**The efficacy of body condition indices in predicting body composition is dependent on natural history and phenology**

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**Abstract**

1. *To be written*

**Key words:** body composition, body condition, fat, lean, natural history, phenology

**Introduction**

Body mass is a frequently measured physical trait of organisms (a Web of Science topic search for “body mass” returned over 7,000 entries when refined for the topic ‘ecology’ alone; 8 February 2021). In mammals, body mass is positively correlated with a number fitness-related traits, including female fecundity (e.g., house mice *Mus musculus* (Ruff et al. 2017)), intraspecific competition among males (e.g., Southern elephant seals *Mirounga leonina* (McCann 1981)), social dominance (multiple taxa, e.g., (French and Smith 2005)), and dispersal (Ozgul et al. 2014). Despite these reasons to expect body mass to be under strong directional selection, body mass is subject to constraints, as evidenced by within-organism trade-offs (e.g., energetic trade-offs (Gearty et al. 2018)) and environmental selection agents (Bonnet et al. 2017). Intraspecific variation in body mass has a heritable component, but within-individual variation demonstrates the plasticity of the phenotype. Adult body mass in determinate-growth organisms shifts within individuals across their lifetimes (e.g., Jebb et al. 2021), including across seasons (e.g., (Boag and Murie 1981; Dobson et al. 1992; Réale et al. 1999; Lázaro et al. 2017). Such phenotypic plasticity in body mass has been shown to be adaptive (Pelletier et al. 2007), suggesting that physiological correlates of optimal phenologies for a given species must be considered when inferring functional relationships involving body mass.

For processes in which energetic reserves, rather than structural size, are the currency of interest, body mass is typically a proxy metric for a specific element of what mass comprises; i.e., body composition. Specifically, fat and lean mass are the significant components as they are the readily-metabolizable energy reserves (Krebs and Singleton 1993a). Measuring the components directly can be challenging, and so body condition indices (BCIs) are frequently used to assess energy reserves relative to structural size in individuals. Organisms that have relatively higher non-skeletal:skeletal mass ratios are considered to be in ‘better’ condition than individuals that are lower in body mass for their skeletal size, as they have relatively more energetic reserves than an organism of similar structural size but lower metabolizable fraction (Schulte-Hostedde et al. 2001). Accurate measures of condition are widely applicable to scenarios such as livestock breeding programs (e.g., maximizing meat or offspring production), conservation biology (e.g., measuring response to habitat degradation (Stevenson and Woods 2006)), and in exploring drivers of patterns in evolutionary and ecological research (e.g., condition-dependent dispersal (Bonte and De La Peña 2009) and migration (Andersen et al. 2000)).

The dynamic nature of body condition over time within individuals (Krebs and Singleton 1993) underscores the importance of considering the correlates and effects of variable body condition within the context of a given species’ natural history (Molnár et al. 2009). The particular body composition comprising ‘good condition’ in an adaptive sense (i.e., that which is associated with increased survival and/or fitness) is therefore expected to vary across species and across time. For example, fat reserves are an important source of metabolizable energy (with ~39.6 kJ/g for dry fat compared with 5.3 kJ/g for wet lean mass, (Jenni and Jenni-Eiermann 1998)) that fuel organisms through energetically-expensive behaviours, such as migration, hibernation, and reproduction. Individuals with higher fat fractions going into hibernation are more likely to survive over winter and breed successfully the following spring (Boyer and Barnes 1999), yet during the active season, carrying more body mass can decrease predator evasion (Kullberg et al. 1996) and alter circulating hormones (Taylor et al. 1982). For organisms that primarily store energy off-body as external food caches, a higher lean fraction may be favoured over fat, as cached food essentially replaces fat as the animal’s primary energy reserves. The optimal body ‘condition’ is therefore likely to be highly dependent on natural history, as well as time of year.

Assessing body condition as it pertains to the relative quantities of metabolizable tissue types is most accurate when animals are sacrificed and chemically analyzed, but this precludes repeated measures of individuals across time (Lindström and Piersoma 1993). Chemical analyses may be undesirable when studying species at risk or other scenarios in which sacrificing individuals is not permitted. Body condition indices are most often calculated as model residuals obtained by regressing body mass on some linear measure of structural size (e.g., body length, tarsus length, foot length, etc. or a combination thereof; (Jakob et al. 1996; Green 2001; Schulte-Hostedde et al. 2001)). Because body mass and structural size can be measured from live animals, BCIs are less invasive compared with chemical analyses, while also allowing for repeated measures. Such condition indices have been confirmed against direct quantification of body composition vertebrates including birds (Chang and Wiebe 2016) and reptiles (Weatherhead and Brown 1996), and invertebrates including arthropods (Jakob et al. 1996; Moya-Laraño et al. 2008; Kelly et al. 2014). However, BCIs are not without their drawbacks: Krebs and Singleton (Krebs and Singleton 1993b) warned of low repeatability attributable to daily fluctuations in animal condition and to measurement bias across different observers. Choice of linear metrics must consider feasibility as well. Length of long bones, for example, yield the most accurate estimates when measured from museum specimens (e.g., (Dobson 1992)), but are challenging to measure on live, unanesthetized animals in field conditions (Green 2001). Finally, skeletal morphology reflects natural history, as selection favours certain shapes for different lifestyles (e.g., arboreal vs. fossorial), limiting applicability of a one-BCI-fits-all approach.

Technological developments now allow body composition to be measured relatively non-invasively in live animals. Methods such as dual-energy X-ray absorptiometry (Stevenson and Van Tets 2008) and total-body electrical conductivity (Lyons and Haig 1995) show high accuracy, but have drawbacks (radiation exposure and non-independence of estimates between tissue components, respectively). A relatively non-invasive method to estimate body composition precisely, accurately, independently, and in absolute quantities uses quantitative magnetic resonance (QMR) technology (Tinsley et al. 2004). Quantitative magnetic resonance will be recognizable to those familiar with medical magnetic resonance imaging (MRI) procedures, as both procedures rest on the same underlying principles of physics (relaxation rates of hydrogen atom spins, following application of an electromagnetic stimulus, differ when suspended in fat versus lean tissues). However, instead of providing images as an MRI scan would, a QMR scan will provide absolute measures of lean mass, fat mass, and water mass.

Given that different evolutionary trajectories among species have selected for different morphologies, physiologies, and breeding phenologies, we expect the relative fractions of metabolizable tissues (fat and lean) will differ with natural history, and therefore the utility of morphometrically-derived BCIs to predict body composition to vary with natural history as well. Furthermore, we expect the relationships between BCIs and body composition to vary seasonally as well, reflecting the dynamic nature of energy budgets in the annual patterns of seasonally-dependent activity. Here, we evaluate the efficacy of BCIs obtained from field-based morphometric measurements in predicting fat and lean mass derived from QMR analyses. We take a comparative approach to investigate these patterns in context phenologically-related shifts in energy balances within natural history context by studying three species of the same family with different patterns in energy storage and metabolic demands (North American red squirrels *Tamiasciurus hudsconicus*, black-tailed prairie dogs *Cynomys ludovicianus*, and Columbian ground squirrels *Urocitellus columbianus*; family: Sciuridae). We hypothesized that the relationship between body condition index and body composition (lean mass and fat mass), varies in accordance with the extent to which each species relies on on-body energetic reserves for overwinter survival, such that BCI will be more informative of body composition in fat-gaining hibernating species, and least informative of body composition for food-caching non-hibernating species. We also expect that the relationships between BCI and composition will change seasonally as energy budgets and body mass shift in accordance with individual species’ phenologies.

**Materials and Methods**

The populations studied are all within in the more northern regions of their respective ranges: food-caching, non-hibernating North American red squirrels (tribe Tamiasciurini; hereafter, red squirrels) in the southwest Yukon, Canada, non-caching hibernating Columbian ground squirrels (tribe Marmotini; hereafter, ground squirrels) in Alberta, Canada, and non-caching hibernating black-tailed prairie dogs (tribe Marmotini; hereafter, prairie dogs) in Saskatchewan, Canada. These species rely on energy stored (either as fat or as food caches) in late summer/early autumn to support overwinter survival and help finance reproductive efforts the following breeding season (Broussard et al. 2005; Fletcher et al. 2013; Grassel et al. 2016).

Since the divergence of these lineages (estimated to have occurred near the end of the Oligocene (Hafner 1984), distinct differences in physiology, morphology, and behaviour have arisen, even between the two Marmotini species. Red squirrels are arboreal (most likely the ancestral trait in Sciuridae, with terrestrial being derived; Steppan et al 2004), primarily granivorous mammals that store cached food items in a central larder called a midden (Smith 1968b), and weigh between 230 and 250 g on average as adults. Red squirrels remain euthermic throughout winter without using torpor (Brigham and Geiser 2012), and are not known to gain significant amounts of fat prior to winter, during which they rely on seeds stored in cached conifer cones for energy. In contrast, prairie dogs are semi-fossorial, herbivorous rodents that weigh up to 1500 g (Hoogland 1995). Throughout most of their range, prairie dogs are active throughout the winter; however, in southern Saskatchewan where we study them (the northern edge of the species distribution), they are known to hibernate (Gummer 2005; Lehmer et al. 2006). Columbian ground squirrels, like prairie dogs, are also semi-fossorial and herbivorous. They show remarkable variation in body mass across their active season (~400 g at emergence in spring, up to ~700 g just prior to immergence in late summer; (Dobson et al. 1992). They are obligate hibernators notable for their relatively short active season (~4 months) and extended time spent metabolically depressed in hibernation (~8 months) each year. Individuals must build sufficient energy stores during the active season to sustain long bouts of hibernation. Correspondingly, they experience their lowest body mass at emergence from hibernation in the spring and their maximum body mass just prior to immergence into hibernation in late summer (Dobson et al. 1992).

*General methods*

For all populations, we collected data via live-trapping free-ranging individuals in their natural habitats (population-specific details below) in 2018 and 2019. Individuals in these systems are typically followed from birth to death/disappearance (for detailed descriptions of population and reproductive monitoring, see (McAdam et al. 2007) for red squirrels, (Kusch et al. 2020) for prairie dogs, and (Lane et al. 2019) for Columbian ground squirrels). Briefly, all individuals received permanent aluminum alphanumeric ear tags (National Band and Tag Company, Newport, Kentucky) uniquely numbered within each population upon first trapping. We assessed pregnancy in all species through abdominal palpations, and females were identified to be lactating if we could express milk from the nipples. We only included adult animals in the present analyses to remove effects of skeletal growth dynamics. We further excluded all pregnant or lactating females to remove composition variance related to maternal investment in offspring.

*North American red squirrel study area and population*

            We have monitored a population of red squirrels for over 30 years at a site within Champagne and Aishihik First Nations traditional lands along the Alaska Highway in the southwest Yukon (61° N, 138° W, elevation ~ 850 m). White spruce (*Picea glauca* (Moench) Voss) is the sole conifer in this region of boreal forest (see (Krebs & Boonstra, 2001) for detailed description of this region), and provides the primary food source for squirrels at this site (seeds contained within mature female cones; (LaMontagne and Boutin 2007; Fletcher et al. 2013)). Squirrels harvest and cache spruce cones from trees from late summer through autumn (Fletcher et al. 2010) in a central hoard called a midden (Smith 1968a). Red squirrels are generally sexually monomorphic, although males are slightly heavier than females on average (Larsen and Boutin 1994), experience a higher reproductive skew (Wishart et al. 2018), and tend to cache more cones than females (Fisher et al. 2019), which may be reflected in relationships between body condition indices and body composition. Red squirrels included in the present study were trapped in an area monitored since 2017, so for many individuals, exact age was unknown, but we could confidently identify and remove young-of-the-year from this dataset based on size (under 200 g at first trapping) and being untagged individuals in the years of the present study.

*Prairie dog study area and population*

We monitored a colony of black-tailed prairie dogs in Grasslands National Park, Saskatchewan (49°N, 107°W *elevation?*) in October 2018. This colony, along with 18 other colonies that exist in and around the park, demarcates the northern edge of the black-tailed prairie dog range. Vegetation in this region primarily consists of needle and thread grass (*Hesperostipa comata*), blue grama (*Bouteloua gracilis*)and western wheat grass (*Agropyron smithii*; (Stephens et al. 2018). Prairie dogs of both sexes increase fat mass prior to winter, even in a population lower in latitude than the Saskatchewan colony studied here (Lehmer and Van Horne 2001). The endogenous energy reserves built up during this fattening period are then used to support metabolism during hibernation as well as reproduction the following spring (Boyer and Barnes 1999). *Need: statement on male vs female size.*

*Columbian ground squirrel area and population*

           We have continuously monitored and censused a population of Columbian ground squirrels in Sheep River Provincial Park, Alberta (50°N, 114°W, elevation: 1500 m) since 2008. Ground squirrels are herbivorous, obligate hibernators that reside in alpine and subalpine grassy meadows in the Rocky Mountains of North America (Elliott and Flinders 1980). They exhibit male-biased sexual dimorphism, and individuals experience significant mass changes across time, though the relative mass of males to females appears maintained across the active season (Boag and Murie 1981). Due to short vegetation growing seasons, ground squirrels spend the majority of the year (8-9 months) hibernating in individual subterranean burrows (Dobson 1992). During their brief active season (3-4 months), ground squirrels must meet the energetic requirements for breeding, moulting, survival and finally fattening before resuming the following hibernation period (Dobson et al. 1992; Lane et al. 2019). Although males tend to emerge from hibernation earlier than females (~1-2 weeks), plant quality and availability is low when both sexes emerge in the spring (Young 1990; Lane et al. 2012). Consequently, body fat stores that remain after hibernation are important for supporting reproduction (Broussard et al. 2005).

*Morphometric measurements*

During handling following live-trapping, we measured body mass (g), zygomatic width (ZW; mm), and right hind foot length (RHF; mm). These measurements were taken during the same handling occurrence as body composition scans (described below) for all red squirrels and most prairie dogs and ground squirrels. For the remaining prairie dogs and ground squirrels, we used skeletal measurements taken closest in time to when body composition scans were completed. Columbian ground squirrels that were younger than 3 years of age were dropped from the analysis if their skeletal measurements were taken more than two weeks before or after the date of body composition and body mass measurements, as younger squirrels are still experiencing skeletal growth (Dobson 1992). This left a single yearling (female) in the spring dataset, so we removed all yearlings from both seasons.

We weighed each prairie dog to the nearest 5 g using a Pesola spring scale and subtracted the handling bag weight. We weighed red squirrels and ground squirrels to the nearest 1 g on an electronic balance at the site of body composition scanning. We measured zygomatic width to the nearest millimeter using calipers (analogue for red squirrels and prairie dogs; digital for ground squirrels), and measured RHF from heel to longest toe (excluding the claw) to the nearest millimeter using a ruler fit with a perpendicular heelstop at 0 mm. We measured both ZW and RHF three times per handling, and used the mean value per handling in all subsequent analyses.

*Body composition analysis*

We measured body composition in autumn (hereafter, pre-winter) only for a total of n = 71 red squirrels (40 females and 31 males total between late-September and mid-October 2018 and 2019) and n = 31 prairie dogs (21 females and 19 males; in late October 2018). For Columbian ground squirrels, we measured body composition in the spring (within a week of emergence from hibernation for most animals) and late summer (within a week prior to immergence for most animals). In spring, we measured n = 105 ground squirrels (70 female, 35 male, between mid-April and early May in 2019) and in late summer (hereafter, pre-winter), we measured n = 37 ground squirrels (32 females and 5 males, between late July and late August across 2018 and 2019).

We used a quantitative magnetic resonance (QMR) body composition analyzer (Echo Medical Systems, Houston, TX) to measure absolute lean and fat mass (g). Studies of body composition readings from QMR indicates that readings correlates well with carcass-derived data for numerous species across multiple taxa, including passerines (house sparrows *Passer domesticus*, European starlings *Sturnus vulgaris*, and zebra finches *Taeniopygia guttata* (Guglielmo et al. 2011)), snakes (Northern watersnakes *Nerodia sipedon* and Eastern Massasaugas *Sistrurus catenatus* (Riley et al. 2016)), bats (little brown bats *Myotis lucifugous*, hoary bats *Lasiurus cinereus*, and silver-haired bats ﻿*Lasionycteris noctivagans* (McGuire and Guglielmo 2010)), fish (zebrafish *Danio rerio* (Fowler et al. 2016)), domesticated mammals such as house cats *Felis catus* (Zanghi et al. 2013a), dogs *Canis familiaris* (Zanghi et al. 2013b), pigs *Sus scrofa domesticus* (Andres et al. 2010), and, most relevant here, rodents (including laboratory rats *Rattus* *norvegicus domestica* (Johnson et al. 2009) and house mice *Mus musculus domesticus* (Jones et al. 2009). Quantitative magnetic resonance also allows for repeated measures of live animals, both awake and sedated, without sacrificing them (Tinsley et al. 2004; Mcguire and Guglielmo 2010; Zanghi et al. 2013b,a). Additionally, QMR offers portability for field applications when housed in a temperature-controlled mobile unit (Guglielmo et al. 2011), which we use here to ensure the same apparatus was used for all three species *in situ*.

Our QMR system was housed in a custom-designed mobile trailer to enable transportation to our study sites. The trailer is climate-controlled to stabilize the temperature at which we calibrated the magnet for at least five hours prior to scanning animals. We targeted an ambient temperature of 21°C, although field conditions widened the range of stabilized temperature to ± 7°C. Our machine was custom outfitted with an additional antenna to measure animals from 100 g up to 1600 g to accommodate the range of body size of the three species under study. Details of similar apparatus and its capabilities are described elsewhere (McGuire and Guglielmo 2010), and we followed similar protocols here. Briefly, each day we calibrated the machine using a canola oil standard (943 g) at the stabilized temperature. Squirrels were live-trapped and transported to the mobile laboratory to be measured. We placed each squirrel in a clear plexiglass holding tube with perforations to allow ample airflow to the live animal, then inserted the tube into the QMR chamber. We recorded body composition through a minimum of two scans and report the average values for each individual. Beginning in 2019, we gave a mild sedative to red squirrels prior to scanning and taking morphometric data (100 µg/kg of dexmedetomidine, reversed by 1 mg/kg atipamezole) to minimize stress and movement while the animal was in the chamber. This was not necessary for the semi-fossorial prairie dogs or ground squirrels, who were comfortable enough in the dark, enclosed space that they frequently fell asleep during scans.

*Calculating body condition indices (BCIs)*

We retained data for individuals for which we had measurements for all of the following: zygomatic width, right hind foot length, body mass, and body composition (fat mass and lean mass). We selected the most appropriate BCI for each species by first exploring correlation between RHF and ZW in each species. If positive for both sexes, we proceeded to estimate structural size by log-transforming and scaling variables to a mean of zero (within sex within specie) and then conducting principal components analysis using the transformed skeletal measurements. We then regressed body mass on the resulting PC1 and retained the residuals to serve as the body condition index (BCI) used for further analyses, following (Schulte-Hostedde et al. 2005). When there was a negative relationship or no relationship between skeletal measures for one or both sexes, we selected the single skeletal measure that positively correlated with body mass with the greatest coefficient of variation (CV; see Supplementary Material). We used this to generate a single skeletal measure index by taking the residuals from regressing either RHF alone on body mass (generating the RHF index), or ZW alone on body mass (generating the ZW index). All BCIs were calculated within each species independently, and further specialized by calculating BCIs independently for males and females within each species to account for sex differences in morphometric values (see Results).

*Statistical analyses*

We initially calculated coefficient of variation for all variables and analyzed relationships between morphometric variables (body mass, RHF, and ZW) in absolute, non-transformed values to explore initial relationships. We performed all analyses of body composition variables expressed as absolute quantities in grams. We regressed all body condition indices against lean (g) and fat (g) using mixed models with individual identity specified as a random effect, as some individuals were measured multiple times within each dataset. All analyses were completed in R (v.4.0.3, (R Core Team 2020)).

**Results**

*Morphology and sexual dimorphism*

We confirmed that red squirrel males are slightly heavier than females (Boutin and Larsen 1993) and found this species sexually monomorphic in skeletal measures, while prairie dogs and ground squirrels showed sexual dimorphism in body mass and skeletal measures (Table 1). The relationship between right hind foot length and zygomatic width was not consistent across the three species. Red squirrels showed a slightly negative but non-significant relationship between RHF and ZW, while ground squirrels showed a positive relationship between these variables, with higher values for males than females (Figure 1). The relationship between RHF and ZW was markedly different between male and female prairie dogs; no relationship in females, while there was a significant positive relationship in males (R2 = 0.64, t = 3.20, df = 15, *p* = 0.006).

**Table 1.** Summary of morphometric data (body mass, right hind foot length, and zygomatic width) for male and female North American red squirrels, black-tailed prairie dogs, and Columbian ground squirrels (values reported as mean ± SEM).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Species | Body mass (g) | | | | Right hind foot length (mm) | | Zygomatic width (mm) | |
|  | *Male* | *Female* | *Male* | | | *Female* | *Male* | *Female* |
| North American red squirrels | 251.8 ± 2.7 \* | 243.8 ± 2.8 \* | | 45.8 ± 0.3 | | 45.6 ± 0.2 | 28.9 ± 0.2 | 28.3 ± 0.1 |
| Black-tailed prairie dogs | 1597.6 ± 20.5\* | 1448.1 ± 20.5\* | | 56.9 ± 0.3\* | | 55.9 ± 0.2\* | 50.2 ± 0.4\* | 47.9 ± 0.2\* |
| Columbian ground squirrels  *Pre-winter*  *Spring* | 705.6 ± 26.2 \*  483.5 ± 8.6 \* | 589.5 ± 12.3 \*  395.9 ± 6.2 \* | | 47.0 ± 0.6 \*  46.8 ± 0.4 \* | | 44.9 ± 0.3 \*  45.1 ± 0.2 \* | 33.7 ± 0.4  35.4 ± 0.2\* | 33.1 ± 0.2  33.5 ± 0.2 \* |

*\*denotes significant differences between males and females; one-way ANOVA with alpha = 0.05)*

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| *A B* Chart, scatter chart  Description automatically generated *Chart, scatter chart  Description automatically generated*  *C D*  Chart, scatter chart  Description automatically generatedChart, scatter chart  Description automatically generated  **Figure 1.** Relationship between zygomatic width (mm) and right hind foot length (mm) for A) North American red squirrels, B) black-tailed prairie dogs, and Columbian ground squirrels pre-winter (C) and in spring (D). Density plots on each axis show distribution of values corresponding to each sex (females in orange, males in blue) to illustrate the degree of overlap. |

*Body composition*

Sex differences in fat and lean mass were found only in ground squirrels and prairie dogs except for pre-winter fat values in ground squirrels (Table 2). Prairie dogs showed the highest percent body fat pre-winter of all species, with 31.3% and 35.2% in males and females, respectively, while red squirrels showed the lowest body fat at 2.4% and 2.9% in males and females, respectively. Ground squirrels showed their highest body fat levels pre-winter before entering hibernation, and were significantly leaner post-emergence in the spring, with both sexes halving their percent body fat over winter.

**Table 2.** Summary of body composition data (fat and lean mass) quantified using quantitative magnetic resonance (QMR) for male and female North American red squirrels, black-tailed prairie dogs, and Columbian ground squirrels (values reported as mean ± SEM, mean values expressed as percent body mass in parentheses).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Species | Fat (g) | | Lean (g) | |
|  | Male | Female | Male | Female |
| North American red squirrels | 6.1 ± 0.5  (2.4%) | 7.2 ± 0.6 (2.9%) | 217.8 ± 2.9 (86.5%) | 214.0 ± 2.3 (87.8%) |
| Black-tailed prairie dogs | 50.20 ± 15.5 (31.3%)\* | 510.7 ± 14.9 (35.2%)\* | 915.8 ± 13.4\* (57.4%)\* | 717.6 ± 13.0\*  (49.5%)\* |
| Columbian ground squirrels  *Pre-winter*  *Spring* | 163.4 ± 9.7  (23.1%)  55.3 ± 3.5 \* (11.5%) \* | 136.6 ± 5.5 (23.0%)  33.3 ± 2.3 \*  (8.2%) \* | 478.0 ± 15.3\* (67.8)  388.8 ± 7.6\*  (80.4%) | 396.2 ± 7.3\*  (67.4%)  322.5 ± 4.9\*  (81.6) |

*\*denotes significant differences in absolute tissue mass or percent body mass  
 between males and females; one-way ANOVA with alpha = 0.05)*

*Selection of BCIs*

Given that red squirrels showed no relationship between RHF and ZW, we selected to use ZW (CV of ZW for females = 2.78, males = 4.22; compared with CV of RHF for females = 2.77, males = 3.09; Supplementary Material) to generate the BCI for this species (‘ZW index’). Prairie dogs had a sex-dependent relationship between RHF and ZW, and ZW was more positively correlated with body mass in both sexes than RHF. Therefore, we also selected ZW (CV of ZW for females = 2.38, males = 3.27; compared with CV of RHF for females = 1.99, males = 2.34) to generate the ZW index for prairie dogs. Columbian ground squirrels showed positive but sex-dependent slopes between RHF and ZW in pre-winter, and showed no relationship between RHF and ZW for either sex in spring. Therefore, we also selected to use ZW index for ground squirrels as well. As all individuals in the present analyses were adults, we could be confident that skull size would not be disproportionately larger for overall body size as may be the case in juvenile animals.

*Relationship between BCI and body composition*

Using the ZW index generated from the residuals of regressing scaled body mass on scaled ZW within sex for each species, we found that lean mass was better predicted by ZW index than fat mass in red squirrels and ground squirrels (spring), but fat was better predicted by ZQ than lean mass in prairie dogs (Figure 2). Pre-winter, both fat and lean mass of ground squirrels were reasonably correlated with body condition index and, like prairie dog lean mass, the two sexes could be distinguished in the data. By spring, the relationship between ground squirrel BCI and fat mass had decreased while lean mass was still correlated with BCI.

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| A B  *Chart, scatter chart  Description automatically generated* Chart, scatter chart  Description automatically generated  C  *Chart, scatter chart  Description automatically generated Chart, scatter chart  Description automatically generated*  C D  Chart, scatter chart  Description automatically generated Chart, scatter chart  Description automatically generated  E F  *Chart, scatter chart  Description automatically generated* Chart, scatter chart  Description automatically generated  **Figure 2.** Fat (g) and lean (g) mass regressed on zygomatic-width derived body condition index for North American red squirrels (A,B), black-tailed prairie dogs (B,C) and Columbian ground squirrels pre-winter (D,E) and in spring (F,G). |

**Discussion**

As predicted, the food-caching North American red squirrels showed the lowest fat reserves, both in absolute and proportional values. Black-tailed prairie dogs had the highest pre-winter fat reserves, with Columbian ground squirrels more closely resembling prairie dogs pre-winter and more closely resembling red squirrels in spring, after energy reserves have been largely consumed supporting hibernation. While prairie dogs in this population do enter torpor during winter, they are not known to be efficient hibernators and are known to arouse frequently (Hawkshaw, unpublished data). Such arousal bouts are energetically costly and may help explain why prairie dogs build significant energy stores (represented as high pre-winter percent body fat) even compared with the obligate hibernating Columbian ground squirrels. In contrast, red squirrels rely heavily on external stores of cached cones to support overwinter survival, and so their low levels of body fat are perhaps not surprising; however, whether or not these low levels of fat reserves are consistent across seasons remains to be shown in this species.

Previous studies on small-non hibernating mammals have found that BCIs are better at predicting lean dry mass and water, rather than fat mass (Schulte-Hostedde et al. 2001). Here we have demonstrated that predicting fat in species with higher proportions of adipose tissue also present challenges. Given the dynamic nature of body composition in hibernating species, estimating fat levels can be particularly difficult since BCIs based on residuals assume that lean mass scales with body size, while fat mass varies according to body condition (McGuire et al. 2018a). However, in some hibernating species, an increase in lean mass can also account for the total increase in body mass during the pre-hibernation phase (Sheriff et al. 2013).

In general, we have shown that the lower amount of body fat an animal is carrying, the less predictive power the body condition index will have in estimating it, even when the coefficient of variation within each species is similar for the same traits. This suggests that direct methods of fat quantification, such as QMR, are better suited than BCIs to estimate body composition on animals expected to have low body fat fractions at a given point in the annual cycle, particularly if fat is central to the research question. If the low degree of variation in measurements of structural size were insufficient to explain the higher degree of variation in body composition (McGuire et al. 2018b), then we would not expect to see relatively strong seasonal signals for the same populations of adult organisms.

**Conclusions**

The variation in relative morphometric measurements across these three species likely reflects the adaptive radiation from their shared ancestor across evolutionary history from divergence of a common ancestor, and we echo previous studies in the literature that call for such morphological adaptations to be considered when selecting informative traits for condition analyses. For example, the hind foot length of red squirrels is relatively large for their overall body size, reflecting morphological adaptations for arboreal lifestyles that would no longer have been under selection in the terrestrial species. Even between the two hibernating Marmotini species that show sexual dimorphism in size and composition, the patterns differ. Furthermore, calculating BCIs within-sex should be done when there is demonstrated sexual dimorphism in both body mass and structural size metrics. The decision to use a PCA-based BCI versus a BCI derived from a single linear metric regressed on mass will depend on species and, as we show here, should also depend on the seasonal dynamics of body composition, but more importantly, whether the selected BCI is a good predictor of the variable of interest at all should prevail.

**Ethics.** All procedures were approved by the University of Saskatchewan Animal Care and Use Committee. Fieldwork was completed under permits issued by Champagne and Aishihik First Nations Renewable Resource Council and Yukon Territorial Government (red squirrels); Grasslands National Park and the Saskatchewan Ministry of the Environment (prairie dogs); and Alberta Parks (ground squirrels).

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**Competing Interests.** We have no competing interests.

**Author Contributions.** *AEW designed the study, collected data, performed data and statistical analysis, and drafted the manuscript. JEL participated in the design. All authors assisted in data collection and provided valuable discussion and contributions to the writing of the manuscript.*

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**Data availability:** Data are available in *(Dryad/Figshare – depends on where submitted)*

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