the inset panel. The inset panel indicates the criteria used to define an individual torpor bout, red arrows point to data points that represent the start and end of torpor as well as the minimum body temperature achieved during the torpor bout. This figure was developed in BioRender.com.

Table 2.1: Summary of temperature-sensitive data loggers implanted during each hibernation year. Sample sizes for males (M) and females (F) are indicated within parentheses.

Hibernation year	T_b sampling frequency	Number of data loggers implanted	Number of data loggers retrieved	Number of data loggers with complete Hibernation records
2017	2h	16 (M=9, F=7)	12 (M=7, F=5)	12 (M=7, F=5)
2019	3h	19 (M=10, F=9)	14 (M=8, F=6)	8 (M=6, F=2)
2020	3h	48 (M=22, F=26)	30 (M=10, F=20)	28 (M=9, F=19)

Table 2.2: Definition of hibernation traits used to investigate the effects of sex, hibernation year, and body condition on black-tailed prairie dog hibernation expression. Units for each trait are indicated in parentheses.

Hibernation trait	Definition
Duration of hibernation (d)	Length of time from the start of first multiday torpor bout to the end of the last multiday torpor bout. Start of multiday torpor was defined as date and time when T_b first decreased to <30 ˚C and remained <30 ˚C for >24 h. End of multiday torpor was defined as the date and time when T_b first increased to ≥30 ˚C after remaining <30˚C for >24 h.
Total time in torpor (d)	Sum of duration of each torpor bout (daily and multiday) used during hibernation. Duration of a torpor bout was defined as date and time when T_b first decreased to <30 ˚C until the date and time when T_b first increased to ≥30 ˚C.
Total time in arousal (d)	Sum of the duration of each arousal bout used during hibernation. Duration of an arousal bout was defined as the date and time when T_b first increased to ≥30 ˚C until the date and time when T_b first decreased to <30 ˚C.
Proportion of hibernation spent torpid (%)	Proportion of hibernation that was spent in torpor (daily and multiday) expressed as a percent (%). Measured as the sum duration of all torpor bouts divided by the duration of the hibernation period multiplied by 100. Duration of a torpor bout was defined as date and time when T_b first decreased to <30 ˚C until the date and time when T_b first increased to ≥30 ˚C.
Number of torpor bouts	Total number of torpor bouts (daily and multiday) used during hibernation. A torpor bout was defined the period when T_b first decreased to <30 ˚C and remained <30 ˚C until T_b first increased to ≥30 ˚C.
Mean torpor bout duration (h)	Mean duration of all torpor bouts (daily and multiday) used during hibernation. Measured as the sum each torpor bout's duration divided by the total number of torpor bouts. Duration of a torpor bout was defined as date and time when T_b first decreased to <30 ˚C until the date and time when T_b first increased to ≥30 ˚C.
Mean torpor bout depth (˚C)	Mean minimum T_b reached during all bouts of torpor (daily and multiday). Measured as sum of each torpor bout's minimum T_b divided by the number of torpor bouts. A torpor bout was defined as the period when when T_b first decreased to <30 ˚C until T_b first increased to ≥30 ˚C.
Minimum T_b (˚C)	Minimum T_b reached during hibernation.
Mean arousal bout duration (h)	Mean duration of all arousal bouts used duration hibernation. Measured as the sum of all arousal bout durations divided by the total number of arousal bouts. Duration of an arousal bout was defined as the date and time when T_b first increased to ≥30 ˚C until the date and time when T_b first decreased to <30 ˚C.
Mean T_b during arousal bouts (˚C)	Mean T_b during all bouts of arousal. Measured as the sum of the mean T_b in each arousal bout divided by the number of arousal bouts. An arousal bout was defined as period when T_b first increased to ≥30 ˚C until T_b first decreased to <30 ˚C.
Hibernation onset (day of year)	Day of year of the first multiday torpor bout when T_b first decreased to <30 ˚C and then remained <30 ˚C for >24 h.
Hibernation emergence (day of year)	Day of year of the last multiday torpor bout when T_b first increased to ≥30 ˚C after being <30 ˚C for >24 h.

Table 2.3: Summary of male and female black-tailed prairie dog hibernation expression across three years of hibernation. Descriptive statistics are presented as the mean ± standard error of the mean (SEM) and (Range). Dates of hibernation onset and emergence are presented as a day of the year, where 31 December is day 365 in non-leap years and day 366 in leap years.

	Male	Female
	$n=13$	$n=14$
Duration of hibernation (d)	51.4 ± 4.6 (33.6 - 99.9)	86.9 ± 4.7 (48.4 - 104.1)
Total time in torpor (d)	37.8 ± 2.8 (25.9 - 65.5)	54.9 ± 3.0 (38.9 - 83.9)
Total time in arousal (d)	13.6 ± 2.0 (6.8 - 34.4)	32.0 ± 3.4 (9.5 - 50.4)
Proportion of hibernation spent torpid (%)	74.6 ± 1.4 (65.4 - 80.1)	64.2 ± 2.6 (50.6 - 80.6)
Number of torpor bouts	7.4 ± 0.5 (5 - 10)	16.1 ± 1.4 (8 - 24)
Mean torpor bout duration (h)*	126.4 ± 7.6 (81.6 - 160.0)	90.3 ± 9.3 (55.7 - 183.1)
Mean torpor bout depth (℃)*	15.2 ± 0.5 (12.3 - 18.6)	18.4 ± 0.9 (12.0 - 22.1)
Minimum T_b (℃)	11.4 ± 0.2 (9.5 - 12.5)	11.0 ± 0.2 (9.5 - 12.5)
Mean arousal bout duration (h)*	51.2 ± 5.0 (28.3 - 91.7)	51.1 ± 3.4 (32.6 - 74.1)
Mean T_b during arousal bouts (℃)*	33.7 ± 0.1 (33.0 - 34.3)	33.6 ± 0.1 (33.2 - 34.4)
Onset of hibernation (day of year)	338.2 ± 1.3 (329 - 346)	329.5 ± 2.8 (314 - 342)
Emergence from hibernation (day of year)	24.1 ± 4.3 (6 - 70)	50.9 ± 3.7 (26 - 78)

* Based on mean value for each individual.
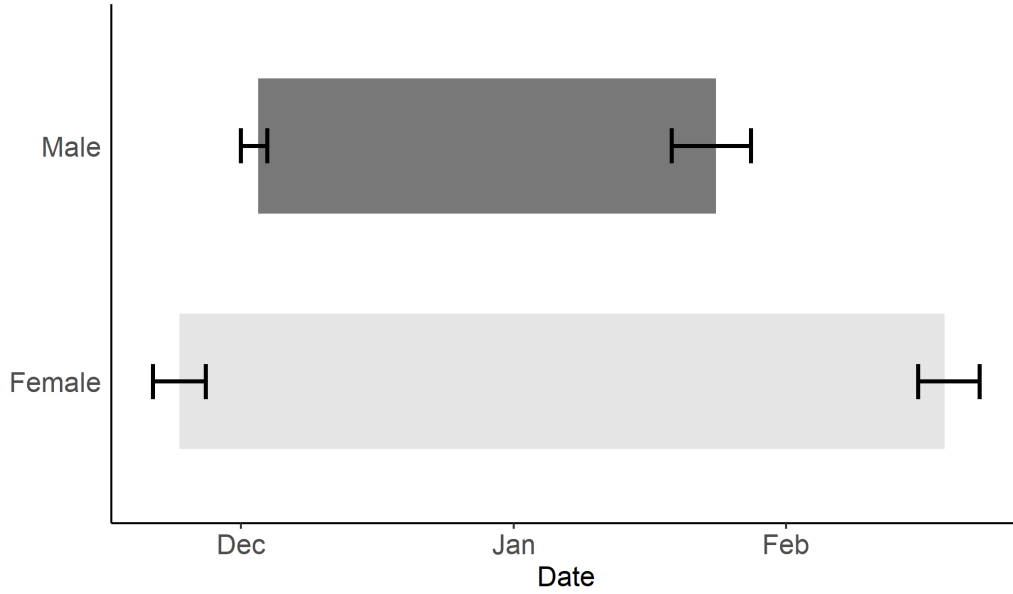
Figure 2.2: Mean hibernation phenology of male and female black-tailed prairie dogs. The left side of each bar represents the mean date that prairie dogs began hibernation, and the right end of the bar represents the mean date that prairie dogs emerged from hibernation. Error bars represent the standard error of the mean.
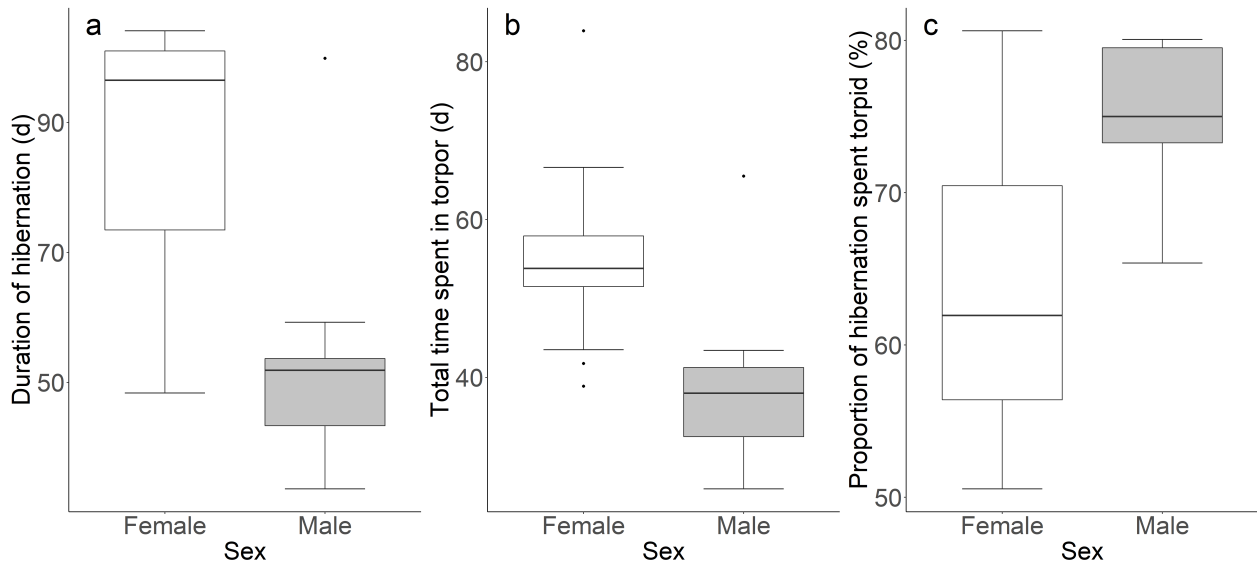
Figure 2.3: Boxplot comparing (a) duration of hibernation, (b) total time spent in torpor and (c) proportion of hibernation spent in torpor in male and female black-tailed prairie dogs. The thick horizontal bar represents the median for each box, and the lower and upper bounds represent the 25th and 75th percentile, respectively. Lower and upper whiskers represent the minimum and maximum values, respectively, and black dots represent outliers.

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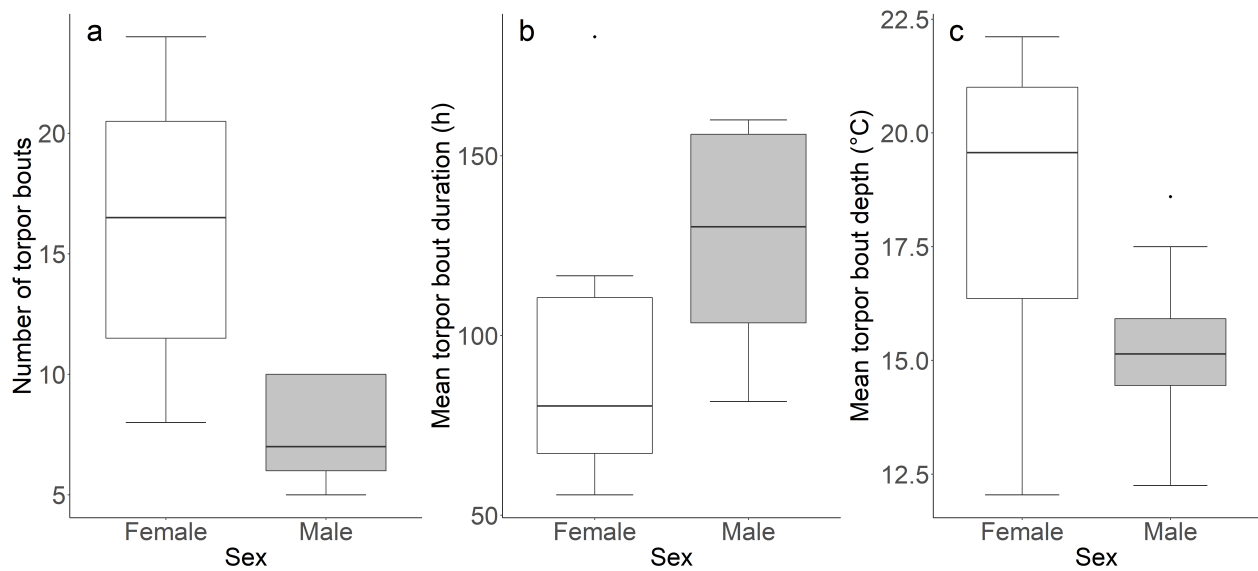
Figure 2.4: Boxplot comparing (a) number of torpor bouts, (b) mean torpor bout duration and (c) mean torpor bout depth in male and female black-tailed prairie dogs. The thick horizontal bar represents the median for each box, and the lower and upper bounds represent the 25th and 75th percentile, respectively. Lower and upper whiskers represent the minimum and maximum values, respectively, and black dots represent outliers.
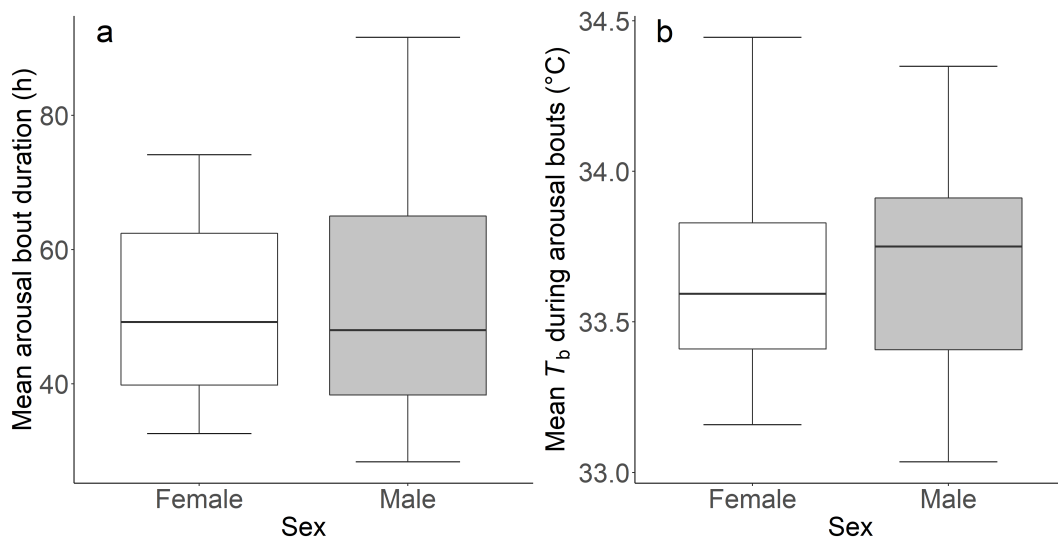
Figure 2.5: Boxplot comparing (a) mean arousal bout duration and (b) mean arousal bout T_b in male and female black-tailed prairie dogs. The thick horizontal bar represents the median for each box, and the lower and upper bounds represent the 25th and 75th percentile, respectively. Lower and upper whiskers represent the minimum and maximum values, respectively, and black dots represent outliers.
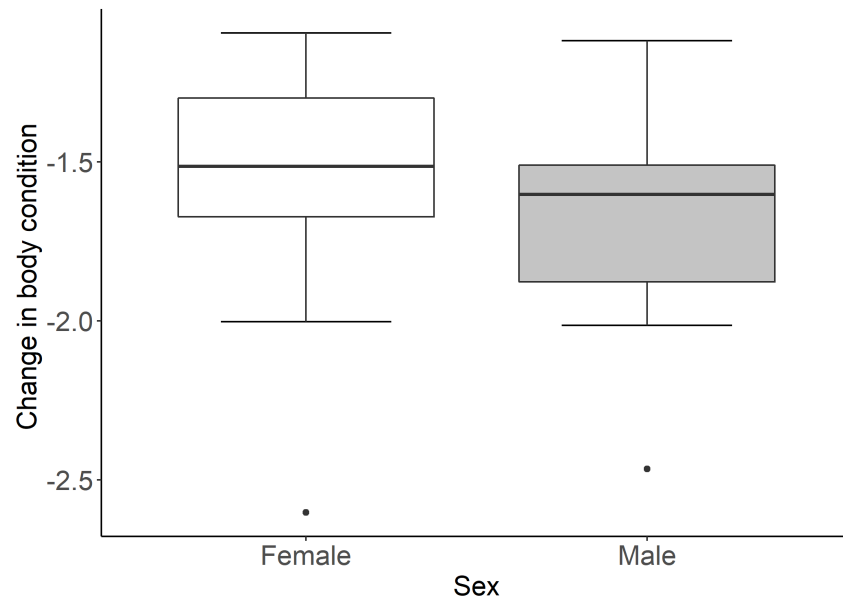
Figure 2.6: Boxplot comparing the change in body condition male and female black-tailed prairie dogs experience over winter. The thick horizontal bar represents the median for each box, and the lower and upper bounds represent the 25th and 75th percentile, respectively. Lower and upper whiskers represent the minimum and maximum values, respectively. Negative values indicate a decrease in body condition over winter, while positive values indicate an increase in body condition.

Chapter 3

Investigation of the role of body condition in black-tailed prairie dog hibernation expression

3.1 Introduction

Hibernation is an effective adaptation used by species across the globe to survive unfavourable environmental conditions, such as those experienced during winter at northern latitudes. Hibernation enhances survival during adverse conditions by reducing an individual's energy expenditure (Wang, 1979; Wilz & Heldmaier, 2000; Geiser & Ruf, 1995) and predation pressure (Turbill et al., 2011). The reduction in energy expenditure results from recurring bouts of torpor, where metabolic rate and body temperature (T_b) are lowered for multiple days (Geiser & Kenagy, 1988; Geiser & Ruf, 1995). Despite its energetic benefits, torpor use has been associated with several physiological costs (e.g., Buzadžic et al., 1990; Popov et al., 1992; Burton and Reichman, 1999; Carey et al., 2000; Millesi et al., 2001). The costs of torpor are thought to be mitigated by arousal bouts (brief returns to euthermia) that intersperse torpor bouts (Humphries et al., 2003b). While arousals may offset the costs of torpor, returning metabolism and body temperature to euthermic levels is energetically expensive (Wang, 1979; Thomas et al., 1990).

Before hibernation, individuals build up energy reserves to support the metabolism during hibernation and energetic costs post-hibernation. Some hibernators build up these energy reserves as food caches, while others build up reserves in the form of fat. Mechanisms fat-storing hibernators use to build up energy stores before hibernation include increased food intake (Körtner & Heldmaier, 1995; McGuire et al., 2009), reduced sensitivity to leptin (Kronfeld-Schor et al., 2000; Concannon et al., 2001), maintaining lower T_b (Sheriff et al., 2012), and supporting metabolism with non-fat-based energy stores (Sheriff et al., 2013). Regardless of the mechanism, fat-storing hibernators drastically increase their fat mass before

hibernation. For example, most of the mass gain Arctic grounds squirrels (*Urocitellus parryii*) experience before hibernation results from a 7-8-fold increase in fat mass (Sheriff et al., 2013). The fat stores acquired before hibernation serve as the primary fuel to support metabolism (Galster & Morrison, 1976; Boyer & Barnes, 1999; Dark, 2005).

Given the cost-benefit trade-offs associated with hibernation and the importance of energy reserves, individuals are thought to optimize torpor and arousal expression according to their energetic condition (Humphries et al., 2003b; "hibernation optimization hypothesis"; *sensu* Boyles et al., 2007). A central prediction of the hibernation optimization hypothesis is that individuals in better body condition have a reduced need to save energy and, therefore, can afford to limit both torpor use and its associated physiological costs. Indeed, several hibernators adjust their use of torpor and arousal during hibernation according to the size of their energy reserves (Bieber et al., 2014; Zervanos et al., 2014; Humphries et al., 2003a; Munro et al., 2005; Siutz et al., 2018; Czenze et al., 2017). For example, Eastern chipmunks (*Tamias striatus*) spent more time aroused when food was supplemented (Humphries et al., 2003a). To date, the hibernation optimization hypothesis has only been investigated in either food-caching, facultative hibernators (Humphries et al., 2003a; Munro et al., 2005; Siutz et al., 2018) or fat-storing obligate hibernators (Bieber et al., 2014; Zervanos et al., 2014; Czenze et al., 2017).

Few, if any, studies have investigated the hibernation optimization hypothesis in fat-storing, facultative hibernators, such as the black-tailed prairie dog (*Cynomys ludovicianus*; Harlow and Menkens Jr., 1986; Harlow, 1995, 1997; Harlow and Frank, 2001; Lehmer et al., 2001; Lehmer et al., 2003; Lehmer and Biggins, 2005. In contrast to obligate hibernators that enter hibernation annually regardless of environmental conditions, facultative hibernators only hibernate when cold, stressed, and/or food is limited. As such, facultative hibernators may show more flexibility in torpor expression compared to obligate hibernators. However, facultative hibernators that support metabolism with fat stores may be more limited in their ability to adjust torpor since their energetic reserve size is more constrained than food caching hibernators (Siutz et al., 2017; Humphries et al., 2003b).

Black-tailed prairie dogs are gregarious, small mammals with the most extensive distribution among species of prairie dogs (Hoogland, 1995). Black-tailed prairie dogs demonstrate extensive variation in over-winter thermoregulatory strategies throughout their range. In some locales, black-tailed prairie dogs have above-ground activity year-round (King, 1955; Koford, 1958; Smith, 1958; Tileston & Lechleitner, 1966; Hoogland, 1995) where body temperatures do not drop below 31℃ (Bakko et al., 1988), while in others shallow short-term and deep long-term torpor bouts have been observed (Lehmer 2001). Additionally, hibernation-like thermoregulatory patterns have been observed in colonies in northern Colorado, USA

(Lehmer et al., 2006) and southwestern Saskatchewan, Canada (Gummer, 2005).

Black-tailed prairie dog colonies located within southwestern Saskatchewan comprise the entire Canadian population and demarcate the northern limit of the species' range. Black-tailed prairie dogs within the Canadian population are the only black-tailed prairie dogs known to consistently hibernate during the winter (Gummer, 2005). Extensive individual variation in hibernation expression within the Canadian population has been observed (Figure 1.1), yet little is known about what factors drive this variation. Canadian black-tailed prairie dogs may hibernate according to the size of pre-hibernation energetic reserves (body condition) as predicted by the hibernation optimization hypothesis. Sex-specific responses to pre-hibernation energy reserve size have been observed in common hamsters (*Cricetus cricetus*; Siutz et al., 2018, which likely stem from sex differences in correlates of individual fitness. In Chapter 2, I presented evidence of sexual variation in hibernation expression, which likely results from differences in the timing and cost of reproduction. Therefore, the effects of body condition on hibernation expression may be due to sex-specific reproductive requirements.

3.1.1 Objectives and Hypotheses

The purpose of this chapter was to test the hibernation optimization hypothesis and determine whether Canadian black-tailed prairie dogs adjust their hibernation expression based on their pre-hibernation body condition. Specifically, I hypothesized that if the quantity of energetic resources affects an individual's need to conserve energy, individuals with fewer energetic resources will employ more energy-conservative hibernation expressions than individuals with more energetic resources. I predicted prairie dogs in better pre-hibernation body condition would decrease the duration of hibernation and reduce the number, depth, and duration of torpor bouts, and correspondingly spend a greater proportion of hibernation aroused. Accordingly, prairie dogs in better pre-hibernation condition should reduce their overall use of heterothermy during hibernation. Further, in relation to the sex-specific effect of pre-hibernation body condition, I predicted that male prairie dogs would decrease the duration of hibernation and reduce the frequency, depth, and duration of torpor bouts to a lesser degree (shallower slope) compared to females as the elevated energy requirements of reproduction for males (for securing mates and gonadal recrudescence) occur sooner after emergence than for females (primarily during lactation). In addition, I explored how pre-hibernation body condition influenced over-winter change in body condition and body condition in the spring, following emergence. Following the hibernation optimization hypothesis and the predicted decrease in torpor use, I predicted that individuals in better pre-hibernation condition would experience a greater change in body condition over winter.

I also predicted that despite experiencing a greater change in body condition, individuals in better pre-hibernation condition would emerge in better condition to use additional energy stores for energetic requirements in the spring.

This chapter extends on Chapter 2 by evaluating the influence of pre-hibernation body condition on hibernation expression and over-winter energy expenditure, as measured by over-winter change in body condition. Additionally, this chapter builds on the sex differences in hibernation expression found in Chapter 2 by determining if there are sex-specific responses to pre-hibernation body condition. To investigate the effect of pre-hibernation body condition, I conducted a supplementary feeding trial in the autumn of one hibernation year during which a subset of prairie dogs had access to a supplementary diet (fed) while another subset did not (unfed/control). These individuals subsequently had their hibernation expression and changes in body condition monitored following the supplementary feeding trial. Food supplementation allowed me to experimentally expand variation in pre-hibernation body condition to help tease apart its influence on hibernation expression and energy expenditure. In addition to the individuals from the food supplementation year, I incorporated unfed individuals from two previous hibernation years to increase the available sample size and add additional variation in pre-hibernation body condition. Unfed individuals across the three hibernation years were the same individuals as those used to investigate sex variation in hibernation expression and energy expenditure in Chapter 2, while the fed individuals from the food supplementation year were not included in Chapter 2 due to their experimental manipulation.

3.2 Methods

3.2.1 Data collection

3.2.1.1 Study site, live-trapping and individual identification

All research activities described below were approved by the University of Saskatchewan Animal Research Ethics Board (AUP-20140042) and conducted under research permits from Parks Canada (GRA-2014-16101 and SAR-GRA-2014-16101) and the Ministry of Environment (20AR028W). All prairie dogs used in this study inhabited a single colony ('Walker') within Grasslands National Park, southwestern Saskatchewan, Canada (49° 3' 46.8"N, 107° 21' 28.8"W). I captured prairie dogs using Tomahawk live-traps (Tomahawk Live Trap Company, Tomahawk, WI, USA) and a bait comprised of peanut butter and rolled oats. The Walker Colony was a part of a long-term study where individuals were identified with unique alpha-numeric ear tags (National Band Tag Company, Newport, Kentucky, US) and sym-

bols comprised of letters and non-alphanumeric characters. Throughout my study, if any new individuals were captured, I followed the previously established convention and tagged the pina of each ear with a unique alpha-numeric tag and assigned the individual a unique symbol. I painted a unique symbol on the dorsal pelage of each individual with Nyanzol-D (Greenville Colorants, Clifton, New Jersey, USA), which allowed me to identify and observe prairie dogs at a distance.

As described in Chapter 2, I restricted my study to adult prairie dogs. Several studies have found that hibernation expression differs between subadults and adults (French, 1990; Kart Gür & Gür, 2015; Siutz et al., 2016; Bieber & Ruf, 2012). The differences in hibernation expression result, in part, from age-related differences in reproductive potential (French, 1990; Williams et al., 2014; Bieber et al., 2018). Given that the primary interest of my study was to determine if an individual's pre-hibernation body condition influenced their hibernation expression and energy expenditure, including subadults, could have reduced my ability to identify any effects of body condition. Further, I restricted my study to relatively trappable individuals to increase the likelihood of retrieving data loggers and measuring post-hibernation condition in the spring.

3.2.1.2   Pre-hibernation food supplementation

To test whether prairie dogs hibernate according to the size of their pre-hibernation energy reserves, I supplementally fed a sample of individuals during the pre-hibernation fattening period to expand variation in the pre-hibernation body condition that existed within the colony. I predicted that individuals who had access to the supplemental diet would gain more body mass over the feeding trials and enter hibernation in better condition than individuals who did not have access to the diet. As both male and female prairie dogs would be trying to increase the quantity of their energy reserves at this time, I did not expect males and females to differ in their response to food supplementation.

I targeted supplementary feeding to specific individuals through Passive Integrated Transponder (PIT) tag-activated feeders (Surefeed feeders Sure Petcare, Clearwater, Florida, USA). Surefeed feeders provide a novel supplementary feeding method for prairie dogs and have been successfully used to target-feed specific individuals in Columbian ground squirrels (*Urocitellus columbianus*, Guererro-Chacon and Lane, *unpublished data*) and snowshoe hares (*Lepus americanus*, Majchrzak, 2016). Before starting the feeding trials, I subcutaneously implanted prairie dogs (13 male and 13 female) with unique Passive Integrated Transponder (PIT) tags. Each PIT tag transmits a unique identification number which I programmed the Surefeed feeders to recognize. Feeders were programmed and arranged on the colony in the following way: if only one prairie dog in a coterie was to receive the supplementary diet, I

placed one feeder within that coterie and programmed it to recognize that individual. If two or three prairie dogs within a coterie were included in my experimentally fed group, I placed two feeders within the coterie. I programmed both feeders to recognize the PIT-tags of each experimental individual within a given coterie. I fed no more than three individuals within a coterie the number of adults on the colony and within coteries was low due to a population crash in 2017-2018 (Kusch et al., 2021).

I conducted the supplementary feeding trials from 3 September to 22 October 2020. During this time, prairie dogs implanted with PIT tags were offered a standard laboratory rodent chow, ad libitum, for six out of seven days a week. Feeders were monitored throughout the day to assess which individuals were eating the supplementary diet and to refill the feeders when the diet provided had been consumed.

In addition to feeding and monitoring the individuals I selected to receive the supplementary diet (fed group), I also attempted to capture and monitor male and female prairie dogs that did not receive the supplemental diet as a control (unfed group). I attempted to measure the body mass of fed and unfed prairie dogs with a 3000 g spring scale (Pesola; Präzisionswaagen AG, Schindellegi, Switzerland) within three days of the start and end of the trial so that I could determine how body mass changed in fed and unfed individuals. I selected three days as my cutoff for body mass measurements so that the body mass I recorded would reflect pre-and post-feeding trial body mass, and changes in body mass would reflect changes that occurred during the feeding trial.

Upon the conclusion of the feeding trial, I attempted to capture and implant fed and unfed prairie dogs with temperature-sensitive data loggers (See details in the Hibernation expression section below). One male that was selected to be fed disappeared at the start of the feeding trial and was not captured again that year. I was unable to replace this male with another male on the colony as I was limited in the number of trappable adult males. Additionally, even with the fed male missing the number of fed males still exceeded the number of unfed. One female that was selected to be fed also disappeared during the feeding trial, but she was replaced with an unfed female as I had a surplus of unfed and trappable females in the colony. The new female was added into the fed group on 1 October and was observed consuming the supplemental diet. I included this female as a fed individual when implanting data loggers; however, I did not include her in the assessment of the effectiveness of the feeding trial as she did not receive the diet for the entire duration of the feeding trial. See Table 3.1 for a summary of samples sizes.

3.2.1.3 Hibernation expression

To quantify prairie dog hibernation expression, I implanted temperature-sensitive data loggers (Model no. DS1921GF50 Thermochron iButtons Maxim Integrated, California, USA) and adopted definitions of hibernation described in Chapter 2. Below, I briefly describe the surgical procedure and hibernation definitions that I used.

3.2.1.3.1 Surgical procedure

For surgical implantation of data loggers, I captured prairie dogs in late October 2020 upon the conclusion of the supplementary feeding trials. Once a prairie dog was captured, I recorded the location of the capture site and transported the prairie dog to a nearby Parks Canada facility. I then measured body mass using a Pesola spring scale (Prazisionswaagen AG, Schindellegi, Switzerland)) and began preparation for the surgical procedure.

All surgical procedures described herein were conducted or supervised by a licensed veterinarian. We anesthetized prairie dogs with Isoflurane (Abbott Laboratories, Saint-Laurent, Quebec) and subcutaneously injected them with lactated Ringer's solution, buprenorphine (0.02 mg kg^{-1}) and ketoprofen (2 mg kg^{-1}) to prevent dehydration and provide analgesia. We placed prairie dogs in a dorsal recumbent position and shaved a small area on the abdomen to accommodate the length of the incision. We then carefully sterilized and draped the incision site and surrounding area. We made a small incision to open the skin and dissected the underlying subcutaneous tissue to expose the abdominal wall. We then made another incision to open the abdominal cavity and inserted the data logger. After the data logger was implanted, we closed the abdominal cavity, subcutaneous tissue and skin. While prairie dogs were recovering from anesthesia, we administered another dose of lactated Ringer's solution to provide additional fluids. Upon recovery from anesthesia, we transferred prairie dogs to small animal cages and monitored them for 24 h. Before release at their capture site, we visually inspected the incision site of each prairie dog and administered a final dose of buprenorphine and ketoprofen. I attempted to capture prairie dogs for several days after their surgery date to continue monitoring the healing of the incision.

I implanted a total of 48 data loggers in October 2020, of which 23 were implanted into individuals that received the supplemental diet (see Table 3.1 for sample sizes). In late July 2020, I attempted to recapture the prairie dogs I had previously implanted to retrieve the data loggers. I followed the same protocol outlined above, except that data loggers were removed from the abdominal cavity as opposed to inserted.

3.2.1.3.2 Defining hibernation

I defined bouts of torpor as the period from when body temperature first dropped below 30 ℃ until body temperature first returned to 30 ℃ or higher (inset panel Figure 2.1), which is a common temperature cutoff used to delineate torpor from euthermia in ground-dwelling Sciurids (Young, 1990; Michener, 1992; Healy et al., 2012; Kart Gür & Gür, 2015). In addition, following the convention of Geiser and Ruf (1995), I distinguished short, daily torpor from multiday torpor by classifying torpor bouts ≤24 h in duration as daily torpor and torpor bouts >24 h in duration as multiday torpor. I further defined hibernation as the period between the start of the first bout of multiday torpor to the end of the last bout of multiday torpor (Figure 2.1), except for one individual that was captured before their last multiday torpor bout during an extensive period of euthermia (details provided in Chapter 2).

For each individual, I determined the date and time that hibernation began and ended as well as the overall duration of the hibernation. I further quantified an individual's use of torpor and arousal during hibernation by quantifying several hibernation traits, including the proportion of time spent torpid, the number of torpor bouts, the mean length and depth of torpor (minimum T_b during torpor), the mean arousal bout length as well as the minimum T_b reached during hibernation. For a complete description of hibernation traits, see Table 2.2. Since most prairie dogs in my study used daily torpor between bouts of multiday torpor, I quantified overall torpor use as opposed to daily torpor and multiday torpor separately. Energy-conservative hibernation patterns are generally considered to be those where individuals hibernate for long periods and use more prolonged, colder bouts of torpor. While hibernation onset and emergence may not directly influence the energy savings accrued during hibernation, they could have implications for annual fitness (Lane et al., 2012).

Lastly, as another method to quantify an individual's hibernation expression, I calculated the Heterothermy Index (HI, Boyles et al., 2011) for both an individual's specific hibernation period and a common period. I defined the common hibernation period as the time from the earliest date a monitored prairie dog in the population began hibernating (11 November) to the latest date a monitored prairie dog stopped hibernating (19 March in non-leap years and 18 March in leap years). Heterothermy Index is a measure of how far and for how long an individual's T_b deviates from their "optimum" or euthermic T_b over a specific period. Specifically, the formulation for is similar to that of a standard deviation and is given by the formula:

$$HI = \sqrt{\frac{\sum (T_{b-mod} - T_{b-i})^2}{n-1}} \tag{3.1}$$

Where T_{b-mod} is the most common euthermic T_b an individual experiences (a proxy for optimal performance T_b while active, T_{b-i} is an individual's T_b at a given time, i , and n is the number of times T_b was sampled in a given period. Using HI allowed me to summarize hibernation expression into a single metric rather than evaluating several (potentially inter-dependent) hibernation traits. It is possible that while hibernation traits can vary between individuals, the overall use of heterothermy during hibernation may be more relevant in regard to energy savings accrued over winter.

3.2.1.4   Quantifying over-winter change in body condition

To quantify body condition, I used the same methods described in Chapter 2. In the sections below, I briefly describe the methods I used but for complete descriptions, see Chapter 2.

3.2.1.4.1   Pre- and post-hibernation body condition

I quantified the pre-and post-hibernation body condition of all individuals implanted with data loggers using a zygomatic arch-derived body condition index (BCI), which has been shown to positively correlate with fat mass in prairie dogs (Wishart et al., 2021 in prep). To calculate BCI, I captured prairie dogs before and following hibernation (October and March, respectively) and measured body mass and zygomatic arch at these captures. I used a Pesola spring scale (either a 1000 g or 3000 g Pesola spring scale; note: The 1000 g Pesola measured mass in increments of 10g and measurements between increments were taken to the nearest 5 g and the 3000 g Pesola measured mass in increments of 20 g and measurements between increments were taken to the nearest 10 g) to measure body mass and analogue calipers (Mastercraft) to measure zygomatic arch breadth. For zygomatic arch breadth, I recorded three measurements during each measurement period (pre-and post-hibernation) and then averaged the pre-and post-hibernation measures.

For each dataset used in a set of analyses, I constructed sex-specific BCIs to account for the sexual dimorphism in prairie dogs (Hoogland, 1995, 2003). To construct the index, I used the residuals that resulted from a linear regression of standardized body mass (z-score; mean=0, standard deviation (SD)=1) on standardized zygomatic arch breadth. Lower BCI values indicate individuals in poorer condition (low energy reserves) relative to individuals with high BCI values. To measure body condition change over winter, I determined the difference between an individual's post-hibernation body condition and pre-hibernation body condition. I also determined the rate of body condition change over winter by dividing an individual's change in body condition by the duration of their hibernation period.

See the statistics section below for details on analyses, but briefly, I analyzed BCI for individuals from 2020 alone and individuals across all three hibernation years. This allowed

me to assess the effect of food supplementation in 2020 while also evaluating the effect of BCI on hibernation with a larger dataset. I calculated separate BCIs for these datasets since I had individuals in 2020 that were represented in the earlier hibernation years (i.e., repeated measures across years). For 2020, I had pre-and post-hibernation measurements for all individuals. When calculating BCI for the 2020 dataset, I included both pre-and post-hibernation measures in the linear regression, as this allowed me to determine an individual's pre-and post-hibernation body condition and the difference between the two time periods, using a consistent metric. For analyses involving individuals across all three hibernation years, I had pre- and post-hibernation measurements for all but four individuals. As the individuals I could use in specific analyses differed, I constructed two BCIs; one using only pre-hibernation measures and one using pre-and post-hibernation measures. By constructing the pre-hibernation measures only BCI, I could include the three individuals with only pre-hibernation measures in subsequent analyses that only required pre-hibernation condition. As mentioned above, by constructing a BCI with both pre-and post-hibernation measures, I could determine how an individual's body condition changed between the two time periods.

Prior to calculating BCI, I applied a correction to my body mass measurements to account for any differences in body mass that may have been a result of when I measured an individual relative to when they began or terminated hibernation. The number of days I measured a prairie dog prior to hibernation ranged from 14 to 46 d, while the number of days I measured a prairie dog after hibernation ranged from 3 to 71 d. As such, differences in pre-and post-hibernation body mass between individuals could have resulted from differences accumulated between when I measured an individual and when they entered/terminated hibernation, as opposed to individual pre-/post-hibernation variation. To determine the appropriate correction to apply, I performed linear regression analyses to quantify population-level rates of body mass change in the days prior to hibernation onset or following emergence from hibernation. This again assumes that body mass changes in prairie dogs immediately before and after emergence are linear which may not be accurately capture what happens to body mass during these periods. For a complete description of the analyses and corrections applied, see Appendix B. But briefly, for each individual, I corrected body mass to reflect what body mass would have been 14 days prior to hibernation and three days after hibernation as these were the closest dates to hibernation upon which I had captured an individual. As in Chapter 2, non-rounded corrected pre- and post-hibernation body mass values were subsequently used to calculated the body condition index described above.

3.2.2 Statistical analyses

I performed all analyses in the R statistical environment (R Core Team 2021; version 1.4.1103). To assess the effectiveness of the pre-hibernation food supplemental feeding trial conducted in 2020, I used Type II Analysis of Variance's (ANOVAs). I first constructed a general linear model with pre-feeding trial body mass as the response variable to assess any pre-existing differences in the body mass of fed and unfed individuals prior to food supplementation. I then constructed separate general linear models with body mass post-feeding and percent change in body mass during the feeding trial as dependent variables. For each model, I included sex and feeding status as independent variables. I validated model assumptions by assessing plots of residuals (residuals versus fitted values, normal Q-Q plots, and conditional plots of residuals for each factor) and determining the ratio of the largest error variance to the smallest error variance. If this ratio was >4, I followed the recommendation of Zuur et al. (2009) and used a generalized least squares (gls) model, as gls models allow for the specification of variance structure. I also used a Welch's *t*-test to determine if pre-hibernation body condition differed between fed and unfed individuals.

I constructed general linear models to assess the effect of pre-hibernation body condition on hibernation expression and overwinter change in condition and post-hibernation body condition. More specifically, I constructed separate models with the duration of hibernation, the proportion of hibernation spent torpid, mean duration of torpor bouts, mean torpor bout depth, Minimum T_b, mean arousal duration, hibernation onset, hibernation emergence, HI over a common and individual-specific hibernation period, change in body condition, rate of change in body condition and post-hibernation body condition as my dependent variables. A caveat for the models assessing the effect of pre-hibernation body condition on change in body condition and the rate of change in body condition experienced over winter is that pre-hibernation body condition appears both as a independent variable and is incorporated into the dependent variable in the models (i.e. Change in body condition = Post-hibernation body condition - Pre-hibernation body condition and Rate of change in body condition = Post-hibernation body condition - Pre-hibernation body condition/Duration of the hibernation period). In all models, I assessed model assumptions by assessing plots of residuals and determining the ratio of largest error variance to smallest error variance. Similar to the recommendations of Zuur et al. (2009), when homogeneity of variance appeared violated (error variance ratio > 4; Fox, 2008), I used gls model with a variance structure specified according to error variance ratio. I compared the linear model to the gls model using a likelihood ratio test, and if the gls model was better I assessed that model assumptions were improved. If the gls model was not better (p≥0.05), I continued with the linear model, as the

error variance ratio cut-off of 4 is considered conservative (Fox, 2008). When assessing the effect of pre-hibernation body condition on the number of torpor bouts, I used a generalized linear model with a Poisson distribution given that this was count data and assessed the model for over-dispersion as well as assessed plots of residuals.

In addition to data collected in 2020, I included any available data collected in the 2016-2017 and 2018-2019 hibernation years in my analyses to increase my sample size as well as add additional variation in pre-hibernation body condition (see Chapter 2 for details). Since supplemental feeding trials were only conducted in 2020, fed animals only occurred in one hibernation year, while unfed animals occurred across all three-hibernation years. Therefore, variation in unfed animals included variation across hibernation years, while variation in fed animals did not. Given this, I first constructed models exclusively on 2020 data to determine if feeding status influenced hibernation expression. In 2020, I retrieved 30 previously implanted temperature-sensitive data loggers; however, two data loggers failed to record any T_b data (Table 2.1). For all data loggers that were retrieved and contained usable data, I had the corresponding pre- and post-hibernation body mass of the prairie dogs in which the data loggers were implanted. The sample size for all models was $n=28$. I included pre-hibernation body condition, sex and feeding status as independent variables in each model. I also included an interaction between pre-hibernation body condition and sex and pre-hibernation body condition and feeding status. The interaction term between pre-hibernation body condition and feeding status was included to account for the potential effect of feeding status over and above that which it had on pre-hibernation body condition on hibernation expression. Model summaries from these analyses are presented in Appendix C, but briefly, there was not a significant interaction between feeding status and pre-hibernation body condition in each model. Subsequently, I did not include feeding status in models, including data across all three hibernation years.

Across all hibernation years, I retrieved 47 data loggers with complete hibernation traces. Included in these traces were two individuals, for whom I had hibernation records from two different years. However, since I had insufficient replication to include individual identity as a random effect in my models, I excluded the second hibernation year of record for both individuals. As such, the sample size was $n=45$ for all hibernation traits and HI models. For 43 of the 47 data loggers I retrieved, I had measured both pre- and post-hibernation body mass of the prairie dogs the data loggers were implanted in. I had records from two different hibernation years for one individual, so I chose to exclude the second year of data for this individual since I could not include a random effect of identity in my analyses. As such, the models for over-winter change in body condition and post-hibernation body condition have a sample size of $n=42$. In each model, I included sex, pre-hibernation body

condition and hibernation year as independent variables, and the interaction between sex and pre-hibernation body condition and pre-hibernation body condition and hibernation year. I reduced each model to the minimally adequate model via stepwise deletion of non-significant terms ($p<0.05$) assessed using an F-test (χ^2-test for Poisson model). All results were considered significant at $p<0.05$.

3.3 Results

3.3.1 Effectiveness of supplemental feeding trials

The mean body mass of male and female prairie dogs, respectively, before food supplementation was 1351 ± 17 g and 1169 ± 18 g (Male range: 1160 to 1480 g; Female range: 1010 to 1490 g). Body mass before the feeding trial significantly differed between the sexes (Type II ANOVA, $F_{(1, 45)}=50.04$, $p<0.001$) but did not differ between individuals in the fed and unfed groups (Type II ANOVA, $F_{(1, 45)}=0.07$, $p=0.80$; Table 3.2). During the feeding trials, the majority of prairie dogs experienced an increase in body mass, although five unfed individuals experienced body mass loss. The percent change in body mass of prairie dogs (fed and unfed) during the supplemental feeding trials was on average a $9.2 \pm 1.4\%$ increase (Range: -8.3 to 28.3%). Fed individuals experienced a significantly greater percent increase in body mass compared to unfed individuals (Type II ANOVA, $F_{(1, 32)}=16.13$, $p<0.001$). On average, fed individuals experienced a $14.2 \pm 1.7\%$ increase in body mass, while unfed individuals only experienced a $5.0 \pm 1.6\%$ increase in body mass. Male and female prairie dogs did not differ in their percent increase in body mass (Type II ANOVA, $F_{(1, 32)}=0.40$, $p=0.53$; Table 3.2).

At the end of the supplementary feeding trials, the mean body mass of individuals was 1470 ± 36 g (males; Range: 1135 to 1660g) and 1280 ± 26 g (females; Range: 1050 to 1470 g). The body mass of males and females significantly differed following the feeding trial (Type II ANOVA, $F_{(1, 33)}=16.84$, $p<0.001$), and there was a difference in the body mass of fed individuals and unfed individuals (Type II ANOVA, $F_{(1, 33)}=6.93$, $p=0.01$; Table 3.2). Similarly, body condition prior to hibernation was greater in supplementally fed individuals compared to those that did not have access to the supplemental diet ($t_{(9.78)}=4.50$, $p=0.001$; Figure 3.1).

3.3.2 Influence of pre-hibernation body condition on hibernation expression

Tables summarizing the estimates, standard error, and significance values of full and final models are presented in Appendix D.

3.3.2.1 Hibernation phenology

The onset of hibernation ranged from 28 November to 11 December in 2016, from 25 November to 5 December in 2018 and 10 November to 9 December 2019. There was no significant interaction between sex and pre-hibernation body condition ($F_{(1, 39)}$=0.42, p=0.52) or hibernation year and pre-hibernation body condition ($F_{(2, 37)}$=0.95, p=0.39). The onset of hibernation did not vary between the sexes ($F_{(1, 41)}$=3.03, p=0.09), and it was not influenced by a prairie dog's pre-hibernation body condition ($F_{(1, 40)}$=0.27, p=0.60). Hibernation onset was significantly different across hibernation years ($F_{(2, 42)}$=7.92, p=0.001).

Emergence from hibernation ranged from 20 January to 19 March in 2017, from 6 January to 11 March in 2019 and from 15 January to 27 February 2020. There was no significant interaction between hibernation year and pre-hibernation body condition ($F_{(2, 37)}$=0.13, p=0.88) or sex and pre-hibernation body condition ($F_{(1, 41)}$=0.10, p=0.75). Pre-hibernation body condition prior to hibernation did not significantly influence a prairie dog's emergence date ($F_{(1, 42)}$=0.31, p=0.58). Hibernation emergence did not significantly differ across hibernation years ($F_{(2, 39)}$=0.16, p=0.86). Emergence dates were significantly different between males and females ($F_{(1, 43)}$=48.56, p<0.001); males on average emerged 22.9 $\pm$ 3.3 d earlier in the spring than females.

3.3.2.2 Hibernation duration

Prairie dogs, on average, hibernated for 71.6 $\pm$ 3.6 d (Range: 33.6 to 108.5 d). There was no significant interaction between sex and pre-hibernation body condition ($F_{(1, 41)}$=0.49, p=0.49) or hibernation year and pre-hibernation body condition ($F_{(2, 37)}$=0.005, p=0.99). Pre-hibernation body condition and hibernation year did not affect hibernation duration in prairie dogs ($F_{(1, 42)}$=0.15, p=0.70 and $F_{(2, 39)}$=0.60, p=0.55, respectively). Hibernation duration significantly differed between males and females ($F_{(1, 43)}$=44.46, p< 0.001), with males hibernating for an overall shorter period compared to females.

3.3.2.3 Torpor use

The proportion of hibernation that a prairie dog spent in torpor was on average $66.4 \pm 1.6\%$ (Range: 42.4 to 80.6%). There was no significant interaction between sex and pre-hibernation body condition ($F_{(1, 39)}=1.26$, p=0.27) or hibernation year and pre-hibernation body condition ($F_{(2, 37)}=0.83$, p=0.44). The proportion of the hibernation period spent torpid did not vary across hibernation years ($F_{(2, 40)}=1.33$, p=0.28). Sex significantly affected the proportion of hibernation spent torpid ($F_{(1, 42)}=27.89$, p<0.001), with males on average spending 73.4% of hibernation torpid and females spending only an average 60.8% of the hibernation period torpid. Pre-hibernation body condition significantly affected the proportion of hibernation a prairie dog spent torpid ($F_{(1, 42)}=7.82$, p=0.008) with prairie dogs in better body condition, reducing the proportion of time they spent torpid (Figure 3.2). On average, per unit increase in body condition, a prairie dog reduced the proportion of time they spent torpid by $4.7 \pm 1.7\%$.

During hibernation, prairie dogs used between 5 and 24 bouts of torpor. There was no significant interaction between pre-hibernation body condition and hibernation year ($\chi^2_{(2)}=0.31$, p=0.86) and between pre-hibernation body condition and sex ($\chi^2_{(1)}=0.001$, p=0.98). Sex and hibernation year had a significant effect on the number of torpor bouts a prairie dog used during hibernation ($\chi^2_{(1)}=59.94$, p<0.001 and $\chi^2_{(2)}=6.61$, p=0.04, respectively) but pre-hibernation body condition did not ($\chi^2_{(1)}=0.35$, p=0.56).

The mean duration of torpor bouts a prairie dog used during hibernation was 107.7 ± 5.1 h (Range: 54.0 to 183.1 h). Neither pre-hibernation body condition and hibernation year nor pre-hibernation body condition and sex had a significant interaction ($F_{(2, 38)}=0.92$, p=0.41 and $F_{(1, 37)}=0.86$, p=0.36, respectively). Hibernation year did not have a significant effect on the mean duration of torpor bouts ($F_{(2, 41)}=2.99$, p=0.06) and was not influenced by their pre-hibernation body condition ($F_{(1, 40)}=0.02$, p=0.88). Consistent with results reported in Chapter 2, the mean duration of torpor bouts a prairie dog used during hibernation was influenced by their sex ($F_{(1, 43)}=21.16$, p<0.001).

During hibernation, the average depth of torpor bouts was 16.9 ± 0.5 °C (Range: 12.0 to 22.4 °C). There was no significant interaction between hibernation year and body condition ($F_{(2, 38)}=1.86$, p=0.17), and hibernation year alone did not influence the average depth of T_b during torpor bouts ($F_{(2, 41)}=2.78$, p=0.07). There was also no significant interaction between sex and body condition ($F_{(1, 37)}=2.20$, p=0.15). Pre-hibernation body condition did not significantly affect the average depth (T_b) of torpor bouts ($F_{(1, 40)}=0.56$, p=0.46). The depth of torpor bouts was significantly colder in males than females ($F_{(1, 43)}=29.39$, p<0.001). Males had a mean torpor bout depth that was on average 4.0 ± 0.7 °C colder than females. Minimum T_b during hibernation ranged from 9.5 to 13.0°C. There was no

significant interaction between sex and pre-hibernation body condition ($F_{(1,\,37)}$=0.01, p=0.92) or hibernation year and pre-hibernation body condition ($F_{(2,\,39)}$=0.86, p=0.43). Sex did not significantly affect the minimum T_b a prairie dog reached during hibernation ($F_{(1,\,38)}$=0.06, p=0.81). Pre-hibernation body condition did have a significant effect on minimum T_b during the hibernation period ($F_{(1,\,41)}$=14.29, p<0.001), with prairie dogs in poorer condition prior to hibernation having colder minimum T_b's compared to prairie dogs in better condition (Figure 3.3). Minimum T_b during hibernation also differed across hibernation years ($F_{(2,\,41)}$=5.02, p=0.01).

3.3.2.4 Arousal use

During the hibernation period, the mean duration of arousals bouts was 58.8 ± 2.8 h (Range: 28.3 to 109.7 h). There was no significant interaction between sex and pre-hibernation body condition ($F_{(1,\,39)}$=1.56, p=0.22) or hibernation year and pre-hibernation body condition ($F_{(2,\,37)}$=0.13, p=0.88). Duration of arousal bouts did not vary between males and females ($F_{(1,\,40)}$=0.07, p=0.79) or across hibernation years ($F_{(2,\,41)}$=1.96, p=0.15). Body condition prior to hibernation had a significant effect on the mean duration of arousal bouts ($F_{(1,\,43)}$=8.07, p=0.007), as prairie dogs in better condition increased the duration of their arousal bouts compared to prairie dogs in poorer condition (Figure 3.4). More specifically, for each per unit increase in body condition, prairie dogs increased the mean duration of their arousal bouts by 10.7 ± 3.8 h.

3.3.2.5 Heterothermy Index

The mean HI over the common hibernation period was 12.5 ± 0.3 (Range: 9.2 to 18.9). There was no evidence of a significant interaction between sex and pre-hibernation body condition ($F_{(1,\,39)}$=0.80, p=0.38) or hibernation year and pre-hibernation body condition ($F_{(2,\,37)}$=0.69, p=0.51). Neither hibernation year nor pre-hibernation body condition significantly affected the HI over the common hibernation period ($F_{(2,\,40)}$=1.31, p=0.28 and $F_{(1,\,42)}$=1.68, p=0.20, respectively). Sex had a significant influence on HI over the common hibernation period ($F_{(1,\,43)}$=12.59, p<0.001), with females having a higher HI than males. The effect of hibernation year and sex reported here are consistent with those reported in Chapter 2.

HI over an individual's specific hibernation period was on average 12.5 ± 0.6 (Range: 7.0 to 20.7). There was no significant interaction between hibernation year and pre-hibernation body condition ($F_{(2,\,39)}$=1.52, p=0.23). Neither sex, pre-hibernation body condition, nor their interaction significantly affected HI over an individual's hibernation period.

($F_{(1, 38)}$=0.79, p=0.38, $F_{(1, 41)}$=3.62, p=0.06 and $F_{(1, 37)}$=0.06, p=0.80, respectively). Consistent with results reported in Chapter 2, hibernation year did affect HI over an individual's hibernation period ($F_{(2, 42)}$=202.21, p<0.001).

3.3.3 Influence of pre-hibernation body condition on over-winter change in body condition

There was no significant interaction between pre-hibernation body condition and sex ($F_{(1, 34)}$=0.94, p=0.34) or between pre-hibernation body condition and hibernation year ($F_{(2, 36)}$=2.20, p=0.13). Change in body condition did not significantly differ across hibernation years or between sexes ($F_{(2, 38)}$=1.48, p=0.24 and $F_{(1, 35)}$=0.14, p=0.71, respectively). Change in body condition over winter was significantly affected by pre-hibernation body condition ($F_{(1, 40)}$=80.83, p<0.001), with individuals in better body condition experiencing a greater decrease in body condition over winter compared to individuals with lower body condition (Figure 3.5). There was no significant interaction between pre-hibernation body condition and hibernation year on the rate of change in body condition ($F_{(2, 34)}$=2.35, p=0.11) and the rate of change in body condition did not differ across hibernation years ($F_{(2, 36)}$=0.15, p=0.86). There was a significant interaction between sex and pre-hibernation body condition ($F_{(1, 38)}$=6.13, p=0.02). For each unit increase in body condition, male prairie dogs lost more body condition per day compared to female prairie dogs (Figure 3.6)

3.3.4 Influence of pre-hibernation body condition on post-hibernation body condition

Post-hibernation body condition was significantly affected by an individual's pre-hibernation body condition ($F_{(1, 40)}$=32.51, p<0.001). Individuals in better body condition prior to hibernation emerged in better condition compared to individuals with lower body condition prior to hibernation (Figure 3.7). Neither the interaction between pre-hibernation body condition and sex ($F_{(1, 34)}$=0.94, p=0.34) nor the interaction between pre-hibernation body condition and hibernation year ($F_{(2, 36)}$=2.20, p=0.13) significantly influenced post-hibernation body condition. Post-hibernation body condition did not significantly differ between the sexes ($F_{(1, 35)}$=0.14, p=0.71) or across hibernation years ($F_{(2, 38)}$=1.48, p=0.24). The similarity in post-hibernation body condition between males and females is the result of constructing sex-specific BCIs, so conclusions as to whether the body condition of males and females can not be made.

3.4 Discussion

I investigated whether pre-hibernation body condition influenced hibernation expression and over-winter energy expenditure in Canadian prairie dogs. To do so, I used previously recorded data and conducted a supplementary feeding experiment during the pre-hibernation fattening period to expand variation in pre-hibernation body condition. This allowed me to test predictions from the hibernation optimization hypothesis in a facultative, fat-storing hibernator which, to my knowledge, had yet to be investigated. Facultative hibernators differ from other hibernators in that they only enter hibernation when resources are limited in the environment and/or ambient temperature is cold. Due to the influence of environmental resource availability and ambient temperature on hibernation expression in facultative hibernators, I assumed that their expression of hibernation may be more sensitive to the size of their energy stores. In addition to investigating the influence of pre-hibernation body condition on hibernation expression, I also investigated if its effect was sex-specific, specifically whether males would show a decreased response to pre-hibernation body condition relative to females.

Prairie dogs demonstrated adjustments in hibernation expression with respect to body condition in some but not all hibernation traits. Prairie dogs entering hibernation in better pre-hibernation condition (larger energy reserves) did not adjust the total time they spent hibernating or the timing of hibernation compared to individuals in poorer condition. However, individuals with larger energy stores before hibernation decreased the proportion of time they spent in torpor by increasing the length of arousal bouts, not by adjusting the number or mean depth of torpor. Though prairie dogs in better pre-hibernation body condition did not reach as cold and minimum T_b during hibernation when compared to prairie dogs in poorer condition. Additionally, prairie dogs in better condition before hibernation experienced a greater loss of body mass over winter but emerged in better condition in the spring. There was no evidence that prairie dogs demonstrated sex-specific adjustments to hibernation expression based on pre-hibernation body condition. However, I found evidence that males experience a greater increase in the amount of body condition lost per day during hibernation as their pre-hibernation body condition increases compared to females.

The supplemental feeding trial I conducted was successfully generated variation in pre-hibernation body condition. In response to food supplementation, prairie dogs gained more body mass during the pre-hibernation fattening period compared to individuals that did not receive the supplemental diet. On average, fed prairie dogs increased their body mass by 14.23% (approx. 178 g), while unfed prairie dogs increased their body mass by 4.96% (approx. 60 g). The increase in body mass before hibernation aligns with findings by Kusch et al. (2021) and is likely reflective of an increase in fat mass before hibernation, as has

been found in a more southern population of black-tailed prairie dogs (Lehmer & Van Horne, 2001) and other fat-storing sciurid hibernators (Jameson & Mead, 1964; Galster & Morrison, 1976; Sheriff et al., 2013). As a result of the food supplementation, fed prairie dogs entered hibernation in better condition (i.e., larger fat reserves) than unfed prairie dogs.

The hibernation optimization hypothesis predicts that individuals with larger energy stores (better body condition) upon hibernation onset should reduce their expression of torpor over-winter (Humphries et al., 2003b), which can be achieved by a reduction in the total amount of time spent hibernating and/or by limiting the use of torpor (i.e., number, depth, duration of torpor bouts, the proportion of time spent torpid) within the hibernation period. I found that prairie dogs did not reduce the overall duration of their hibernation period. While this finding does not support this prediction from the hibernation optimization hypothesis, it does align with findings that edible dormice (*Glis glis*) do not reduce the length of hibernation when they have larger energy reserves (Bieber et al., 2014). Collectively, the findings presented in Bieber et al. (2014) and this chapter suggest that the energy savings and physiological costs of hibernation are not the only factors that influence the duration of hibernation. As suggested for edible dormice (Bieber et al., 2014), prairie dogs may maintain the duration of their hibernation period regardless of their body condition to reap the benefits of predator avoidance. Conversely, while hibernating prairie dogs may be particularly susceptible to predation via North American badgers (*Taxidea taxus*) and other burrow excavators. Michener (2004) found that peak hunting of Richardson's ground squirrels (*Spermophilus richardsonii*) by badgers occurs during a portion of the hibernation period and over a 15 year study period 83% of 64 confirmed badger predation events were of hibernation ground squirrels. Nonetheless, as prairie dogs function as prey to various grassland species (COSEWIC, 2011), reducing predation risk may be an important influence on their hibernation duration. Investigating how hibernation duration varies under differing levels of predation risk would improve our understanding of its role in optimal hibernation expression. Additionally, given that prairie dogs hibernate facultatively, the duration of the hibernation season may be more dependent on weather conditions. Prairie dogs are also a gregarious species that are arranged in coteries within a colony (Hoogland, 1995). Gummer (2005) found that prairie dogs that were captured in similar locations on a colony exhibited similar T_b traces compared to those that were not, which may be the result of prairie dogs within the same coterie attempting to hibernate communally. Therefore, individuals that vary in body condition within a colony may synchronize hibernation expression and dilute any influence of pre-hibernation body condition on the duration of hibernation.

In support of the hibernation optimization hypothesis, I found that prairie dogs did reduce torpor use by decreasing the proportion of the hibernation period spent in torpor.

Woodchucks (*Marmota monax*) in southeastern Pennsylvania also decrease the percentage of the hibernation spent torpid when heavier in mass (Zervanos et al., 2014). The decrease in the proportion of time spent torpid observed in individuals in better pre-hibernation body condition season resulted from increases in the length of their arousal bouts. On average, for each unit increase in body condition, prairie dogs increased the length of the arousal bouts by 10.88 h. Prairie dogs in better condition before hibernation also appear to decrease the length and depth of torpor (i.e., have shorter and warmer torpor bouts); however, these findings were not significant as body condition was not included in the final models. Additionally, overall, prairie dogs did not reach as cold a minimum T_b over the hibernation period when in better body condition. The reduction in torpor use during hibernation observed in individuals with larger energy reserves likely results from individuals attempting to minimize the physiological costs associated with torpor use (Humphries et al., 2003b). For example, torpor use and hibernation have been linked to reduced memory retention, which could be particularly important in highly social species, like black-tailed prairie dogs. Memory is essential for maintaining social bonds, recognizing kin, and defending territories (Ferguson et al., 2002). As such, if prairie dogs with larger energy stores can reduce their use of torpor during the hibernation season, they could benefit from an improved ability to maintain social bonds. In addition to mitigating the physiological costs of torpor, prairie dogs that spend a greater proportion of the hibernation period in arousal may benefit from detecting whether conditions outside their hibernaculum are suitable for emergence, and thereby synchronize their emergence with more favourable environmental conditions, as has been suggested for woodchucks (Zervanos & Salsbury, 2003).

In addition to investigating the expression of various hibernation traits in relation to pre-hibernation body condition, I also investigated how an individual's overall use of heterothermy (HI) was affected by pre-hibernation body condition. Synthesizing an individual's hibernation expression into a single measure of their use of heterothermy over the hibernation period can allow for an assessment of the energy conservation of hibernation expression. If individuals are predicted to reduce their use of torpor during hibernation when they have access to larger energy stores, how much their T_b during hibernation deviates from their optimal euthermic T_b should be reduced (greater energy expenditure). Despite decreases in the proportion of time spent torpid, there was no significant effect of pre-hibernation body condition on HI over an individual's hibernation or common period. Variation in HI across hibernation years could be obscuring the ability to detect an effect of pre-hibernation body condition on HI during an individual's hibernation period. As suggested in Chapter 2, future studies exploring the effect of pre-hibernation body condition on HI within a single year may help elucidate these relationships. With respect to HI calculated over a common

hibernation period, given that I calculated HI from the earliest date of hibernation use to the last date of hibernation for many individuals, HI was calculated over periods when they were no longer hibernating. As such, this may have dampened my ability to detect an effect of pre-hibernation body condition.

Prairie dogs in better condition before hibernation did not differ in the timing of hibernation relative to individuals in poorer body condition. Hibernation onset may not vary between prairie dogs different quantities of energetic resources due to their high degree of sociality. Prairie dogs, particularly those that reside within the same coterie, may try to synchronize the onset of hibernation to maximize the amount of time available to interact socially. If prairie dogs hibernate communally, individuals in poor condition may enter hibernation at similar times as individuals in better pre-hibernation conditions to benefit from social thermoregulation, which can reduce energy expenditure and mortality (Arnold, 1988, 1993). Similar to hibernation onset, prairie dog emergence was not influenced by their body condition before hibernation. This could be the result of emergence dates being tightly regulated due to reproductive events following closely after (i.e., gonadal maturation and copulation). Several hibernating species have been found to emerge from hibernation earlier when they have access to additional energy reserves (Bieber et al., 2014; Siutz et al., 2018). However, given that prairie dogs reside in coteries that generally are comprised of one breeding male, a harem of breeding females, non-reproductive juveniles, and yearlings (Hoogland, 1995), body condition may not have the same effect. Males with fewer fat stores may emerge at similar times as males with larger fat stores to defend their territory (and females) from neighbouring males. Female prairie dogs may also emerge at similar times regardless of pre-hibernation body condition to establish and defend high-quality burrows to rear pups, particularly in cases where there are large numbers of breeding females. Reproductive competition has been found to influence emergence dates in yellow-bellied marmots, another social hibernating species (*Marmota flaviventris*; Blumstein, 2009). Furthermore, since prairie dogs are facultative hibernators, environmental conditions may have a greater influence on their hibernation phenology than body condition. The onset of prairie dog hibernation was significantly different across hibernation years, which may result from annual variation in environmental conditions.

In accordance with the decreased use of torpor and prolonged arousal bouts, individuals in better pre-hibernation condition experienced a greater over-winter change in body condition as compared to individuals in poorer condition. Additionally, prairie dogs in better pre-hibernation body condition also body condition at a faster rate (more body condition per day of hibernation) compared to those of a poorer pre-hibernation body condition. Therefore, prairie dogs appeared to mitigate some of the adverse effects of torpor use by limiting torpor

use at the cost of increased energy expenditure, as predicted by the hibernation optimization hypothesis (Humphries et al., 2003b). Despite prairie dogs with better pre-hibernation body condition using more energetic resources over winter, they still emerged in better condition upon emergence. This suggests that prairie dogs are not using all their additional energy stores to limit the costs of torpor but are conserving a portion of their energy for energetic requirements in the spring. Female edible dormice and male common hamsters with larger pre-hibernation energy stores also decrease their torpor use during hibernation but still emerge in better condition (Bieber et al., 2014; Siutz et al., 2016; Siutz et al., 2018). Collectively, these findings suggest that the costs of torpor may not be as "costly" as originally predicted as individuals in good condition are still inclined to enter torpor to conserve energy despite having the ability to reduce the costs of torpor further. However, by reducing torpor expression but conserving surplus energy for emergence, individuals likely benefit from decreased physiological damage and increased emergent energy stores upon emergence. Future research investigating the direct effects the physiological costs of torpor have on individual fitness would provide further insight into the importance of the physiological cost of torpor in driving optimal hibernation expression. Female prairie dogs in better body condition have been found to benefit from increased litter sizes (Kusch & Lane, 2021a), and heavier males also benefit from an increased number of offspring (Hoogland, 2001). Thus, prairie dogs with large pre-hibernation energy reserves may improve their reproductive success by conserving a portion of their surplus energy for the spring.

Interestingly, despite not finding sex-specific effects of pre-hibernation body condition on hibernation expression, I found that male prairie dogs experienced a greater increase in the rate of body condition lost overwinter as their pre-hibernation body condition improved compared to females. This discrepancy may result from males making slightly larger adjustments to several hibernation traits than females, such that sex-specific responses for any one hibernation trait were not detected. For example, in terms of the effect of pre-hibernation body condition on the duration of hibernation, in the full model, males appeared to decrease the length of hibernation as their body condition improved while females appeared to increase the length of hibernation slightly. This difference, however, was not significant, and the interaction term between sex and body condition was dropped from the final model. Regardless, despite having an earlier reproductive investment, males appear to adjust the rate of body condition lost over winter to a greater extent compared to females. In Chapter 2, I found that males use longer and deeper bouts of torpor and spend proportionally more time in torpor compared to females. As such, when males are in better condition prior to hibernation, they may attempt to reduce their use of torpor to a greater extent compared to females to reduce the increased physiological damage they typically accrue. However, as discussed in Chapter

2, the body mass correction I applied to a prairie dog's body mass may not have accurately captured how body mass changed in the weeks prior to and following hibernation for males and females. As such, results, particularly regarding over-winter change in body condition between the sexes, should be interpreted with caution, as they may have included changes in body condition that occurred outside of hibernation.

It is possible that some differences in hibernation expression I report as a result of pre-hibernation body condition are in part the result differences in polyunsaturated fat (PUFA) content, which have be found to influence torpor expression in black-tailed priarie dogs from more southern populations and white-tailed prairie dogs (*Cynomys leucurus*, Harlow and Frank, 2001). The food supplementation trials I conducted could have altered the PUFA content in the prairie dogs that received the supplemental diet, however, I did perform analyses with the 2020 data alone to account for a pre-hibernation body condition: feeding status interaction. In these analyses, the interaction term was always insignificant which indicates that the effect of pre-hibernation body condition did not differ between unfed and fed prairie dogs and, as such, it is likely the PUFA content did not differ between these two groups. It is possible that the prairie dog used in the study exhibited natural variation in PUFA content that would contribute to the differences in hibernation expression I report. In the present study I was unable to account for this, but future work investigating the influence of PUFAs on prairie dogs hibernation expression in the northern population (i.e. feeding prairie dogs diets both high and low in PUFAs or measuring PUFA content prior to hibernation) is warranted.

Prairie dogs demonstrate the ability to adjust their use of torpor during hibernation according to the size of their energy reserves. This suggests that the energetic state of the individual influences their need to use energy-saving, but physiologically costly, bouts of torpor over-winter. As resource availability can fluctuate annually, plasticity in hibernation expression and over-winter energy expenditure with respect to pre-hibernation body condition is likely important for population persistence. For example, if resource availability is low during the active season and individuals enter hibernation with fewer energetic resources, the ability to increase torpor use could allow individuals to survive over-winter and potentially reproduce upon emergence. The ability to adjust hibernation expression based on energetic needs could be particularly important in the face of climate change. Climate change is predicted to alter precipitation patterns and increase the risk of drought (IPCC, 2013), which could influence vegetation availability and, in turn, the size of pre-hibernation energy stores. Given that hibernation duration and phenology were not influenced by pre-hibernation body condition understanding how other factors (i.e., predation, sociality, climate, environmental resource availability) influence these traits could aid our understanding of the evolutionary

and ecological consequences of hibernation expression.

3.5   Conclusion

In this chapter, I present the first evidence that black-tailed prairie dogs adjust hibernation expression in response to the size of their pre-hibernation energy reserves. This finding adds to a growing body of work demonstrating the flexible nature of hibernation and factors that influence an individual's use of hibernation and over-winter energy use. In response to increased pre-hibernation energy stores, prairie dogs reduced the proportion of the hibernation period spent torpid and increased the length of arousal bouts, which is consistent with my predictions and supports the hypothesis that hibernators balance the expression of hibernation/torpor according to their energetic condition (Humphries et al., 2003b). However, prairie dogs in good pre-hibernation body condition did not limit the duration of the hibernation season, which suggests that other non-energetic related factors are missing from the hibernation optimization hypothesis (Bieber et al., 2014; Boyles et al., 2020). In this chapter, I also demonstrated the effectiveness of a novel feeding method to improve pre-hibernation body condition and post-hibernation body condition. Given that body mass (Hoogland, 2001) and condition (Kusch & Lane, 2021a) have been linked to improved reproductive output, improving pre-hibernation body condition is likely an effective conservation strategy. Further, given that climate change is likely to affect the availability of food resources could be of particular importance in coming years.

Table 3.1: Summary of the number of fed and unfed black-tailed prairie dogs used in the supplementary feeding trial conducted from 3 September to 22 October 2020.

	Unfed		Fed	
	Male	Female	Male	Female
Number captured within 3 days of feeding trial start date (3 September 2020)	9	15	12	12
Number captured within 3 days of feeding trial end date (22 October 2020)	7	13	9	7
Number capture within 3 days of feeding trial start and end date	6	13	9	7
Number implanted with data loggers post-feeding trial	10	13	12	13

Table 3.2: Summary of body mass in fed and unfed black-tailed prairie dogs before and following feeding trials conducted from 3 September to 22 October 2020. Sample sizes are indicated for each sex within group, and descriptive statistics are presented as the mean ± standard error of the mean (SEM).

	Unfed				Fed			
	Male		Female		Male		Female	
	n	Mean±SEM	n	Mean± SEM	n	Mean± SEM	n	Mean± SEM
Pre-feeding trial body mass (g)	9	1351±36	15	1175±28	12	1352±16	12	1163±22
Post-feeding trial body mass (g)	7	1390±60	13	1252±35	9	1532±34	7	1330±28
Change in body mass (%)	6	3.1±3.1	13	5.8±1.8	9	14.1± 2.8	7	14.4±1.6
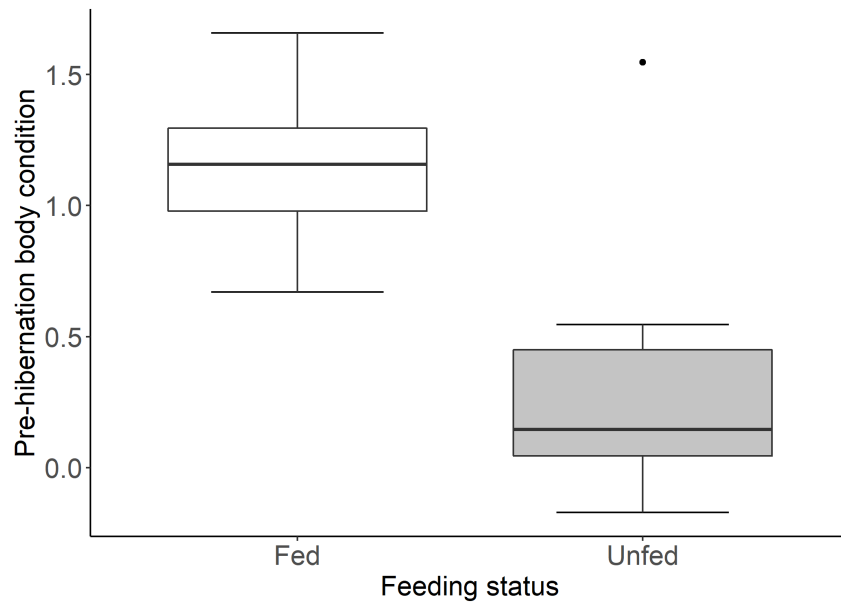
Figure 3.1: Boxplot of pre-hibernation body condition for supplementally fed and unfed male and female black-tailed prairie dogs. The thick horizontal bar represents the median for each box, and the lower and upper bounds represent the 25th and 75th percentile, respectively. Lower and upper whiskers represent the minimum and maximum values, respectively, and the black dots represent outliers. Higher body condition values indicate better body condition compared to lower body condition values.
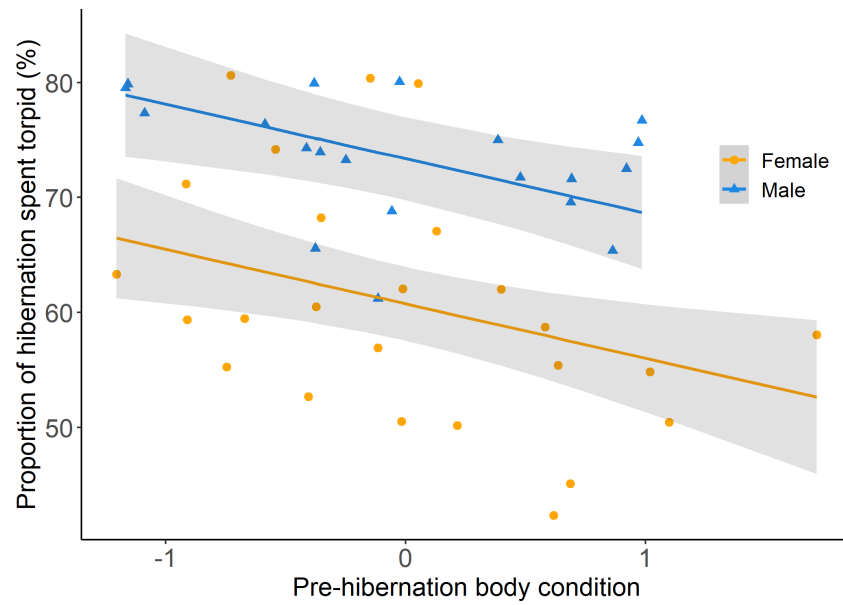
Figure 3.2: The influence of pre-hibernation body condition on the proportion of hibernation spent torpid in male and female black-tailed prairie dogs. Orange circles represent female prairie dogs, and blue triangles represent male prairie dogs. The grey area around the fitted line for each sex represents the 95% confidence interval. Higher body condition values indicate individuals in better body condition compared to lower body condition values.
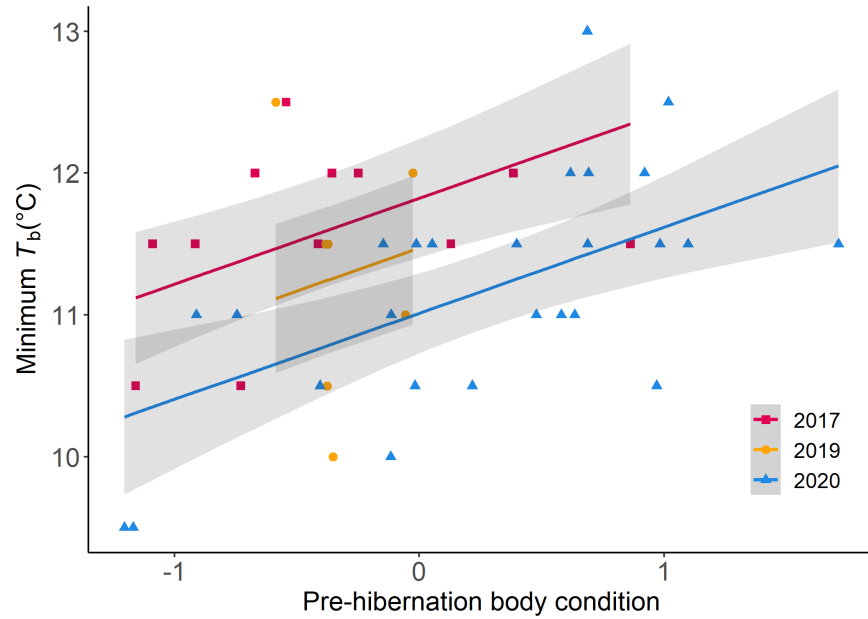
Figure 3.3: The influence of pre-hibernation body condition on minimum T_b (°C) during hibernation in black-tailed prairie dogs in 2017, 2019 and 2020. Red squares represent prairie dogs in 2017, orange circles represent prairie dogs in 2019, and blue triangles represent prairie dogs in 2020. The grey area around the fitted line for each hibernation year represents the 95% confidence interval.
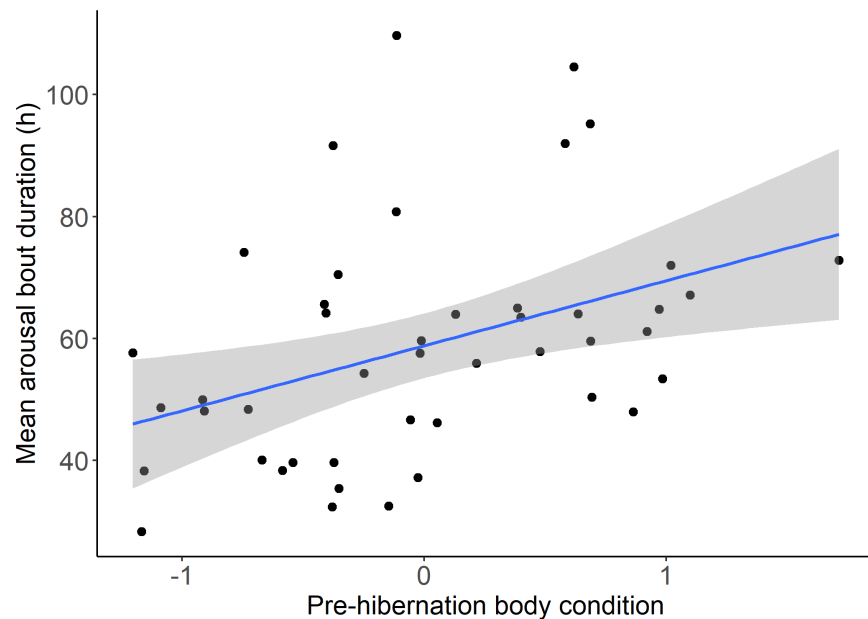
Figure 3.4: The influence of pre-hibernation body condition on mean arousal bout duration in black-tailed prairie dogs. Black dots represent individual prairie dogs. The grey area around the fitted line represents the 95% confidence interval.
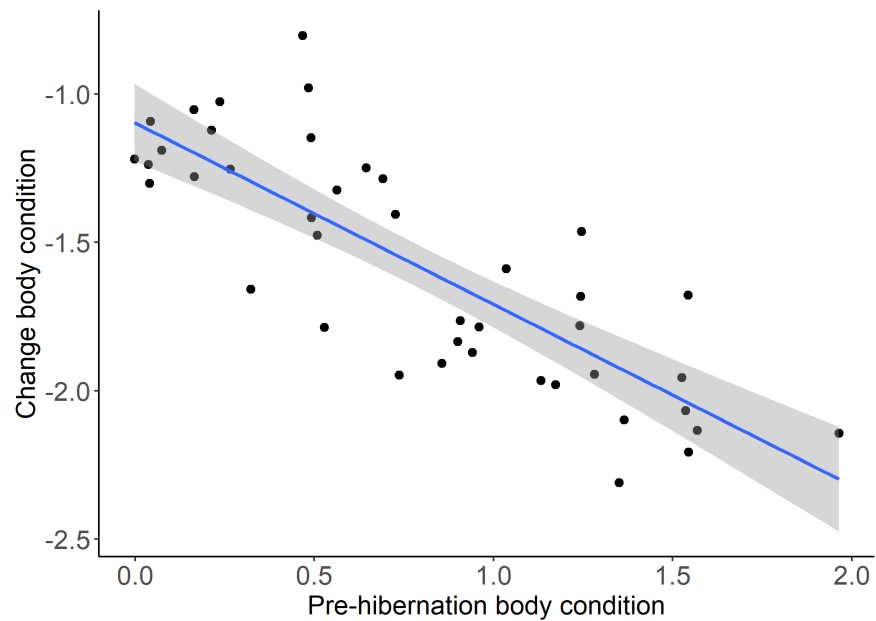
Figure 3.5: The influence of pre-hibernation body condition on change in body condition over winter in black-tailed prairie dogs. The grey area around the fitted line represents the 95% confidence interval. Note: The model assessing the influence of pre-hibernation body condition on the rate of change in body condition has a caveat in that pre-hibernation body condition appears a independent variable and is incorporated into the dependent variable (i.e. Change in body condition = Post-hibernation body condition - Pre-hibernation body condition) in the model.
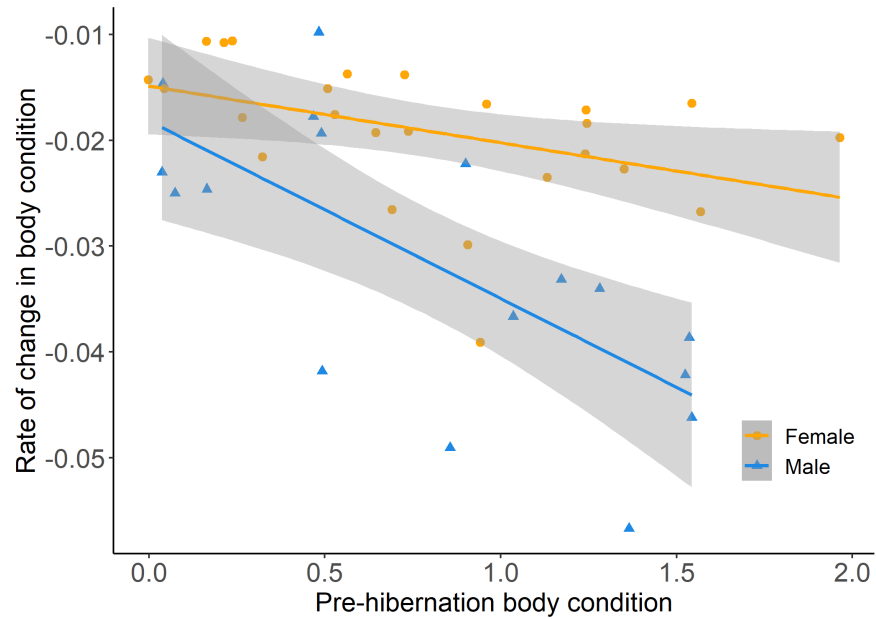
Figure 3.6: The influence of pre-hibernation body condition on the rate of change in body condition over winter in black-tailed prairie dogs. Orange circles represent female prairie dogs and blue triangles represent male prairie dogs. The grey area around the fitted line for sex represents the 95% confidence interval. Note: The model assessing the influence of pre-hibernation body condition on the rate of change in body condition has a caveat in that pre-hibernation body condition appears a independent variable and is incorporated into the dependent variable (i.e. Rate of change in body condition = Post-hibernation body condition - Pre-hibernation body condition/Duration of the hibernation period) in the model.
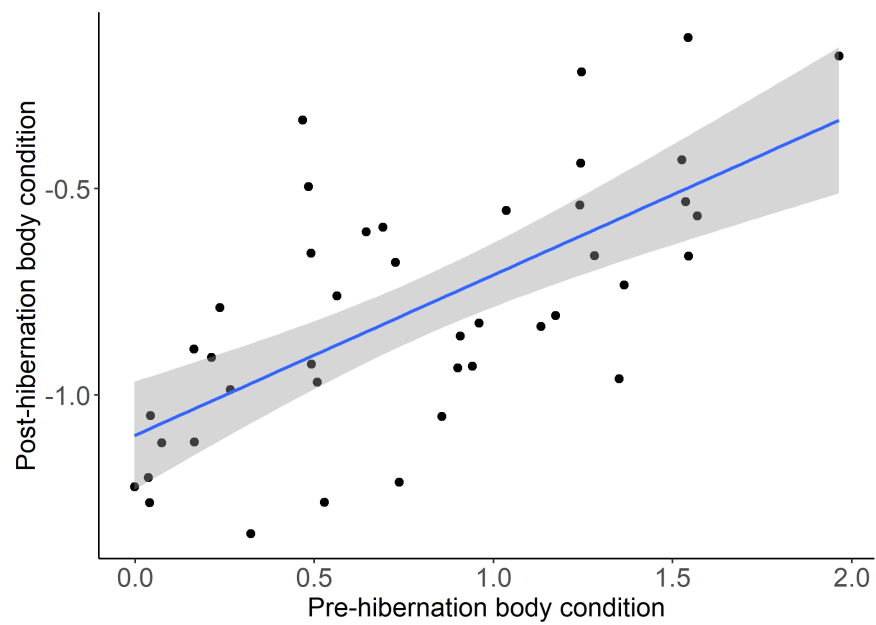
Figure 3.7: The influence of pre-hibernation body condition on post-hibernation body condition in black-tailed prairie dogs. The grey area around the fitted line represents the 95% confidence interval.

Chapter 4

General Discussion

4.1 Summary

Black-tailed prairie dogs (*Cynomys ludovicianus*) are social, fat-storing, facultative hibernators that exhibit tremendous variation in their thermoregulatory strategies not only across their geographic range but also within single populations (Figure 1.1). In this thesis, I investigated factors that contribute to this intraspecific variation in the northernmost wild population. Specifically, I explored the role of sex and pre-hibernation body condition on over-winter change in body condition, hibernation phenology, and duration, as well as the specific characteristics of torpor and arousal use.

Hibernation expression is hypothesized to result from optimizing the trade-offs between energy-saving, but physiologically costly, bouts of torpor and energetically expensive bouts of arousal (Humphries et al., 2003b). This optimization is predicted to be governed by an individual's energetic state (pre-hibernation body condition) and their energetic requirements following emergence (Humphries et al., 2003b). While this hypothesis had been investigated in fat-storing obligate hibernators and food caching facultative hibernators, it had yet to be studied in a fat-storing, facultative hibernator. Hibernation expression in fat-storing, facultative hibernators may be more sensitive to the energetic state of the individual, given that their hibernation expression is assumed to occur only when they are cold-stressed and/or food-restricted.

Furthermore, since both the timing and overall cost of spring energy requirements can differ between the sexes, sexual variation in hibernation expression can exist. In the prairie dog system, males and females invest in reproduction post-emergence, and males typically invest earlier (gonadal recrudescence, securing mates) in the active season than females. Given this, one may predict that males may express more energy-conservative expressions of hibernation relative to females. However, a recent study found that male prairie dogs experience a more substantial decline in body mass over winter compared to females (Kusch et al., 2021). This suggests that prairie dogs may hibernate according to the thrifty female

hypothesis (Jonasson & Willis, 2011) and that female prairie dogs may employ hibernation expressions that conserve more energy than males.

In Chapter 2, I determined that black-tailed prairie dogs demonstrate sexual variation in hibernation expression. Male and female prairie dogs entered hibernation at similar times of the year, but males emerged from hibernation approximately three weeks earlier than females (Figure 4.1). As a result, females hibernated for longer durations, underwent more torpor bouts and spent an overall greater amount of time in torpor over-winter than males. However, during hibernation, their respective hibernation periods, males spent proportionally more time in torpor and exhibited torpor bouts that were, on average, longer and colder than females. Collectively these findings highlight how sexual selection and reproductive requirements during the active season may influence hibernation expression. Male prairie dogs have an earlier reproductive investment relative to females and may benefit from an increase in reproductive success when they emerge earlier (Michener, 1983b; Hasselquist, 1998; Morbey, 2000; Myers, 1981; Francis and Cooke, 1986, see Morbey and Ydenberg, 2001 for a review on selection for protandry). For male prairie dogs to be reproductively ready to mate, they need to undergo spermatogenesis, which is inhibited during torpor use (Barnes et al., 1986) which likely advances their emergence date and shortens their hibernation period relative to females. Female prairie dogs may also benefit from waiting until males are reproductive ready and environmental conditions are favourable (Michener, 1983b). As male prairie dogs hibernate for a shortened period, they may maximize their energy savings to support reproduction by using torpor to a greater extent. Conversely, female prairie dogs that hibernate for an overall more extended period may attempt to decrease the physiological costs of torpor use by using shorter and warmer bouts of torpor. Despite males using what appears to be a more energy-conservative expression of hibernation, I found that they lost approximately the same amount of body condition as females in a shorter amount of time. As discussed in Chapter 2, the results regarding their over-winter change in body condition likely capture body condition dynamics that occurred outside of hibernation (i.e., breeding, gonadal recrudescence). Thus, male prairie dogs likely conserve more energy during hibernation than their female counterparts and then expend more energy immediately after hibernation as they prepare for and participate in the breeding season.

In Chapter 3, I determined that pre-hibernation body condition does, in part, explain some of the variation in black-tailed prairie dog hibernation expression that has been observed. I found that prairie dogs reduced their torpor expression during hibernation; however, they did not reduce the overall duration or adjust the timing of their hibernation. The reduction in torpor use and increased length of arousal bouts indicates that prairie dogs do optimally express hibernation according to their energetic state. Yet, I found that prairie

dogs do not limit the total amount of time they spend hibernating. The timing and overall duration of hibernation in the population are likely driven by other non-energetic factors, such as predation risk or sociality. Despite prairie dogs that entered hibernation in better body condition limiting their use of torpor and losing more body condition over winter, they were still able to emerge in better condition relative to individuals who entered hibernation in poorer condition. As such, while prairie dogs limit their use of torpor when they have sufficient energy to do so, they use torpor enough to ensure they benefit from relatively higher condition upon emergence. This suggests that while there are physiological costs of torpor use, the costs of torpor may not outweigh its energetic benefits and the fitness benefit of emerging in good body condition.

4.2   Broader relevance

My thesis offers unique insight into the role of spring energy requirements on hibernation expression. Within a species, the sex with increased reproductive energy requirements is predicted to express torpor to a greater extent and conserve more energy over winter (Humphries et al., 2003b). Despite male prairie dogs having an earlier reproductive investment than females, female prairie dogs have previously been reported to lose less body mass over winter than males (Kusch et al., 2021), suggesting they have higher spring energy requirements. I found that male prairie dogs spent proportionally more time in torpor and used longer and colder bouts of torpor but experienced a similar change in body condition over-winter and, when accounting for their short hibernation period, lost body condition at a faster rate relative to females. While this finding suggests that the hibernation expression of males is less thrifty than that of females, this may be an artifact of when I measured the prairie dogs relative to their hibernation onset and emergence. Although I attempted to account for lags between when I measured a prairie dog and when they emerged from hibernation, the correction I applied may not accurately represent how body mass changes early in the active season. Future research investigating body mass dynamics and energy expenditure early in the active season is warranted before drawing more definitive conclusions. It would also be of interest to collect body condition data immediately before and after hibernation, as this would increase the understanding of prairie dog over-winter energy expenditure in relation to various hibernation expressions.

Although the results regarding sexual variation in over-winter change in body condition need to be interpreted with caution, it highlights that other aspects of hibernation use should be considered when evaluating the energy savings provided from a given hibernation expression. It is possible that while an individual uses a more energy-conservative hibernation,

other factors (i.e., using food caches in addition to fat reserves, micro-climate, social hibernation, length of pre and post-hibernation euthermia) could result in an individual using more or less of their energy reserves than would be expected. For example, individuals with similar hibernation expressions but use cache resources to varying extents could emerge from hibernation with very different amounts of energy expenditure. Studies investigating how conservative an expression of hibernation relative to another will need to consider all aspects of hibernation, not just the extent of torpor use. Investigation of hibernation in natural, as opposed to laboratory conditions, will be beneficial as under natural conditions, how an individual selects a hibernaculum microclimate or uses food caches relative to hibernation expression and energy use could be assessed or, at a minimum, be captured in the energy use that is measured.

Identifying and determining the strength of factors that influence hibernation expression and the flexibility of those factors could prove crucial in the face of climate change. As precipitation patterns are predicted to change and the risk of drought is expected to increase (IPCC, 2013), the amount of vegetation available to hibernators during the active season may be limited. The ability of hibernators to adjust torpor use and the amount of energy savings accrued over winter according to their energy reserve size (body condition) may allow them to survive and reproduce in years where energetic resources are limited before hibernation. This ability may be particularly relevant for black-tailed prairie dogs as droughts have been identified as a key factor that threatens their persistence (COSEWIC, 2011), and it has been suggested that their body condition entering hibernation may be influential in determining their survival and reproduction (Stephens et al., 2018). I found that prairie dogs alter their torpor use in response to their body condition before hibernation. As such, prairie dogs may be more resilient to drought and poor pre-hibernation body condition than originally predicted. However, there is likely a minimum amount of pre-hibernation energy reserves that an individual requires to survive the harsh winter months and successfully reproduce in the spring. Compared to obligate hibernators, facultative hibernators, like black-tailed prairie dogs, may be more resilient to changes brought on by climate change. For example, given that hibernation expression in facultative hibernators typically occurs when they are cold-stressed and/or food-limited, they may have a greater ability to adjust their hibernation phenology in response to changes in weather conditions (i.e., re-enter hibernation in the event of a late-winter snowstorm). Long-term monitoring of black-tailed prairie dog hibernation in Canada and comparison to obligate hibernators, particularly those that experience similar over-winter conditions, would provide an opportunity to assess this.

Though tangential to the goal of this thesis, I demonstrated that food supplementation in the months before hibernation effectively improves the pre-hibernation body condition of wild

black-tailed prairie dogs. I also found that prairie dogs in better pre-hibernation body condition increased their arousal use but still emerged in better condition in the spring. Female prairie dogs in better body condition and male prairie dogs that are heavier have been shown to benefit from an increased number of offspring (Hoogland, 2001; Kusch & Lane, 2021a). Thus, given the threatened status of black-tailed prairie dogs (COSEWIC, 2011)(Species at Risk Act, 2018), and that drought risk is expected to increase (Lemmen et al., 1997; Bush & Lemmen, 2019), food supplementation before hibernation may be an effective conservation strategy for this species. Management strategies that focus on increasing pre-hibernation body mass/condition may serve as a more effective strategy to improve reproductive output than directly increasing the post-hibernation body condition, as they would not disrupt the reproductive period of prairie dogs. Additionally, since prairie dogs reproduce immediately following emergence, supplemental feeding in autumn before hibernation likely provides more time to improve body condition. As prairie dogs in better condition also reduce their use of torpor during hibernation, prairie dogs may further benefit from accruing less physiological damage over winter. Regardless, food supplementation before hibernation is likely an effective strategy to improve annual fitness in hibernating species.

4.3   Future Directions

The results presented in this thesis highlight several areas of research that could be explored. I found evidence that sex and pre-hibernation body condition account for some of the observed variation in black-tailed prairie dog hibernation expression. However, there is still a great deal of variation in hibernation expression that was not accounted for. Gummer (2005) did not find evidence of sex differences in hibernation expression and suggested that the use of communal hibernation may be a contributing factor. In Chapter 2, however, I presented evidence of sex-specific hibernation expression that contrasts the study conducted by Gummer (2005). If prairie dogs hibernate communally, then they likely hibernate with individuals within their coterie as burrow sharing occurs between individuals within the same coterie (Hoogland, 1995). Investigating whether prairie dogs within the same coterie exhibit similar expressions of hibernation will provide insight into whether prairie dogs hibernate communally and if the sex differences in hibernation expression I found holds when accounting for an individual's coterie. Previously, males and females in species that hibernate communally have been found to exhibit similar hibernation expressions (Ruf & Arnold, 2000). If prairie dogs hibernate communally but still exhibit sex-specific hibernation expression, this would offer new insight into the relationships between sex, hibernation and sociality. Communal hibernation may also dampen any effects of pre-hibernation body condition, and other factors (i.e., ambient

temperature) may have on hibernation expression and torpor use. If communal hibernation reduces energy expenditure via social thermoregulation, individuals in poor condition that hibernate in a large social group may not express torpor to the same extent as individuals in poor condition that hibernate solitarily or in smaller social groups.

My thesis also highlights that when assessing how individuals express hibernation, consideration of factors not related to energy savings is warranted. Edible dormice (*Glis glis*; Bieber et al., 2014), and now prairie dogs have been found to maintain the length of the hibernation season when they have larger energy reserves before hibernation. This suggests that other non-energetic costs and benefits may influence hibernation expression. Predation risk has been suggested as a factor that can affect the duration of an individual's hibernation period (Bieber et al., 2014; Turbill et al., 2011; Constant et al., 2020). As such, it would be interesting to test whether individuals hibernate for different durations according to their perceived predation risk. Prairie dogs in Canada have been found to increase the time they spend vigilant in response to increased perceived predation risk (Crill Matzke et al., 2021 unpublished manuscript) and as such, represent an opportunity to investigate how predation risk influences hibernation. In addition, comparisons of prairie dog predation risk or more specifically predation rates during the active and the hibernation season and compared to other forms of mortality (i.e. natural death) could shed light on how predation risk could influences hibernation expression. As mentioned in Chapter 2, in prairie dogs, sociality may also affect the duration of their hibernation season. Hibernation can limit the time individuals have available to interact and develop social bonds (Michener, 1983a, 1984), which is one of the previous explanations for why prairie dogs were non-hibernating. Prairie dogs are clearly capable of hibernating, but the length of their hibernation season may be constrained by the need to maintain their social bonds. Future work involving intraspecific comparison of individuals that are more social (i.e., more connected to other individuals) relative to those that are less social could shed light on how the degree of sociality influences hibernation expression.

In this thesis, I have demonstrated that increasing pre-hibernation body condition leads to a decreased use of torpor during hibernation while allowing individuals to still emerge in improved post-hibernation body condition. Kusch and Lane (2021a) have demonstrated that females in better condition have an increased number of offspring. Therefore, improving pre-hibernation body condition could increase reproductive success. However, I was unable to investigate this relationship directly. Future studies investigating the relationships between pre-hibernation body condition, hibernation expression, post-hibernation body condition and reproductive success collectively will be required to fully understand the consequences of intraspecific variation on hibernation expression.

The results presented in this thesis provide an account of how sex and body condition can influence hibernation in a social, facultative, fat-storing hibernator. Prairie dogs offer support for the hibernation optimization hypothesis, where individuals limit their use of torpor when they have sufficiently sized pre-hibernation energy reserves. However, prairie dogs also provide a unique opportunity to test how other non-energetic factors may influence hibernation expression (i.e., sociality, predation risk). Sexual variation in the timing of spring energy requirements is an important factor in prairie dogs' hibernation expression, as it not only influences the length of hibernation season but the expression of torpor within. While sex differences in hibernation emergence and duration are similar to other sciurid hibernators, prairie dogs differ in that males are the sex that spends proportionally more time in torpor and that uses torpor bouts that are on average longer and colder. This thesis adds to a growing body of work investigating the factors that influence hibernation expression under natural conditions and, in collection with other studies, demonstrates the diversity of optimally hibernation expressions across species. Investigations of key hypotheses regarding hibernation expression in a variety of hibernators from various geographical locations will collectively demonstrate how individuals use hibernation across a variety of ecological contexts and, potentially, how they will respond to changing environments.
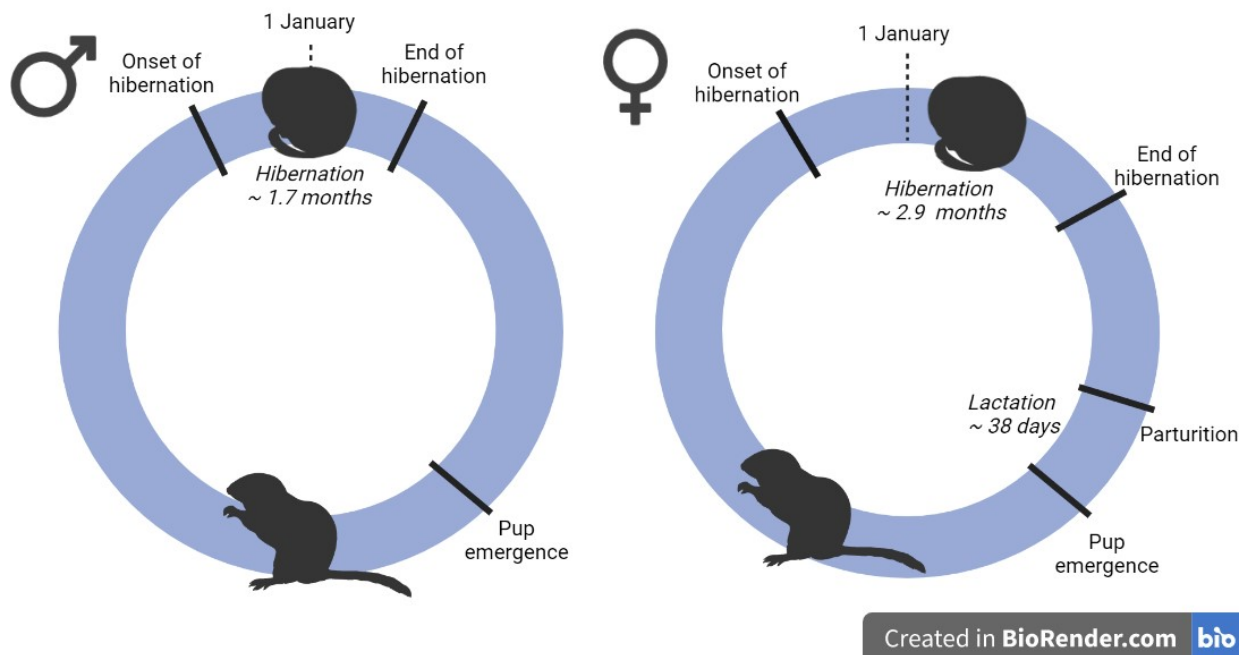
Figure 4.1: Annual cycle of male and female black-tailed prairie dogs at the northern edge of their range. This figure was created in BioRender.com.

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Appendix A

Chapter 2: Pre- and post-hibernation body mass correction analysis

To assess how the rate of body mass change before or after hibernation affected the pre-hibernation and post-hibernation body mass I recorded, I constructed general linear models for each period (i.e. pre-hibernation and post-hibernation). For one individual, I had pre-hibernation body mass measurements from two different hibernation years. Since I did not have enough individuals with repeated measures to use mixed-effects modelling, I excluded the second year of measurement. For this individual, I only had a record of their autumn body mass in their first year of measurement, while in their second year of measurement, I had measured both their pre-and post-hibernation body mass. As such, I applied the rate of body mass change determined from the linear model to their second year of measurement so they could be included when I determined the post-hibernation rate of body mass change. This also allowed me to include them in subsequent analyses regarding over-winter change in body condition.

I constructed the model for pre-hibernation body mass with the number of days measured before hibernation, sex, hibernation year as a fixed effect, and an interaction term between hibernation year and the number of days measured before hibernation. I included an interaction term as weather conditions and vegetation availability before hibernation could vary from year to year and thus affect how an individual's body mass may change in the days prior to hibernation. Given that I included an interaction term between hibernation year and the number of days measured before hibernation, the model produced year-specific coefficients which estimate the effect of the number of days measured before hibernation for each hibernation year. Table A.1 summarizes model estimates, standard error, test statistics, and significance values for this model. Using the estimates for the number of days measured before hibernation presented in Table A.1, I determined the rate of change in body mass to be 3.198g/day, 11.716g/day, -1.928g/day in 2017, 2019 and 2020, respectively. I only corrected an individual's body mass to approximate what it would have been 15 days before hibernation. I chose 15 days as this was the fewest number of days I measured an individual prior to hibernation. Additionally, by correcting each individual's body mass to 15 days before hibernation, I still accounted for any body mass differences that may have occurred as a result of differences in the number of days I measured an individual before hibernation. For each individual, I determined the number of days I needed to correct for, multiplied that

number by the estimate that corresponded to each year, and then subtracted or added that value from the body mass I recorded. Summary statistics for the autumn body mass, the correction I applied, and corrected body mass are presented in Table A.2.

To determine how the number of days measured after hibernation influenced the post-hibernation body mass I recorded, I constructed a linear model with the number of days after hibernation, sex, hibernation year, and pre-hibernation body mass as fixed effects. In the model, I also included interaction terms between sex and the number of days measured after hibernation, sex and pre-hibernation body mass, and hibernation year and pre-hibernation body mass. As such, the linear regression produced sex-specific coefficients to estimate the effect of the number of days captured after hibernation for each sex. Table A.3 summarizes the model estimates, standard error, test statistics, and significance values for the regression. Using the estimates for the number of days measured after hibernation, I determined the rate of body mass change for females and males to be 2.48633g/day and 0.55686g/day, respectively. Using the sex-specific coefficients, I corrected an individual's body mass to approximate what it would have been three days after hibernation. I chose three days as this was the closest to hibernation emergence that I measured a prairie dog. For each individual, I determined the number of days I needed to correct for, multiplied that number by 2.48633 and 0.55686, and then subtracted the resulting value from the body mass I recorded depending on the sex of the individual. Summary statistics for the spring body mass, the correction I applied, and corrected body mass are presented in Table A.4.

Table A.1: Model estimates, standard error, test statistics, and significance values for the general linear model predicting autumn body mass based on the number of days a prairie dog was measured before the onset of hibernation. Coefficient estimates for Days measured before hibernation, Days measured before hibernation: Hibernation year (2019) and Days measured before hibernation: Hibernation year (2020) were used to determine the correction to apply to a prairie dogs body mass measured in 2017, 2019 and 2020 to estimate their pre-hibernation body mass. The estimate Days measured before hibernation represents the coefficient for 2017, the estimate for Days measured before hibernation: Hibernation year (2019) represents the difference between the coefficient for 2017 and 2019 and the estimate for Days measured before hibernation: Hibernation year (2020) represent the differences between coefficients from 2017 and 2020.

Predictor variable	Estimate	Std. Error	t-value	p-value
Intercept	921.23	195.46	4.71	<0.001
Days measured before hibernation	3.20	9.22	0.35	0.73
Sex (Male)	229.55	59.24	3.88	<0.001
Hibernation year (2019)	39.22	574.28	0.07	0.95
Hibernation year (2020)	298.22	226.05	1.32	0.20
Days measured before hibernation: Hibernation year (2019)	8.52	17.60	0.48	0.63
Days measured before hibernation: Hibernation year (2020)	-5.13	9.80	-0.52	0.61

Table A.2: Summary of autumn body mass and corrections applied to estimate pre-hibernation body mass black-tailed prairie dogs. Summary statistics are presented as the mean $\pm$ SEM for each sex in each year. The number of days corrected for reflects the number of days accounted for to estimate an individual's body mass 15 days prior to hibernation. The number of days corrected for was multiplied by the estimated year-specific estimates in Table A.1 to determine the correction applied. Pre-hibernation body mass reflects the body mass after applying the correction to autumn body mass.

Sex	Hibernation year	n	Autumn body mass (g)	Number of days corrected for	Correction applied (g)	Pre-hibernation body mass (g)
F	2017	5	974 $\pm$ 31	4.8 $\pm$ 1.5	-15.4 $\pm$ 4.9	959 $\pm$ 32
M	2017	7	1231 $\pm$ 55	7.9 $\pm$ 1.8	-25.1 $\pm$ 5.6	1206 $\pm$ 55
F	2019	2	1410 $\pm$ 30	21.0 $\pm$ 2.0	-246.0 $\pm$ 23.4	1164 $\pm$ 53
M	2019	5	1596 $\pm$ 55	20.6 $\pm$ 1.8	-241.3 $\pm$ 20.8	1355 $\pm$ 49
F	2020	7	1171 $\pm$ 53	9.7 $\pm$ 4.0	18.7 $\pm$ 7.7	1190 $\pm$ 52
M	2020	2	1250 $\pm$ 115	16.0 $\pm$ 14.0	30.8 $\pm$ 27.0	1281 $\pm$ 142

Table A.3: Model estimates, standard error, test statistics, and significance values for a general linear model predicting spring body mass based on the number of days a prairie dog was measured after hibernation emergence. Estimates for Days measured after hibernation and Days measured after hibernation: Sex (Male) were used to determine the correction to apply to a male's and female's spring body mass to estimate their post-hibernation body mass. The estimate for Days measured after hibernation represented the coefficient for females. The estimate for Days measured after hibernation: Sex (Male) represented the difference between the coefficient for females and males.

Predictor variable	Estimate	Std. Error	t-value	p-value
Intercept	123.12	222.17	0.55	0.59
Days measured after hibernation	2.49	1.54	1.62	0.13
Sex (Male)	-72.47	265.55	-0.27	0.79
Pre-hibernation body mass	0.57	0.20	2.80	0.01
Hibernation year (2019)	487.91	455.60	1.07	0.30
Hibernation year (2020)	226.20	255.81	0.88	0.39
Days measured after hibernation: Sex (Male)	-1.93	2.08	-0.93	0.37
Pre-hibernation body mass: Sex (Male)	0.07	0.21	0.33	0.74
Pre-hibernation body mass: Hibernation year (2019)	-0.38	0.36	-1.05	0.31
Pre-hibernation body mass: Hibernation year (2020)	-0.16	0.23	-0.71	0.49

Table A.4: Summary of spring body mass and corrections applied to estimate post-hibernation body mass for black-tailed prairie dogs. Summary statistics are presented as the mean $\pm$ SEM for each sex in each year. The number of days corrected reflects the number of days to estimate an individual's body mass three days post-hibernation. The number of days corrected was multiplied by the estimated sex-specific estimates in Table A.3 to determine the correction applied. Post-hibernation body mass reflects the body mass after applying the correction to autumn body mass.

Sex	Hibernation year	n	Spring body mass (g)	Number of days corrected for	Correction applied (g)	Post-hibernation body mass (g)
F	2017	5	738 $\pm$ 24	26.0 $\pm$ 7.1	-64.6 $\pm$ 17.6	673 $\pm$ 31
M	2017	5	824 $\pm$ 39	47.8 $\pm$ 3.8	-26.6 $\pm$ 2.1	797 $\pm$ 39
F	2019	2	845 $\pm$ 75	8.0 $\pm$ 1.0	-19.9 $\pm$ 2.5	825 $\pm$ 73
M	2019	3	915 $\pm$ 26	45.3 $\pm$ 20.2	-25.2 $\pm$ 11.3	890 $\pm$ 19
F	2020	7	889 $\pm$ 28	17.1 $\pm$ 4.0	-42.6 $\pm$ 9.9	846 $\pm$ 23
M	2020	2	893 $\pm$ 123	32.5 $\pm$ 4.5	-18.1 $\pm$ 2.5	874 $\pm$ 120

Table A.5: Summary of Type II Analysis of Variances (ANOVAs) of the effect of sex and hibernation year on hibernation traits in black-tailed prairie dogs. Type II ANOVA statistics are based on general linear models and general least squares models were applicable. A generalized linear model was used for number of torpor bouts. Adjusted p-values were corrected for false discovery rate using the Benjamini-Hochberg procedure.

Response variable	Predictor variable	df	F-value	p-value	Adjusted p-value
Duration of hibernation (d)	Sex	1	19.90	<0.001	<0.001
	Hibernation year	2	0.70	0.50	0.67
Proportion of hibernation spent torpid (%)	Sex	1	5.67	0.03	0.04
	Hibernation year	2	1.60	0.22	0.35
Mean torpor bout duration (h)*	Sex	1	5.68	0.03	0.04
	Hibernation year	2	3.61	0.04	0.10
Mean torpor bout depth (°C)*	Sex	1	6.78	0.02	0.03
	Hibernation year	2	1.54	0.24	0.35
Minimum T_b(°C)	Sex	1	0.002	0.96	0.96
	Hibernation year	2	4.05	0.03	0.10
Mean arousal bout duration (h)*	Sex	1	0.13	0.72	0.86
	Hibernation year	2	0.59	0.56	0.67
Mean T_b during arousal bouts (°C)*	Sex	1	0.02	0.89	0.96
	Hibernation year	2	1.71	0.20	0.35
Total time in torpor (d)	Sex	1	15.49	<0.001	0.002
	Hibernation year	2	0.29	0.75	0.82
Total time in arousal (d)	Sex	1	27.85	<0.001	<0.001
	Hibernation year	2	3.83	0.04	0.10
Onset of hibernation (day of year)	Sex	1	1.17	0.29	0.39
	Hibernation year	2	3.95	0.03	0.10
Emergence from hibernation (day of year)	Sex	1	20.87	<0.001	<0.001
	Hibernation year	2	0.17	0.85	0.85

Response variable	Predictor variable	df	χ^2	p-value	Adjusted p-value
Number of torpor bouts**	Sex	1	33.76	<0.001	<0.001
	Hibernation year	2	7.34	0.03	0.10

* Based on mean value for each individual.

**Generalized linear model with a Poisson distribution due to response variable being count data.

Appendix B

Chapter 3: Pre- and post-hibernation body mass correction analysis

To assess how the rate of body mass change before or after hibernation affected the pre-hibernation and post-hibernation body mass measurements, I constructed general linear models for each period (i.e., pre-hibernation and post-hibernation). For two individuals, I had pre-hibernation body mass measurements from two different hibernation years. Since I did not have enough individuals with repeated measures to use mixed-effects modelling, I excluded the second year of measurement for both individuals. For one of both individuals, I still applied the rate of body mass change determined for the linear model to their autumn body mass so I could include them in the subsequent analyses in Chapter 3. For one individual, I measured post-hibernation body mass in two different years and thus, removed their second year of measurement in the post-hibernation analysis. Similarly, I still applied the rate of body mass change determined for the linear model to their autumn body mass so I could include them in subsequent analyses in Chapter 3.

For the pre-hibernation body mass model, I constructed a general linear model with data from 2020 separately from the other two hibernation years. As mentioned in the main text, food supplementation was only conducted in 2020. However, given the supplemental feeding trial's effect on autumn body mass (fed individuals were heavier than unfed individuals), I needed to account for the effect of feeding status in 2020. In the 2020 hibernation year model, I included the number of days measured before hibernation, sex, and feeding status as fixed effects. In the model with the remaining two hibernation years, I included the number of days measured before hibernation, sex, hibernation year as fixed effects, and an interaction term between the number of days measured before hibernation and hibernation year. I included the interaction term to account for the fact that 2020 would have a year-specific rate of body mass change. Additionally, how an individual's body mass may change in the days leading up to hibernation could vary based on environmental conditions. Tables B.1 and B.2 summarize the model estimates, standard error, test statistics, and significance values from both regressions. Using the estimates for the number of days measured before hibernation presented in Tables B.1 and B.2, I determined the rate of change in body mass to be 3.229g /day, 11.711g/day, -0.343g/day, in 2017, 2019 and 2020, respectively (unrounded estimates). I only corrected an individual's body mass to approximate what it would have been 14 days before hibernation. I chose 14 days as this was the fewest number of days I measured an

individual prior to hibernation. Additionally, by correcting each individual's body mass to 14 days before hibernation, I still accounted for any differences in body mass that may have resulted from differences in the number of days I measured prairie dogs before hibernation. For each individual, I determined the number of days I needed to correct for, multiplied by the estimate that corresponded to the hibernation year the individual was from, and subtracted or added that value from the body mass I recorded. Summary statistics for the autumn body mass, the correction I applied, and corrected body mass are presented in Table B.3.

I constructed a general linear model to determine how the number of days measured after hibernation influenced the post-hibernation body mass I recorded. I first constructed a general linear model with all data from 2020 to assess whether feeding status influenced post-hibernation body mass. I included the number of days measured after hibernation, sex, autumn body mass, and feeding status as fixed effects in the model. I also included an interaction term between feeding status and pre-hibernation body mass, sex and pre-hibernation body mass and sex and number of days measured after hibernation. The interaction between feeding status and pre-hibernation body mass was to account for the influence feeding status had on pre-hibernation body mass. I found no significant interaction between feeding status and pre-hibernation body mass on spring body mass (Table B.4). As a result, I did not include feeding status in the general linear model constructed with data from all hibernation years. The model with data collected from all hibernation years included the number of days measured after hibernation, sex, hibernation year, and pre-hibernation body mass as fixed effects. In the model, I included interaction terms between sex and the number of days measured after hibernation, sex and pre-hibernation body mass, and hibernation year and pre-hibernation body mass. As such, the linear regression produced sex-specific coefficients to estimate the effect of the number of days captured after hibernation for each sex. Table B.5 summarizes the model estimates, standard error, test statistics, and significance values for the regression. Using the estimates for the number of days measured after hibernation, I determined the rate of body mass change for females and males to be 3.34408g/day and -0.09135g/day, respectively (unrounded estimates). Using the sex-specific coefficients, I corrected an individual's body mass to approximate what it would have been three days after hibernation. I chose three days as this was the closest to hibernation emergence that I measured prairie dogs. For each individual, I determined the number of days I needed to correct for. Depending on the sex of the individual, I multiplied that number by 3.34408 and -0.09135 and added or subtracted the resulting value from the body mass I measured. Summary statistics for the spring body mass, the correction I applied, and corrected body mass are presented in Table B.6.

Table B.1: Model estimates, standard error, test statistics, and significance values for the general linear model predicting autumn body mass based on the number of days a prairie dog was measured before the onset of hibernation in 2020. The coefficient estimate for Days measured before hibernation used to determine the correction to apply to a autumn prairie dogs body mass to estimate its pre-hibernation body mass.

Predictor variable	Estimate	Std. Error	t-value	p-value
Intercept	1347.68	53.17	25.35	<0.001
Days measured before hibernation	-0.34	1.80	-0.19	0.85
Sex (Male)	231.13	47.09	4.91	<0.001
Feeding status (Unfed)	-171.61	41.32	-4.15	<0.001

Table B.2: Model estimates, standard error, test statistics, and significance values for the general linear model predicting autumn body mass based on the number of days a prairie dog was measured before the onset of hibernation in 2017 and 2019. Coefficient estimates for Days measured before hibernation and Days measured before hibernation: Hibernation year (2019) were used to determine the correction to apply to a prairie dogs autumn body mass measured in 2017, 2019 to estimate their pre-hibernation body mass. The estimate Days measured before hibernation represents the coefficient for 2017, the estimate for Days measured before hibernation: Hibernation year (2019) represents the difference between the coefficient for 2017 and 2019.

Predictor variable	Estimate	Std. Error	t-value	p-value
Intercept	920.98	186.98	4.93	<0.001
Days measured before hibernation	3.23	8.86	0.36	0.72
Sex (Male)	228.86	60.58	3.78	0.002
Hibernation year (2019)	40.16	549.68	0.07	0.94
Days measured before hibernation: Hibernation year (2019)	8.48	16.86	0.50	0.62

Table B.3: Summary of autumn body mass and corrections applied to estimate pre-hibernation body mass for each black-tailed prairie dog used in my study. Summary statistics are presented as the mean ± SEM for each sex in each year in each feeding status (if applicable). The number of days corrected for reflects the number of days accounted for to estimate an individual's body mass 14 days prior to hibernation.The number of days corrected for was multiplied by the estimated year-specific estimates in Tables B.1 and B.2 to determine the correction applied. Pre-hibernation body mass reflects the body mass after applying the correction to autumn body mass.

Sex	Hibernation year	Feeding status	n	Autumn body mass (g)	Number of days corrected for	Correction applied (g)	Pre-hibernation body mass (g)
F	2017	unfed	5	974 ± 31	5.8 ± 1.5	-18.7 ± 4.9	955 ± 32
M	2017	unfed	7	1231 ± 55	8.9 ± 1.8	-28.6 ± 5.7	1203 ± 55
F	2019	unfed	2	1410 ± 30	22.0 ± 2.0	-257.6 ± 23.4	1152 ± 53
M	2019	unfed	5	1596 ± 55	21.6 ± 1.8	-253.0 ± 20.8	1343 ± 49
F	2020	unfed	7	1171 ± 53	10.7 ± 4.0	3.7 ± 1.4	1175 ± 53
M	2020	unfed	2	1250 ± 115	17.0 ± 14.0	5.8 ± 4.8	1256 ± 120
F	2020	fed	12	1334 ± 19	13.6 ± 3.5	4.7 ± 1.2	1339 ± 19
M	2020	fed	7	1570 ± 27	22.9 ± 4.0	7.8 ± 1.4	1578 ± 27

Table B.4: Model estimates, standard error, test statistics, and significance values for general linear model used to assess whether feeding status influenced the effect pre-hibernation body mass had on spring body mass in 2020.

Predictor variable	Estimate	Std. Error	t-value	p-value
Intercept	447.70	277.43	1.61	0.12
Days measured after hibernation	4.56	1.11	4.12	<0.001
Sex (Male)	-231.47	236.75	-0.98	0.34
Pre-hibernation body mass	0.31	0.21	1.43	0.17
Feeding status (Unfed)	-133.09	288.71	-0.46	0.65
Days measured after hibernation: Sex (Male)	-9.71	3.85	-2.52	0.02
Pre-hibernation body mass: Sex (Male)	0.35	0.21	1.63	0.12
Pre-hibernation body mass: Feeding status (Unfed)	0.11	0.22	0.51	0.62

Table B.5: Model estimates, standard error, test statistics, and significance values for general linear model used to assess whether feeding status influenced the effect pre-hibernation body mass had on spring body mass in all hibernation years.

Predictor variable	Estimate	Std. Error	t-value	p-value
Intercept	-10.58	154.35	-0.07	0.95
Days measured after hibernation	3.34	0.98	3.40	0.002
Sex (Male)	77.16	136.82	0.56	0.58
Pre-hibernation body mass	0.69	0.14	4.77	<0.001
Hibernation year (2019)	383.56	352.41	1.09	0.28
Hibernation year (2020)	300.90	181.25	1.66	0.11
Days measured after hibernation: Sex (Male)	-3.44	1.52	-2.26	0.03
Pre-hibernation body mass: Sex (Male)	-0.03	0.10	-0.33	0.74
Pre-hibernation body mass: Hibernation year (2019)	-0.30	0.29	-1.05	0.30
Pre-hibernation body mass: Hibernation year (2020)	-0.24	0.16	-1.47	0.15

Table B.6: Summary of spring body mass and corrections applied to estimate post-hibernation body mass black-tailed prairie dogs. Summary statistics are presented as the mean $\pm$ SEM for each sex in each year in each feeding status (if applicable). The number of days corrected for reflects the number of days to accounted for to estimate an individual's body mass three days post-hibernation. The number of days corrected for was multiplied by the estimated sex-specific estimates in Table B.5 to determine the correction applied. Post-hibernation body mass reflects the body mass after applying the correction to autumn body mass.

Sex	Hibernation year	Feeding status	n	Spring body mass (g)	Number of days corrected for	Correction applied (g)	Post-hibernation body mass (g)
F	2017	unfed	5	738 $\pm$ 24	26.0 $\pm$ 7.1	-86.9 $\pm$ 23.7	651 $\pm$ 35
M	2017	unfed	5	824 $\pm$ 39	47.8 $\pm$ 3.8	4.4 $\pm$ 0.3	828 $\pm$ 39
F	2019	unfed	2	845 $\pm$ 75	8.0 $\pm$ 1.0	-26.8 $\pm$ 3.3	818 $\pm$ 72
M	2019	unfed	3	915 $\pm$ 26	45.3 $\pm$ 20.2	4.1 $\pm$ 1.8	919 $\pm$ 27
F	2020	unfed	7	889 $\pm$ 28	17.1 $\pm$ 4.0	-57.3 $\pm$ 13.4	831 $\pm$ 21
M	2020	unfed	2	893 $\pm$ 123	32.5 $\pm$ 4.5	3.0 $\pm$ 0.4	895 $\pm$ 123
F	2020	fed	12	968 $\pm$ 24	20.0 $\pm$ 3.4	-66.9 $\pm$ 11.4	901 $\pm$ 16
M	2020	fed	7	1011 $\pm$ 26	41.6 $\pm$ 1.9	3.8 $\pm$ 0.2	1015 $\pm$ 26

APPENDIX C

Table C.1: Model estimates, standard error, test statistics, and significance values for full model assessing whether feeding status influenced the effect pre-hibernation body condition had on the duration of hibernation. Bolded metric indicates the interaction between pre-hibernation body condition and feeding status was not significant (p$\geq$0.05).

	Full model			
Predictor variable	Estimate	Std. Error	t-value	p-value
Intercept	93.20	22.77	4.09	<0.001
Pre-hibernation body condition	-7.46	19.33	-0.39	0.70
Sex (Male)	-12.78	16.17	-0.79	0.44
Feeding.status (Unfed)	-4.94	23.90	-0.21	0.84
Pre-hibernation body condition: Sex (Male)	-16.70	16.01	-1.04	0.31
Pre-hibernation body condition: Feeding status (Unfed)	**9.44**	**23.62**	**0.40**	**0.69**

Table C.2: Model estimates, standard error, test statistics, and significance values for full model assessing whether feeding status influenced the effect pre-hibernation body condition had on the proportion of hibernation spent torpid. Bolded metric indicates the interaction between pre-hibernation body condition and feeding status was not significant (p$\geq$0.05).

	Full model			
Predictor variable	Estimate	Std. Error	t-value	p-value
Intercept	39.45	8.81	4.48	<0.001
Pre-hibernation body condition	15.13	7.85	1.93	0.07
Sex (Male)	13.66	6.33	2.16	0.04
Feeding.status (Unfed)	22.81	7.55	3.02	0.006
Pre-hibernation body condition: Sex (Male)	1.15	6.16	0.19	0.85
Pre-hibernation body condition: Feeding status (Unfed)	**-14.80**	**9.41**	**-1.57**	**0.13**

Table C.3: Model estimates, standard error, test statistics, and significance values for full model assessing whether feeding status influenced the effect pre-hibernation body condition had on the number of torpor bouts. Bolded metric indicates the interaction between pre-hibernation body condition and feeding status was not significant (p≥0.05).

	Full model			
Predictor variable	Estimate	Std. Error	t-value	p-value
Intercept	2.75	0.33	8.36	<0.001
Pre-hibernation body condition	-0.13	0.28	-0.48	0.63
Sex (Male)	-0.29	0.23	-1.31	0.19
Feeding.status (Unfed)	0.05	0.35	0.16	0.88
Pre-hibernation body condition: Sex (Male)	-0.36	0.24	-1.47	0.14
Pre-hibernation body condition: Feeding status (Unfed)	**0.15**	**0.33**	**0.45**	**0.65**

Table C.4: Model estimates, standard error, test statistics, and significance values for full model assessing whether feeding status influenced the effect pre-hibernation body condition had on the mean torpor bout duration. Bolded metric indicates the interaction between pre-hibernation body condition and feeding status was not significant (p≥0.05).

	Full model			
Predictor variable	Estimate	Std. Error	t-value	p-value
Intercept	88.24	27.82	3.17	0.004
Pre-hibernation body condition	3.71	23.63	0.16	0.88
Sex (Male)	26.13	19.76	1.32	0.20
Feeding.status (Unfed)	-7.32	29.21	-0.25	0.80
Pre-hibernation body condition: Sex (Male)	14.55	19.56	0.74	0.46
Pre-hibernation body condition: Feeding status (Unfed)	**-6.37**	**28.87**	**-0.22**	**0.83**

Table C.5: Model estimates, standard error, test statistics, and significance values for full model assessing whether feeding status influenced the effect pre-hibernation body condition had on the mean torpor bout depth. Bolded metric indicates the interaction between pre-hibernation body condition and feeding status was not significant (p≥0.05).

	Full model			
Predictor variable	Estimate	Std. Error	t-value	p-value
Intercept	19.25	2.95	6.53	<0.001
Pre-hibernation body condition	-0.43	2.56	-0.17	0.87
Sex (Male)	-2.08	1.89	-1.10	0.28
Feeding.status (Unfed)	-0.18	2.83	-0.06	0.95
Pre-hibernation body condition: Sex (Male)	-2.64	1.85	-1.42	0.17
Pre-hibernation body condition: Feeding status (Unfed)	**-0.04**	**3.14**	**-0.01**	**0.99**

Table C.6: Model estimates, standard error, test statistics, and significance values for full model assessing whether feeding status influenced the effect pre-hibernation body condition had on the minimum body temperature (T_{b}) during hibernation. Bolded metric indicates the interaction between pre-hibernation body condition and feeding status was not significant ($p \geq 0.05$).

Predictor variable	Full model			
	Estimate	Std. Error	t-value	p-value
Intercept	10.04	0.79	12.78	<0.001
Pre-hibernation body condition	1.25	0.67	1.87	0.08
Sex (Male)	-0.35	0.56	-0.63	0.53
Feeding.status (Unfed)	0.47	0.83	0.57	0.57
Pre-hibernation body condition: Sex (Male)	0.20	0.55	0.36	0.72
Pre-hibernation body condition: Feeding status (Unfed)	**-0.90**	**0.82**	**-1.10**	**0.28**

Table C.7: Model estimates, standard error, test statistics, and significance values for full model assessing whether feeding status influenced the effect pre-hibernation body condition had on the mean arousal bout duration. Bolded metric indicates the interaction between pre-hibernation body condition and feeding status was not significant ($p \geq 0.05$).

Predictor variable	Full model			
	Estimate	Std. Error	t-value	p-value
Intercept	85.27	23.06	3.70	0.001
Pre-hibernation body condition	-12.47	19.59	-0.64	0.53
Sex (Male)	-12.29	16.38	-0.75	0.46
Feeding.status (Unfed)	-31.04	24.22	-1.28	0.21
Pre-hibernation body condition: Sex (Male)	4.01	16.22	0.25	0.81
Pre-hibernation body condition: Feeding status (Unfed)	**14.54**	**23.93**	**0.61**	**0.55**

Table C.8: Model estimates, standard error, test statistics, and significance values for full model assessing whether feeding status influenced the effect of pre-hibernation body condition had on the hibernation onset. Bolded metric indicates the interaction between pre-hibernation body condition and feeding status was not significant ($p \geq 0.05$).

Predictor variable	Full model			
	Estimate	Std. Error	t-value	p-value
Intercept	325.53	14.04	23.19	<0.001
Pre-hibernation body condition	-1.02	11.92	-0.09	0.93
Sex (Male)	-1.61	9.97	-0.16	0.87
Feeding.status (Unfed)	-0.70	14.74	-0.05	0.96
Pre-hibernation body condition: Sex (Male)	11.11	9.87	1.13	0.27
Pre-hibernation body condition: Feeding status (Unfed)	**-3.31**	**14.56**	**-0.23**	**0.82**

Table C.9: Model estimates, standard error, test statistics, and significance values for full model assessing whether feeding status influenced the effect pre-hibernation body condition had on the hibernation emergence. Bolded metric indicates the interaction between pre-hibernation body condition and feeding status was not significant ($p \geq 0.05$).

	Full model			
Predictor variable	Estimate	Std. Error	*t*-value	*p*-value
Intercept	54.87	9.90	5.54	<0.001
Pre-hibernation body condition	-9.20	8.67	-1.06	0.30
Sex (Male)	-15.07	6.53	-2.31	0.03
Feeding.status (Unfed)	-6.95	9.27	-0.75	0.46
Pre-hibernation body condition: Sex (Male)	-5.52	6.38	-0.87	0.40
Pre-hibernation body condition: Feeding status (Unfed)	**4.80**	**10.58**	**0.45**	**0.65**

Table C.10: Model estimates, standard error, test statistics, and significance values for full model assessing whether feeding status influenced the effect pre-hibernation body condition had on the *HI* over a common hibernation period (10 Nov. to 19 Mar. (18 Mar. in leap years)). Bolded metric indicates the interaction between pre-hibernation body condition and feeding status was not significant ($p \geq 0.05$).

	Full model			
Predictor variable	Estimate	Std. Error	*t*-value	*p*-value
Intercept	12.66	1.38	9.16	<0.001
Pre-hibernation body condition	0.08	1.17	0.07	0.94
Sex (Male)	1.03	0.98	1.05	0.30
Feeding.status (Unfed)	0.88	1.45	0.61	0.55
Pre-hibernation body condition: Sex (Male)	-1.80	0.97	-1.85	0.08
Pre-hibernation body condition: Feeding status (Unfed)	**0.32**	**1.43**	**0.22**	**0.83**

Table C.11: Model estimates, standard error, test statistics, and significance values for full model assessing whether feeding status influenced the effect pre-hibernation body condition had on the *HI* over an individual's hibernation period. Bolded metric indicates the interaction between pre-hibernation body condition and feeding status was not significant ($p \geq 0.05$).

	Full model			
Predictor variable	Estimate	Std. Error	*t*-value	*p*-value
Intercept	9.04	0.99	9.14	<0.001
Pre-hibernation body condition	0.07	0.84	0.08	0.93
Sex (Male)	0.78	0.70	1.12	0.28
Feeding.status (Unfed)	0.60	1.04	0.58	0.57
Pre-hibernation body condition: Sex (Male)	-1.32	0.70	-1.89	0.07
Pre-hibernation body condition: Feeding status (Unfed)	**0.39**	**1.03**	**0.38**	**0.71**

Table C.12: Model estimates, standard error, test statistics, and significance values for full model assessing whether feeding status influenced the effect pre-hibernation body condition had on change in body condition experienced over winter. Bolded metric indicates the interaction between pre-hibernation body condition and feeding status was not significant (p≥0.05).

	Full model			
Predictor variable	Estimate	Std. Error	t-value	p-value
Intercept	-1.47	0.19	-7.56	<0.001
Pre-hibernation body condition	-0.40	0.17	-2.42	0.02
Sex (Male)	-0.07	0.14	-0.47	0.64
Feeding.status (Unfed)	0.31	0.20	1.51	0.14
Pre-hibernation body condition: Sex (Male)	0.09	0.14	0.64	0.53
Pre-hibernation body condition: Feeding status (Unfed)	**-0.41**	**0.20**	**-2.05**	**0.053**

Table C.13: Model estimates, standard error, test statistics, and significance values for full model assessing whether feeding status influenced the effect pre-body condition had on the rate of change in body condition over winter. Bolded metric indicates the interaction between pre-hibernation body condition and feeding status was not significant (p≥0.05).

	Full model			
Predictor variable	Estimate	Std. Error	t-value	p-value
Intercept	-0.015	0.009	-1.72	0.10
Pre-hibernation body condition	-0.008	0.007	-1.14	0.27
Sex (Male)	-0.002	0.006	-0.30	0.77
Feeding.status (Unfed)	0.00002	0.009	0.002	1.00
Pre-hibernation body condition: Sex (Male)	-0.01	0.006	-1.67	0.11
Pre-hibernation body condition: Feeding status (Unfed)	**0.0007**	**0.009**	**0.08**	**0.94**

Table C.14: Model estimates, standard error, test statistics, and significance values for full model assessing whether feeding status influenced the effect pre-body condition had on the post-hibernation body condition. Bolded metric indicates the interaction between pre-hibernation body condition and feeding status was not significant (p≥0.05).

	Full model			
Predictor variable	Estimate	Std. Error	t-value	p-value
Intercept	-1.47	0.19	-7.56	<0.001
Pre-hibernation body condition	0.60	0.17	3.63	0.001
Sex (Male)	-0.07	0.14	-0.47	0.64
Feeding.status (Unfed)	0.31	0.20	1.51	0.14
Pre-hibernation body condition: Sex (Male)	0.09	0.14	0.64	0.53
Pre-hibernation body condition: Feeding status (Unfed)	**-0.41**	**0.20**	**-2.05**	**0.053**

APPENDIX D

Table D.1: Model estimates, standard error, test statistics, and significance values for full and final models predicting the duration of hibernation based on pre-hibernation body condition in black-tailed prairie dogs. Not fit indicates effects and/or interactions that were not retained in the final model. Bolded metrics indicate significance (p<0.05).

	Full model				Final model			
Predictor variable	Estimate	Std. Error	*t*-value	*p-value*	Estimate	Std. Error	*t*-value	*p*-value
Intercept	**80.92**	**7.41**	**10.92**	**<0.001**	**86.71**	**3.41**	**25.44**	**<0.001**
Pre-hibernation body condition	1.38	10.84	0.13	0.90		Not fit		
Sex (Male)	**-33.12**	**5.81**	**-5.70**	**<0.001**	**-34.09**	**5.11**	**-6.67**	**<0.001**
Hibernation year (2019)	7.06	14.71	0.48	0.63		Not fit		
Hibernation year (2020)	7.03	7.66	0.92	0.36		Not fit		
Pre-hibernation body condition: Sex (Male)	-4.81	8.16	-0.59	0.56		Not fit		
Pre-hibernation body condition: Hibernation year (2019)	-0.99	38.05	-0.03	0.98		Not fit		
Pre-hibernation body condition: Hibernation year (2020)	0.98	10.75	0.09	0.93		Not fit		

Table D.2: Model estimates, standard error, test statistics, and significance values for full and final models predicting the proportion of hibernation spent torpid based on pre-hibernation body condition in black-tailed prairie dogs. Not fit indicates effects and/or interactions that were not retained in the final model. Bolded metrics indicate significance (p<0.05).

	Full model				Final model			
Predictor variable	Estimate	Std. Error	*t*-value	*p-value*	Estimate	Std. Error	*t*-value	*p*-value
Intercept	**62.77**	**3.26**	**19.24**	**<0.001**	**60.77**	**1.59**	**38.18**	**<0.001**
Pre-hibernation body condition	**-10.31**	**4.77**	**-2.16**	**0.04**	**-4.73**	**1.69**	**-2.80**	**0.008**
Sex (Male)	**11.44**	**2.56**	**4.48**	**<0.001**	**12.61**	**2.39**	**5.28**	**<0.001**
Hibernation year (2019)	-0.15	6.48	-0.02	0.98		Not fit		
Hibernation year (2020)	-3.73	3.37	-1.11	0.28		Not fit		
Pre-hibernation body condition: Sex (Male)	5.24	3.59	1.46	0.15		Not fit		
Pre-hibernation body condition: Hibernation year (2019)	4.99	16.75	0.30	0.77		Not fit		
Pre-hibernation body condition: Hibernation year (2020)	6.10	4.73	1.29	0.21		Not fit		

Table D.3: Model estimates, standard error, test statistics, and significance values for full and final models predicting number of torpor bouts based on pre-hibernation body condition in black-tailed prairie dogs. Not fit indicates effects and/or interactions that were not retained in the final model. Bolded metrics indicate significance (p<0.05).

Predictor variable	Full model				Final model			
	Estimate	Std. Error	*t*-value	*p-value*	Estimate	Std. Error	*t*-value	*p*-value
Intercept	**2.61**	**0.14**	**19.20**	**<0.001**	**2.59**	**0.10**	**24.99**	**<0.001**
Pre-hibernation body condition	0.06	0.20	0.31	0.76		Not fit		
Sex (Male)	**-0.77**	**0.11**	**-7.24**	**<0.001**	**-0.77**	**0.10**	**-7.43**	**<0.001**
Hibernation year (2019)	0.35	0.27	1.28	0.20	**0.38**	**0.15**	**2.59**	**0.01**
Hibernation year (2020)	0.11	0.14	0.76	0.45	0.12	0.11	1.05	0.29
Pre-hibernation body condition: Sex (Male)	-0.03	0.15	-0.19	0.85		Not fit		
Pre-hibernation body condition: Hibernation year (2019)	-0.05	0.70	-0.08	0.94		Not fit		
Pre-hibernation body condition: Hibernation year (2020)	-0.11	0.21	-0.55	0.58		Not fit		

Table D.4: Model estimates, standard error, test statistics, and significance values for full and final models predicting mean torpor bout duration based on pre-hibernation body condition in black-tailed prairie dogs. Not fit indicates effects and/or interactions that were not retained in the final model. Bolded metrics indicate significance (p<0.05).

Predictor variable	Full model				Final model			
	Estimate	Std. Error	*t*-value	*p-value*	Estimate	Std. Error	*t*-value	*p*-value
Intercept	**96.03**	**11.41**	**8.42**	**<0.001**	**90.21**	**5.69**	**15.86**	**<0.001**
Pre-hibernation body condition	-26.05	16.69	-1.56	0.13		Not fit		
Sex (Male)	**39.76**	**8.94**	**4.45**	**<0.001**	**39.25**	**8.53**	**4.60**	**<0.001**
Hibernation year (2019)	-23.77	22.64	-1.05	0.30		Not fit		
Hibernation year (2020)	-9.94	11.79	-0.84	0.40		Not fit		
Pre-hibernation body condition: Sex (Male)	11.68	12.57	0.93	0.36		Not fit		
Pre-hibernation body condition: Hibernation year (2019)	12.89	58.58	0.22	0.83		Not fit		
Pre-hibernation body condition: Hibernation year (2020)	26.15	16.55	1.58	0.12		Not fit		

Table D.5: Model estimates, standard error, test statistics, and significance values for full and final models predicting mean torpor bout depth based on pre-hibernation body condition in black-tailed prairie dogs. Not fit indicates effects and/or interactions that were not retained in the final model. Bolded metrics indicate significance (p<0.05).

Predictor variable	Full model				Final model			
	Estimate	Std. Error	*t*-value	*p-value*	Estimate	Std. Error	*t*-value	*p*-value
Intercept	**20.43**	**0.88**	**23.10**	**<0.001**	**18.68**	**0.64**	**29.25**	**<0.001**
Pre-hibernation body condition	**2.99**	**1.26**	**2.38**	**0.02**		Not fit		
Sex (Male)	**-4.24**	**0.76**	**-5.57**	**<0.001**	**-4.01**	**0.74**	**-5.42**	**<0.001**
Hibernation year (2019)	**-2.52**	**1.19**	**-2.12**	**0.04**		Not fit		
Hibernation year (2020)	**-1.86**	**0.75**	**-2.49**	**0.02**		Not fit		
Pre-hibernation body condition: Sex (Male)	-1.59	1.07	-1.48	0.15		Not fit		
Pre-hibernation body condition: Hibernation year (2019)	-3.07	3.03	-1.01	0.32		Not fit		
Pre-hibernation body condition: Hibernation year (2020)	**-2.10**	**0.99**	**-2.11**	**0.04**		Not fit		

Table D.6: Model estimates, standard error, test statistics, and significance values for full and final models predicting minimum T_b based on pre-hibernation body condition in black-tailed prairie dogs. Not fit indicates effects and/or interactions that were not retained in the final model. Bolded metrics indicate significance (p<0.05).

Predictor variable	Full model				Final model			
	Estimate	Std. Error	t-value	p-value	Estimate	Std. Error	t-value	p-value
Intercept	**11.75**	**0.29**	**40.88**	**<0.001**	**11.82**	**0.21**	**57.56**	**<0.001**
Pre-hibernation body condition	0.35	0.42	0.84	0.41	**0.61**	**0.16**	**3.78**	**<0.001**
Sex (Male)	-0.05	0.23	-0.24	0.81		Not fit		
Hibernation year (2019)	-0.58	0.57	-1.02	0.31	-0.35	0.32	-1.09	0.28
Hibernation year (2020)	**-0.75**	**0.30**	**-2.52**	**0.02**	**-0.81**	**0.26**	**-3.13**	**0.003**
Pre-hibernation body condition: Sex (Male)	-0.03	0.32	-0.10	0.92		Not fit		
Pre-hibernation body condition: Hibernation year (2019)	-0.84	1.48	-0.57	0.57		Not fit		
Pre-hibernation body condition: Hibernation year (2020)	0.37	0.42	0.90	0.37		Not fit		

Table D.7: Model estimates, standard error, test statistics, and significance values for full and final models predicting mean arousal bout duration based on pre-hibernation body condition in black-tailed prairie dogs. Not fit indicates effects and/or interactions that were not retained in the final model. Bolded metrics indicate significance (p<0.05).

Predictor variable	Full model				Final model			
	Estimate	Std. Error	t-value	p-value	Estimate	Std. Error	t-value	p-value
Intercept	**58.03**	**7.33**	**7.92**	**<0.001**	**58.83**	**2.63**	**22.35**	**<0.001**
Pre-hibernation body condition	15.85	10.72	1.48	0.15	**10.66**	**3.75**	**2.84**	**0.007**
Sex (Male)	-1.32	5.74	-0.23	0.82		Not fit		
Hibernation year (2019)	-12.34	14.54	-0.85	0.40		Not fit		
Hibernation year (2020)	5.89	7.57	0.78	0.44		Not fit		
Pre-hibernation body condition: Sex (Male)	-10.26	8.70	-1.27	0.21		Not fit		
Pre-hibernation body condition: Hibernation year (2019)	-12.84	37.62	-0.34	0.74		Not fit		
Pre-hibernation body condition: Hibernation year (2020)	-4.61	10.63	-0.43	0.67		Not fit		

Table D.8: Model estimates, standard error, test statistics, and significance values for full and final models predicting hibernation onset based on pre-hibernation body condition in black-tailed prairie dogs. Not fit indicates effects and/or interactions that were not retained in the final model. Bolded metrics indicate significance (p<0.05).

Predictor variable	Full model				Final model			
	Estimate	Std. Error	t-value	p-value	Estimate	Std. Error	t-value	p-value
Intercept	**334.83**	**2.39**	**139.96**	**<0.001**	**338.08**	**1.19**	**284.11**	**<0.001**
Pre-hibernation body condition	-3.07	3.78	-0.81	0.42		Not fit		
Sex (Male)	4.48	2.27	1.98	0.055		Not fit		
Hibernation year (2019)	-5.62	3.26	-1.72	0.09	-2.08	1.77	-1.18	0.25
Hibernation year (2020)	**-8.58**	**3.04**	**-2.82**	**0.008**	**-10.54**	**2.65**	**-3.98**	**<0.001**
Pre-hibernation body condition: Sex (Male)	3.39	3.76	0.90	0.37		Not fit		
Pre-hibernation body condition: Hibernation year (2019)	-10.85	8.22	-1.32	0.19		Not fit		
Pre-hibernation body condition: Hibernation year (2020)	1.05	4.18	0.25	0.80		Not fit		

Table D.9: Model estimates, standard error, test statistics, and significance values for full and final models predicting hibernation emergence based on pre-hibernation body condition in black-tailed prairie dogs. Not fit indicates effects and/or interactions that were not retained in the final model. Bolded metrics indicate significance (p<0.05).

Predictor variable	Full model				Final model			
	Estimate	Std. Error	*t*-value	*p-value*	Estimate	Std. Error	*t*-value	*p*-value
Intercept	**45.57**	**4.62**	**9.87**	**<0.001**	**47.28**	**2.07**	**22.80**	**<0.001**
Pre-hibernation body condition	-3.14	6.66	-0.47	0.64		Not fit		
Sex (Male)	**-22.50**	**3.68**	**-6.11**	**<0.001**	**-22.85**	**3.28**	**-6.97**	**<0.001**
Hibernation year (2019)	1.73	20.08	0.09	0.93		Not fit		
Hibernation year (2020)	1.59	4.69	0.34	0.74		Not fit		
Pre-hibernation body condition: Sex (Male)	-0.85	4.90	-0.17	0.86		Not fit		
Pre-hibernation body condition: Hibernation year (2019)	-12.74	55.19	-0.23	0.82		Not fit		
Pre-hibernation body condition: Hibernation year (2020)	2.80	6.58	0.43	0.67		Not fit		

Table D.10: Model estimates, standard error, test statistics, and significance values for full and final models predicting *HI* for a common hibernation period (10 Nov. – 19 Mar.; 18 Mar. in leap years) based on pre-hibernation body condition in black-tailed prairie dogs. Not fit indicates effects and/or interactions that were not retained in the final model. Bolded metrics indicate significance (p<0.05).

Predictor variable	Full model				Final model			
	Estimate	Std. Error	*t*-value	*p-value*	Estimate	Std. Error	*t*-value	*p*-value
Intercept	**12.22**	**0.75**	**16.32**	**<0.001**	**13.33**	**0.36**	**36.57**	**<0.001**
Pre-hibernation body condition	-1.94	1.09	-1.77	0.08		Not fit		
Sex (Male)	**-1.86**	**0.59**	**-3.17**	**0.003**	**-1.94**	**0.55**	**-3.55**	**<0.001**
Hibernation year (2019)	2.28	1.49	1.53	0.13		Not fit		
Hibernation year (2020)	1.20	0.77	1.55	0.13		Not fit		
Pre-hibernation body condition: Sex (Male)	0.92	0.82	1.11	0.27		Not fit		
Pre-hibernation body condition: Hibernation year (2019)	2.93	3.84	0.76	0.45		Not fit		
Pre-hibernation body condition: Hibernation year (2020)	1.11	1.09	1.02	0.31		Not fit		

Table D.11: Model estimates, standard error, test statistics, and significance values for full and final models predicting *HI* for an individual's hibernation period based on pre-hibernation body condition in black-tailed prairie dogs. Not fit indicates effects and/or interactions that were not retained in the final model. Bolded metrics indicate significance (p<0.05).

Predictor variable	Full model				Final model			
	Estimate	Std. Error	*t*-value	*p-value*	Estimate	Std. Error	*t*-value	*p*-value
Intercept	**16.43**	**0.54**	**30.67**	**<0.001**	**16.61**	**0.39**	**43.11**	**<0.001**
Pre-hibernation body condition	-0.93	0.78	-1.19	0.24		Not fit		
Sex (Male)	-0.37	0.42	-0.88	0.39		Not fit		
Hibernation year (2019)	**3.06**	**1.06**	**2.88**	**0.007**	**1.51**	**0.63**	**2.38**	**0.02**
Hibernation year (2020)	**-7.07**	**0.55**	**-12.77**	**<0.001**	**-7.49**	**0.47**	**-16.08**	**<0.001**
Pre-hibernation body condition: Sex (Male)	-0.15	0.59	-0.25	0.80		Not fit		
Pre-hibernation body condition: Hibernation year (2019)	4.63	2.75	1.69	0.10		Not fit		
Pre-hibernation body condition: Hibernation year (2020)	0.50	0.78	0.67	0.51		Not fit		

Table D.12: Model estimates, standard error, test statistics, and significance values for full and final models predicting change in body condition over winter based on pre-hibernation body condition in black-tailed prairie dogs. Not fit indicates effects and/or interactions that were not retained in the final model. Bolded metrics indicate significance (p<0.05). Note: The model assessing the influence of pre-hibernation body condition on change in body condition over winter has a caveat in that pre-hibernation body condition appears a independent variable and is incorporated into the dependent variable in the model (i.e. Change in body condition = Post-hibernation body condition - Pre-hibernation body condition) in the model.

	Full model				Final model			
Predictor variable	Estimate	Std. Error	t-value	p-value	Estimate	Std. Error	t-value	p-value
Intercept	**-1.18**	**0.13**	**-9.31**	**<0.001**	**-1.10**	**0.06**	**-17.01**	**<0.001**
Pre-hibernation body condition	-0.18	0.25	-0.73	0.47	**-0.61**	**0.07**	**-8.99**	**<0.001**
Sex (Male)	0.08	0.13	0.59	0.56		Not fit		
Hibernation year (2019)	0.72	0.44	1.63	0.11		Not fit		
Hibernation year (2020)	-0.04	0.15	-0.29	0.77		Not fit		
Pre-hibernation body condition: Sex (Male)	-0.13	0.13	-0.97	0.34		Not fit		
Pre-hibernation body condition: Hibernation year (2019)	-1.46	0.75	-1.95	0.06		Not fit		
Pre-hibernation body condition: Hibernation year (2020)	-0.32	0.25	-1.26	0.22		Not fit		

Table D.13: Model estimates, standard error, test statistics, and significance values for full and final models predicting rate of change in body condition over winter based on pre-hibernation body condition in black-tailed prairie dogs. Not fit indicates effects and/or interactions that were not retained in the final model. Bolded metrics indicate significance (p<0.05).Note: The model assessing the influence of pre-hibernation body condition on the rate of change in body condition has a caveat in that pre-hibernation body condition appears a independent variable and is incorporated into the dependent variable (i.e. Rate of change in body condition = Post-hibernation body condition - Pre-hibernation body condition/Duration of the hibernation period) in the model.)

	Full model				Final model			
Predictor variable	Estimate	Std. Error	t-value	p-value	Estimate	Std. Error	t-value	p-value
Intercept	**-0.02**	**0.004**	**-3.74**	**<0.001**	**-0.01**	**0.003**	**-5.26**	**<0.001**
Pre-hibernation body condition	-0.002	0.01	-0.23	0.82	-0.005	0.003	-1.78	0.08
Sex (Male)	-0.003	0.004	-0.68	0.50	-0.003	0.004	-0.75	0.46
Hibernation year (2019)	0.03	0.01	1.95	0.06		Not fit		
Hibernation year (2020)	0.001	0.005	0.30	0.77		Not fit		
Pre-hibernation body condition: Sex (Male)	**-0.01**	**0.005**	**-2.42**	**0.02**	**-0.01**	**0.005**	**-2.48**	**0.02**
Pre-hibernation body condition: Hibernation year (2019)	**-0.06**	**0.03**	**-2.16**	**0.04**		Not fit		
Pre-hibernation body condition: Hibernation year (2020)	-0.004	0.01	-0.42	0.68		Not fit		

Table D.14: Model estimates, standard error, test statistics, and significance values for full and final models predicting post-hibernation body condition based on pre-hibernation body condition in black-tailed prairie dogs. Not fit indicates effects and/or interactions that were not retained in the final model. Bolded metrics indicate significance (p<0.05).

Predictor variable	Full model				Final model			
	Estimate	Std. Error	*t*-value	*p*-value	Estimate	Std. Error	*t*-value	*p*-value
Intercept	**-1.18**	**0.13**	**-9.31**	**<0.001**	**-1.10**	**0.06**	**-17.01**	**<0.001**
Pre-hibernation body condition	**0.82**	**0.25**	**3.22**	**0.003**	**0.39**	**0.07**	**5.70**	**<0.001**
Sex (Male)	0.08	0.13	0.59	0.56		Not fit		
Hibernation year (2019)	0.72	0.44	1.63	0.11		Not fit		
Hibernation year (2020)	-0.04	0.15	-0.29	0.77		Not fit		
Pre-hibernation body condition: Sex (Male)	-0.13	0.13	-0.97	0.34		Not fit		
Pre-hibernation body condition: Hibernation year (2019)	-1.46	0.75	-1.95	0.06		Not fit		
Pre-hibernation body condition: Hibernation year (2020)	-0.32	0.25	-1.26	0.22		Not fit		