THESIS

CLIMATE DRIVEN VARIABILITY IN THE DEMOGRAPHY AND PHYSIOLOGY OF THE UINTA GROUND SQUIRREL

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ABSTRACT

CLIMATE DRIVEN VARIABILITY IN THE DEMOGRAPHY AND PHYSIOLOGY OF THE UINTA GROUND SQUIRREL

Climate change is impacting the phenology of many species, ultimately altering their fitness and population dynamics. Shifts in phenology have been documented across a variety of taxa and ecosystems, but few studies have considered the effects of pertinent season-specific climatic variables on phenology and fitness. Hibernators may be particularly susceptible to changes in climate since they have a relatively short active season in which to reproduce and gain enough mass to survive the following winter. To understand whether and how climatic changes may be affecting hibernator fitness, we analyzed historical (1964-1968) and contemporary (2014-2017) mark-recapture data taken from the same population of Uinta ground squirrels (UGS, *Urocitellus armatus*). Although survival of UGS has not changed significantly over time, annual survival seems to fluctuate strongly in response to climate and phenology. Population density also increased, suggesting resources are less limited today than they used to be. Cheatgrass is now dominating low-elevation UGS habitat and seems to provide a better food source than native plants did historically. Although the phenology of UGS has not changed significantly over time with a locally warming climate (3.22ºF over 50 years), season-specific climatic variables were important in determining over-winter survival rates. To understand the role that physiological processes play in shaping the life history of UGS in light of warming temperatures, we studied UGS life history trade-offs near the extremes of their elevation range (6200 ft. versus 8000 ft.) which offer contrasted micro-climatic conditions. Specifically, we quantified trade-offs between body mass dynamics and immune function in two populations of UGS that experience different phenologies and active season lengths. UGS at the high elevation had a shorter active season, largely driven by extended snow cover into spring. UGS at this elevation also weighed less at emergence from hibernation than UGS at the lower elevation. Despite this, UGS at the high elevation gained mass faster than UGS at the low elevation, entering estivation at a similar weight. This accelerated mass gain was associated with a decline in immune function at the higher elevation, but not at the lower elevation where UGS experience a much longer active season and are not food-limited. Juveniles generally gained mass faster than adults and yearlings, but did not demonstrate a decrease in immune function at either elevation, possibly because they do not have to bear the energetic burden of reproduction. Our results improve our understanding of how hibernators respond to climate change, and how these plastic responses lead to contrasted life history strategies. Our results have implications for the conservation of this species and similar ones that are sensitive and responsive to even small changes in climate.

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CHAPTER 1

Introduction

Understanding how climate change is impacting the survival, phenology, and fitness of wildlife is a significant challenge in ecology (Visser and Both 2005; Parmesan 2006). Elucidating which environmental factors are influential in affecting individual fitness and attempting to forecast wildlife responses typically requires long-term data on a population, in conjunction with long-term climate data (Miller-Rushing et al. 2010).

Hibernators may be particularly susceptible to changes in climate through phenological shifts (Humphries et al. 2002), because they have a relatively short active season in which to reproduce and gain enough weight to survive the following winter. For example, data have been collected on yellow-bellied marmots in the Colorado Rocky Mountains for over 30 years to study various aspects of their behavior and life history (e.g., Oli and Armitage 2003; Ozgul et al. 2006, 2007; Borrego et al. 2008). Using extensive demographic and climate data, studies unraveled that population size and survival began increasing around 2001 due to increases in body mass in response to warmer springs, leading to an earlier green-up of vegetation and a longer growing season for the marmots (Ozgul et al. 2010). In Alpine marmots, earlier snowmelt also led to earlier emergence; however, the loss of winter snow cover and its insulation resulted in a decrease in both litter size and juvenile survival (Tafani et al. 2013; Rezouki et al. 2016) because marmots were forced to burn through their fat reserves faster than they would if snow was deeper (Tafani et al. 2013), outweighing the benefits of a longer active season. These conflicting results suggest that the extent to which an organism's phenology relies on climatic cues is important, as different climatic variables can have opposing effects on phenology, making associated fitness outcomes difficult to predict (Bjorkman et al. 2015).

A better understanding of how hibernator phenology is influenced by environmental conditions and how this may affect fitness will be key in addressing why climate change leads to different outcomes, even in closely related species. To address this, long-term individually based data is necessary, but not often available, limiting our ability to study and predict changes in population responses to climate change (Miller-Rushing et al. 2010). Elevational gradients can provide a proxy to climate change and a natural experiment of sorts because climatic variables including season length, temperature, and resource levels can all vary naturally along the gradient. This can be used as a proxy for climatic changes over time when long-term coupled data on climate change and population demography are not available. Elevational gradients are often used in mammals to look at different life history strategies that are adopted relative to different climates. Female golden mantled ground squirrels at higher elevations had higher survival rates, but delayed reproduction and had smaller litters when they did reproduce, demonstrating different strategies at each elevation related to climate and resource levels (Bronson 1979).

For this thesis, both a longitudinal and an elevational approach were employed to try to understand how hibernators (Uinta ground squirrels, *Urocitellus armatus*) respond to a warming climate. In Chapter 1, we analyzed demographic responses to climate over a 50-year period by comparing historical to contemporary demographic capture-mark-recapture records to understand whether climatic changes may be affecting fitness in Uinta ground squirrels (UGS). In northern Utah, where the data were collected, temperatures have been increasing (dos Santos et al. 2011) and winter snowfall has already decreased by nearly ten percent in the last half century, with an increasing proportion of winter precipitation falling as rain instead of snow (Gillies et al. 2012).

Because hibernators may be particularly susceptible to changes in climate, we anticipated changes in survival related to the observed warming and shift in snow dynamics.

In Chapter 2, immune function, growth rate, and reproductive rates of two populations of UGS were compared to study life history strategies of populations at different elevations. Populations of the same species can express contrasted life history traits across elevations, where differences in phenology, reproduction, survival, and growth exist. Higher elevations are typically characterized by a shorter growing season, lower temperatures, and more limited food resources, and individuals that belong to high elevation populations must allocate limited resources among key functions in a shorter period of time, shaping a different life history strategy than that of individuals at low elevations. We measured trade-offs between body mass and immune function in two populations of UGS that experience different phenologies and active season lengths at sites separated by 600-meters in elevation. A greater understanding of how UGS populations allocate resources at different elevations with different climatic conditions will contribute to our understanding of how hibernators respond to changes in climate in the future.

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CHAPTER 2

Climate-driven variability in the survival of Uinta ground squirrels

INTRODUCTION

Climate change is impacting the phenology of many species, ultimately altering their fitness and population dynamics (Visser and Both 2005; Parmesan 2006). For instance, earlier snow melt in montane habitats causes wildflowers to germinate earlier, increasing their susceptibility to mid-June frost-kills, ultimately leading to a reduction in recruitment by phenological mismatch (Inouye 2008). Shifts in phenology have been documented across a variety of taxa and ecosystems, although the direction of these shifts is inconsistent (Parmesan and Yohe 2003). Phenological mismatches can occur among a large range of factors, including how organisms interact with abiotic factors, food resources, conspecifics, and predators, complicating our ability to understand the implications of phenological shifts (Miller-Rushing et al. 2010). In a long-term study of great tits (*Parus major*) in the Netherlands, disparities in the phenological response to climate change across trophic levels have increased the asynchrony between great tit hatching date and peak caterpillar biomass, their preferred source of food for nestlings, which has decreased fledging success (Visser et al. 2006). However, in a long-term study of great tits in Witham Woods, England, the birds have shifted their phenology in response to warming springs while managing to maintain synchrony of their hatch date with peak food abundance by altering their incubation period (Cresswell and McCleery 2003). These studies illustrate how difficult it can be to predict demographic consequences of phenology shifts, particularly because linking phenology and demography requires extensive and individually-based data that are often unavailable (Miller-Rushing et al. 2010). Although there are several studies that have directly quantified the effects of phenological shifts on fitness components (e.g., Møller et al. 2008; Saino et al. 2011; Arlt and Pärt 2017), few pertain to mammals (e.g., Ozgul et al. 2010; Lane et al. 2012).

Hibernating mammals may be particularly susceptible to changes in climate through phenological shifts because they have a relatively short active season in which to reproduce and gain enough weight to survive the following winter (Humphries et al. 2002). In yellow-bellied marmots (*Marmota flaviventris*), for example, years with earlier snowmelt led to an earlier emergence from hibernation and a longer growing season, resulting in an increase in overwinter survival and reproduction (van Vuren and Armitage 1991; Ozgul et al. 2010). In Alpine marmots (*Marmota marmota*), earlier snowmelt also led to earlier emergence, but the loss of winter snow cover resulted in a decrease in both litter size and juvenile survival (Tafani et al. 2013; Rezouki et al. 2016). Thinner snow cover can force marmots to catabolize their fat reserves faster than under the insulation of a deeper snowpack (Tafani et al. 2013), outweighing the benefits of a longer active season. These conflicting results suggest that the extent to which an organism's phenology relies on climatic cues is important because different climatic variables can have opposing effects on phenology, making associated fitness outcomes difficult to predict (Bjorkman et al. 2015).

A better understanding of how hibernator phenology is influenced by seasonal environmental conditions and how this may affect fitness will be key in addressing why climate change leads to different outcomes, even in closely related species (Doak and Morris 2010). Few studies have considered the effects of pertinent season-specific climatic variables on hibernator phenology and fitness, but a study by Dobson et al. 2016 indicates that a focus on seasonality will help predict how hibernators may respond to warming and increased climate variability in the future. For instance, snow depth influences soil temperatures and can prevent the ground from freezing (Happold 1998; Inouye et al. 2000). Since mammalian torpor occurs within a relatively narrow optimal temperature range, with increased energy expenditure required when the ambient temperature is either warmer or colder than average (Kronfeld-Schor and Dayan 2013), winter conditions can affect over-winter survival up to spring emergence. Spring snow dynamics can further affect fitness after emergence because snow melt determines when green-up begins and when food resources become available (Walker 1968; Vuren and Armitage 1991; Inouye et al. 2000; Ozgul et al. 2010; Rezouki et al. 2016). Reproduction and subsequent juvenile emergence is generally timed with peak food abundance in hibernators (Walker 1968), as observed in other taxa (e.g., Visser et al. 2006). Hot and dry summers can cause grasses to senesce earlier, while additional precipitation can delay this process (Walker 1968), affecting whether juvenile emergence is matched with peak food abundance or not.

These seasonal responses are further complicated by demography, since phenological and fitness responses to climate can vary across age classes (Rezouki et al. 2016) and sex (Sheriff et al. 2013b). Juveniles have the least amount of time to gain body mass before hibernation, and they may also expend more energy while hibernating due to their large surface-to-volume ratio (Kortner and Geiser 2000; Sherman and Runge 2002), making them more susceptible to phenological mismatches than older age classes (Miller-Rushing et al. 2010). In relatively short-lived species this may have a large impact on overall abundance and persistence because population growth is quite sensitive to proportional changes in juvenile survival (Oli et al. 2001). In arctic ground squirrels (*Spermophilus parryii*), snow cover affects the emergence of females, while male emergence is more influenced by female emergence (Sheriff et al. 2013a), providing a reason to think that males and females have the potential to be affected differently by specific climatic variables, with consequences for sex-specific fitness.

Because worldwide temperatures are predicted to continue increasing (IPCC 2013), it is essential to broaden our understanding of how hibernators respond to changes in climate. We analyzed mark-recapture data from a population of Uinta ground squirrels (UGS, *Urocitellus armatus*) over a 50-year period to assess how climate change affected the survival of a hibernating species endemic to the western U.S. In northern Utah, where the data were collected, temperatures have been increasing (dos Santos et al. 2011) and winter snowfall has already decreased by nearly ten percent in the last half century, with an increasing proportion of winter precipitation falling as rain instead of snow (Gillies et al. 2012).

We estimated annual survival using historical (1964-1968) and contemporary (2014-2017) mark-recapture data in an effort to understand how season-specific climate affected long-term changes in a key fitness component of the species' life cycle. We expected that changes in snow dynamics could cause a decrease in survival if snowpack declines, or an increase in survival if snow recedes sooner in the spring, leading to earlier green-up. We also predicted that warmer summers may cause food resources (i.e. grasses) to senesce earlier, leading to a shortage of food before estivation which would have a negative impact on UGS over-winter survival, particularly in juveniles. By examining annual changes in survival as they relate to key phenological events in the UGS life cycle (emergence, reproduction, juvenile emergence, and estivation), we hope to determine whether survival has changed over the last 50 years in response to warming, and pinpoint which of the season-specific climatic and phenological variables may affect this key vital rate across age- and sex-classes.

METHODS

Uinta ground squirrel ecology

UGS typically emerge from hibernation between March and April and enter estivation by August, depending on elevation, age, and sex (Eshelman and Sonnemann 2000). UGS reproduce within a week of emergence, and adult and yearling females can produce a single litter each year of four to six juveniles. Maximum life expectancy is seven years in the wild but average life expectancy for juveniles is approximately 1.46 years. Yearling males rarely emerge from hibernation in breeding condition, while yearling females often emerge too late to secure territories and mates (Walker 1968; Eshelman and Sonnemann 2000). Spring climatic conditions influence emergence date and emergence order of each sex and age class: adult males and females emerge before yearlings in warmer springs, but colder springs cause a more synchronous delayed emergence of sex and age classes (Knopf 1973), making it more likely for yearling females to breed (Walker 1968). The diet of UGS is composed of graminoids and forbs, and historically they primarily consumed Kentucky bluegrass (*Poa pratensis*; Walker 1968). Contemporary surveys show that the site is now dominated by cheatgrass (*Bromus tectorum*), but *Poa spp* including *Poa pratensis* are still present (C. Falvo, unpublished data). Extensive data were collected from 1964-1971 on UGS behavior and demography (e.g., Walker 1968; Slade and Balph 1974; Knopf and Balph 1977), but in 1968 the population was reduced by approximately half to study density-dependent dynamics (Slade and Balph 1974), thus we excluded those later years from our analysis. Contemporary data collection began in 2014 up to 2017 in the same location, in order to make comparisons to historical survival probabilities.

Field site and data collection

The contemporary data were collected from 2014-2017 at the Utah State University Forestry Field Station in Logan Canyon, UT, in accordance with IACUC protocol #2220. Historical data from 1964-1968 were collected in the same location by previous researchers detailing the behavior, social, and population dynamics of UGS (for more detailed description of site and capture methods, see Walker 1968; Slade and Balph 1974; Knopf and Balph 1977).

UGS were caught using Tomahawk live-traps (Tomahawk, Wisconsin, USA) that were baited with rolled oats and peanut butter. Traps were set early in the morning before squirrels were active, checked within an hour, and squirrels were processed within 2-3 hours of capture. At initial capture, the age of the animal was determined and individuals were classified as juveniles (young of the year), yearling (1-year-old), or adult (≥ 2-year-old) based primarily on body mass, reproductive status, and comparison with individuals of known age from previous years of trapping. Each individual's mass was determined by placing the animal in a cloth bag and using a Pesola scale (+/-2 g.; Pesola Company, Baar, Switzerland). For contemporary data, additional morphometric variables (body, hind foot, and tail length) were measured using a measuring tape or calipers. The reproductive status of males and females were recorded (males as scrotal or non-scrotal; females as pregnant, lactating, or non-reproductive). Small, numbered, non-corrosive metal ear tags (Monel 1005-1, National Band and Tag Company, Newport, Kentucky) were placed on each squirrel at first capture for identification upon recapture. Passive Integrative Transponders (PIT tags, Biomark) were also injected under the skin to keep track of individuals in case of ear tag loss.

Impact of season-specific climate on UGS viability fitness

Climate data were obtained from a weather station approximately 26 km (16 miles) from the field site. Although a weather station is located < 1 km from the field site, data were only available from 2009-present. Climate variables of interest were compared between these two weather stations for available co-occurring years and were found to be highly correlated (see Appendix 1.1).

Fall. Snow acts as an insulator and can prevent the ground from freezing (Happold 1998; Inouye et al. 2000). In Alpine marmots, when snow depth is greater than 110 cm, the burrow temperatures are thought to be buffered against air temperatures (Tafani et al. 2013, Appendix D). To assess the potential influence of below-freezing temperatures on UGS survival before an insulating layer of snow was present, the number of days below freezing that occurred after estivation began and before permanent snow cover occurred (August – November or December) were tallied up each year t, and the impact of that variable on survival was assessed between year t and $t+1$.

Winter. Since mammalian torpor occurs within a relatively narrow optimal temperature range, with increased energy expenditure required if the ambient temperature is warmer or colder (Kronfeld-Schor and Dayan 2013), winter conditions can affect over-winter survival as well as fitness post-emergence. The average snow depth and temperature from December of the previous year t-1 until emergence the next spring at t were used to examine the influence of winter conditions on survival from $t-1$ to t (cross-seasonal effect) and from t to $t+1$ (direct effect).

Spring. Snow melt determines when green-up begins and food resources become available (Walker 1968; Vuren and Armitage 1991; Inouye et al. 2000; Rezouki et al. 2016). In years when squirrels emerged long before snowmelt (snow melted late relative to other years), their body mass typically declined before increasing again as food became accessible (Knopf and Balph 1977), indicating the importance of spring temperatures as they relate to green-up and body mass at spring emergence. Since temperatures in year t could influence survival from t -1 to t or from t to t +1, we considered the maximum temperatures between March 15th and March 31st (per Knopf and Balph 1977) in our model selection framework.

Summer. Summer precipitation and temperatures influence food availability, with hot and dry summers causing earlier plant senescence (Walker 1968). Precipitation may be positively correlated with survival through improved forage quality (Armitage 1994). Previous studies have shown that timing of UGS estivation roughly coincides with the drying out of vegetation (Knopf 1973), suggesting declines in precipitation have the potential to shorten the active season via changes in plant availability. To examine the influence of summer food conditions in relation to UGS phenology, growing degree days (at 0ºC; (Frank 1996)) were calculated from emergence until the estimated date of juvenile emergence, when forage quality and quantity is essential to this age class. We also considered an aridity index that incorporates average temperature and precipitation from June - August (BGI, Bagnoul-Gaussen Index: daily rainfall (mm) minus twice the average temperature (°C); (Canale et al. 2016; http://leddris.aegean.gr/ses-parameters/295-aridity-index.html), as well as average temperature and average precipitation alone as variables that could influence survival to the following year.

Capture-Mark-Recapture Analyses and Model Selection

To determine whether UGS survival has changed over time, we used Cormack-Jolly-Seber (CJS, (Lebreton et al. 1992)) capture-mark-recapture (CMR) models developed in R (R Core Team 2015, version 3.2.3) using the RMark package (Laake 2013) to estimate annual apparent survival (ϕ) and recapture probability (p) from both historical and contemporary data.

Goodness of fit. We used goodness-of-fit (GOF) tests computed in the RELEASE software implemented in program MARK (Cooch and White 2006), which tests assumptions of the CJS model that every marked animal present at time t has the same probability of recapture, and that every marked animal immediately after t has the same probability of surviving to $t + 1$ (Cooch and White 2006) (see Appendix 1.2 for details). We considered a global model where survival was allowed to vary by age, sex, and time period (historical versus contemporary). Although a global model may pass GOF tests, this does not imply that assumptions of the CMR modeling framework are met perfectly. We therefore estimated $\hat{c}$ to adjust model likelihoods for over-dispersion in the data by dividing the overall χ^2 values by the total degrees of freedom, which is not biased as high as the median $\hat{c}$ approach for CJS models (White 2002).

Detection probability (p). Because of a 46-year gap in the CMR data, appropriate recapture and survival parameters were fixed to 0 between the historical and contemporary study periods. Additionally, data on adult males were not available during the first 2 years of the historical study, and thus these parameters were also fixed to 0. For historical years, we fixed p to 1 because trapping was considered a census in those years (Slade and Balph 1974). Since estimates for p consistently converged to 1 for females, and for both sexes in 2016, we also fixed $p = 1$ for those sex-year combinations. All other recapture probabilities were estimated by comparing models that allowed variability by age, sex, and year using QAICc (AIC adjusted for sample size and over-dispersion) (Burnham and Anderson 2002). The top performing model for p was then used alongside alternative parameterizations for ϕ.

Apparent Survival Probability (ϕ). To keep our model selection process down to a reasonable number of models (Burnham and Anderson 2002), we first examined the influence of demographic categories and time (age, sex, and year) on apparent survival. We carried forward the highest-ranked term(s) into an intermediate set of models where we also considered univariate effects of phenological variables and seasonal climate variables (emergence dates, season length; winter, spring, summer, or fall climate) interacting with age. The top-ranking variables were then used to construct a final set of models where we considered multiple climate and phenology variables in the same model, so long as they were not multicollinear. By avoiding model dredging, we acknowledge we may have overlooked certain variable combinations supported by the data, and of importance in affecting actual UGS survival.

1. Demographic categories. Within each subset of *a priori* models, we only considered models that tested for ecological hypotheses of interest. For instance, we expected survival to be different between males and females because females bear the cost of reproduction, and after gestation and provisioning for their offspring, they have less time to gain mass before estivation begins (Knopf 1973). We also anticipated differences in survival between juveniles and older age classes because juveniles have the shortest amount of time to gain mass prior to estivation. Models with three age classes (juvenile, yearling, and adult) versus two age classes (juvenile, yearling and adult combined) were compared, as we did not anticipate large differences in survival between yearlings and adults (Oli et al. 2001).

2. Phenological variables. We compared models that accounted for the effect of emergence date, season length, and a cross-seasonal effect of either on survival (*t*-1 to *t* and *t* to *t*+1). Emergence date was the calendar date the first squirrel was seen in a given year, and season length was based on the amount of time squirrels were known to be active, from emergence to estivation in a given year. An early emergence date may be beneficial (if green-up and snowmelt are also initiated early), or detrimental (if snow cover persists beyond emergence). Similarly, a

longer season may be beneficial if food resources haven't yet senesced, and nutritional quality is not compromised.

3. Seasonal climate variables. We considered the number of below-freezing days after estivation and before permanent snowpack, winter temperature, winter snowfall, maximum March temperature, growing degree days (GDD), Bagnoul-Gaussen summer drought index (BGI), summer temperature, and summer precipitation. We tested for correlations between climate variables (Appendix 1.3; using Pearson's product moment correlation coefficient) to avoid inclusion of multicollinear variables in a given model. Variables that were significantly correlated or clearly redundant with other variables (e.g., BGI is calculated with precipitation and temperature) were never considered as part of the same model.

Model selection. We used QAICc to score the top model(s) in each tier of our model selection process (see above), while ensuring that each model reflected ecological hypotheses of interest, as per the Lebreton et al. (1992). Because time-varying climate variables are redundant with annual changes in survival, generic (fixed effect) time variation in survival (if selected in our demographic set of models) was never considered along with time-varying covariates in the final model set. Overall, this tiered approach was preferred because it allowed us to consider models that reflected relevant ecological hypotheses while restricting the number of models considered to a reasonable level. Further details regarding model selection, including the complete set of candidate models, are available in Appendix 1.2.

We used analysis of deviance (ANODEV function, MARK; White and Burnham 1999) to estimate the proportion of temporal variance in survival explained by season-specific variables by calculating the proportion of model deviance explained by a particular variable relative to two extremes: a model with full time variation and a model that excluded temporal variation.

RESULTS

Trends in climate and phenological variables

Trends in the climate variables that we considered in our analysis indicated warming over the study-period, consistent with global and regional trends (e.g. IPCC 2013). Specifically, average winter temperatures marginally increased over time (by 1.58 ºC; $p = 0.054$, Adj. $R^2 = 0.051$), and maximum temperatures in March (March 15-31) have increased significantly over time as well (by 3.56ºC; $p = 0.007$, Adj. $R^2 = 0.111$). Unlike regional trends, average winter snow depth did not decline ($p = 0.435$, Adj. $R^2 = -0.007$). Average summer temperatures also increased (1.79 ºC; $p < 0.001$, Adj. $R^2 = 0.199$), while average summer precipitation declined over the study-period (-0.60 mm; $p = 0.012$, Adj. $R^2 = 0.097$). Although the BG drought index declined over time (-4.14; $p < 0.001$, Adj. $R^2 = 0.201$), indicating drier summer conditions in recent years, growing degree days (from average emergence to average juvenile emergence from their natal burrow) did not significantly change over time ($p = 0.692$, Adj. $R^2 = -0.016$) (Figure 2.1). Phenological variables did not exhibit any temporal trend (emergence: Welch's t-test, $t = 0.0258$, df = 5.5412, $p = 0.9803$; season length: $t = 0.9381$, df = 6.4662, $p = 0.3819$): the average emergence dates (historical calendar date = 92.4, contemporary Julian date = 92.3) and season length (historical = 128.6 days, contemporary = 123.4 days) did not change over time either.

Capture-mark-recapture analyses

The GOF test conducted on the most parameterized model indicated that juveniles in 1967 violated the assumptions of Test 3 ($\chi^2 = 74.1728$, df = 19, p = 0). However, the addition of cohort effects for these two groups resolved the issue ($\chi^2 = 15.6503$, df = 16, p = 0.4776) (see Appendix 1.2 for details). Note that Test 3 determines whether individuals tagged that particular year were seen again at the same rate as individuals tagged in previous years. Because the survival interval that violated assumptions was the last for our study period, we are not concerned about this result which simply indicates that our study ended and was reinitiated many years later. We estimated a $\hat{c}$ value of 3.89 and used it to correct for moderate over-dispersion in the data.

The top model for detection probability included an effect of sex and outperformed the next best model by 2.001 QAICc point (p ~ time). We retained this parameterization for p for the remainder of the model selection process. There were several models within 2 QAICc points that explained variation in apparent survival probability among years and demographic categories, but all top models included age class (juveniles versus adults and yearlings combined), so this term was included in the following tiers of model selection. On average, adult and yearling survival was higher (estimate = 0.41 +/- 0.01) than juvenile survival (estimate = 0.29 +/- 0.01).

Next, we considered models with an interaction between age class and each climate variable, including partial interactions. There were several models with climate variables within 2 QAICc points of the top model, including full (i.e. age class) and partial (i.e. juveniles) interactions between age and winter temperature, age and emergence date, and a partial interaction between juvenile and growing degree days. We compared these models to ones with more structure that included additive effects of multiple climate terms (Table 2.1). Overall, the best performing model included an interaction between age class (i.e. juveniles and yearlings/adults) and winter temperatures and an interaction between age class and emergence date (Figure 2.2-2.3, Table 2.1). This model indicates that warmer winter temperatures had a positive effect on juvenile survival (beta estimate = 0.18 +/- 0.06) but a negative effect on adult/yearling survival (beta estimate = -0.37 +/- 0.07), while a later emergence date in the following year had a positive influence on survival (beta estimate juveniles = 0.13 +/- 0.07, beta estimate adults/yearlings = 0.37 +/- 0.07), although the magnitude was larger in adults/yearlings. The second best performing model

(ΔQAICc = 0.35) only differed by inclusion of a partial interaction between juveniles and growing degree days (Table 1.2E), whereby more growing degree days (measured between emergence of UGS from hibernation and emergence of juvenile UGS from their natal burrows) had a positive influence on juvenile survival (beta estimate = 0.16 +/- 0.06).

The top model explained a marginally significant amount of deviance associated with temporal variation relative to the model that only accounted for UGS demographic categories (ANODEV, F = 3.09, $p = 0.082$). Similarly, the second model also explained a marginally significant amount of deviance (ANODEV, F = 2.92, $p = 0.097$).

DISCUSSION

Has survival changed over the past 50 years?

While UGS survival has not changed significantly over time, annual survival fluctuates strongly in response to emergence phenology and climate. Since UGS population growth is quite sensitive to proportional changes in juvenile survival (Oli et al. 2001), changes in juvenile survival likely led to important fluctuations in the population growth rate and UGS density. Although the historical capture area was more heterogeneous in its vegetation than it currently is, and the trapping area today might not be exactly in line with what it used to be, we used the Horovitz-Thompson estimator (Hogben and Cross 1952) to calculate approximate density (Figure 2.4). Since juveniles are more likely to emigrate, density was only calculated for adults and yearlings (i.e., residents). UGS density does appear to fluctuate between years, consistent with our finding that survival is affected by environmental change and warming. Since climate is predicted to become more unpredictable, this may lead to greater fluctuations in population size and negatively impact population growth rate in the future.

Does climate play a role in explaining observed changes in survival?

Although temperatures at the study site increased in both summers and winters over time, and precipitation in summer declined over the span of this study (as expected from larger regional trends), snow depth at our site did not significantly change. Snow depth did not appear to be an important variable in any of the survival models. Interestingly, phenological variables (emergence and season length) did not indicate a significantly linear change over time either, despite changes in climate.

Winter temperatures may affect ages differently, with higher juvenile survival predicted for warmer winters, and adults/yearlings negatively affected by warmer winter temperatures (Figure 2.2-2.3, Table 2.2). Warmer winter temperatures, particularly closer to emergence, may result in earlier snowmelt and green-up that spring. A similar trend is seen in yellow-bellied marmots, where snow melt constrains the beginning of the growing season and has a significant impact on reproduction, litter size, and juvenile body mass, with persistent snow cover leading to less frequent reproduction (Van Vuren and Armitage 1991). If emergence from hibernation also occurs later than average, there may be a better overlap between UGS and their food (i.e. phenological match). Since juveniles are morphologically the smallest age group, they may benefit more from this phenological match (or are less able to survive a mismatch). Colder temperatures could benefit adults/yearling UGS since colder temperatures can reduce the energetic output necessary during hibernation (Kronfeld-Schor and Dayan 2013) and allow UGS to remain in hibernation longer (Turbill and Prior 2016). However, if temperatures continue to warm as predicted and snow depth declines, there may be negative consequences for survival, as seen in alpine marmots (e.g., Tafani et al. 2013; Rezouki et al. 2016).

In the spring, later emergence led to increased survival for all age classes, which may similarly be due to a better match with food resources, since UGS are more likely to find forage instead of snow if they emerge later in the year. Previous research on the same population also found that in early springs, more UGS females failed to produce litters or disappear entirely prior to recruitment (Walker 1968), which supports our results. Earlier springs led to asynchronous emergence across age classes, with adults emerging earlier than yearlings. When this happens, adults act more aggressively towards yearlings, since they are more likely to have already established territories and mated, which often results in yearlings failing to reproduce or emigrating away from their natal colony (Walker 1968). Earlier emergence may also expose UGS to predation for a longer period of time. In other hibernating species, a later emergence led to increased survival since remaining in hibernation provided protection from predation by most predators (Bryant and Page 2005; Turbill et al. 2011; Bieber et al. 2014), with the exception of burrowing predators such as badgers (*Taxidea taxus*) and weasels (*Mustela spp.*) (Walker 1968). Although badgers are present at our site, hawks (e.g., *Buteo jamaicensis*), foxes (*Vulpes vulpes*), and coyotes (*Canis latrans*) are also known predators of UGS (Amend 1970), thus remaining in their burrows for a longer time period in the spring would provide additional protection for UGS.

We had expected hotter and drier summers to have a negative impact on the quality of food or cause estivation to begin earlier; in 1966, the warmest and driest year within the historical study, the active season ended the earliest and was the shortest (even though emergence from hibernation that year was intermediate to the other years), while the cooler and rainier years had seasons that ended later (Knopf 1973). This does not appear to occur in the most recent years of our study; in fact, warmer summers with more growing degree days between emergence from hibernation and juvenile emergence from their natal burrows led to a greater over-winter survival probability for juveniles (Table 1.2E). In UGS, juvenile emergence from their natal burrows typically coincides with peak food abundance (Walker 1968), and growing degree days can reflect the phenology of food resources. The presence of cheatgrass at the study site (C. Falvo, unpublished data), that was never mentioned to be present historically (Walker 1968), may be increasing forage availability to UGS by outcompeting sagebrush (Stewart and Hull 2009). Sagebrush is only consumed in small amounts by UGS (~ 10%) and is not their preferred food (Walker 1968)). Juveniles emerge at a time when grasses are at their most abundant (Walker 1968), further suggesting that juveniles do not eat large amounts of sagebrush. The substitution of cheatgrass for sagebrush may have improved forage availability for juveniles, particularly via massive seed production, and continued warming may allow cheatgrass to further dominate the area (Chambers et al. 2007; Compagnoni and Adler 2014) and benefiting this particular UGS population. Cheatgrass establishment, biomass, and seed production is most strongly constrained by cold temperatures (Chambers et al. 2007), further suggesting that warming temperatures are beneficial for its establishment and persistence. The increase in cheatgrass biomass which persists through the summer at high elevations within its range could outweigh the negative effects of a decline in native grasses for UGS. However, the only years in this study with warmer than average summers were 2016 and 2017. Although we did not detect any changes in the number of growing degree days over the last 50 years, overall summer temperatures are predicted to continue warming, and UGS may experience negative consequences of hotter and drier summer in the future that we are not yet able to detect.

Conclusions

These results add to our understanding of the relationship between climate change, phenology, and demography, as illustrated by changes in survival in hibernating ground squirrels.

Although the phenology of UGS does not appear to have changed over time, UGS survival is affected by climate, but each age class responds differently. Warmer winter temperatures may increase juvenile survival, but decrease adult survival, making it difficult to predict how this population may respond to prolonged and increasing warming. We suspect warmer temperatures in our study area have facilitated the invasion of cheatgrass, and that this relatively new food source is benefiting UGS, especially juveniles whose emergence coincides with leaf and seed production. The potential negative impact of warming on UGS survival might have been tempered by the invasion of cheatgrass, although this buffering effect may be short-lived. Additional work will attempt to quantify how an invasive species might have led to an improvement in juvenile body mass, ultimately leading to a substantial improvement in juvenile survival and overall population abundance.

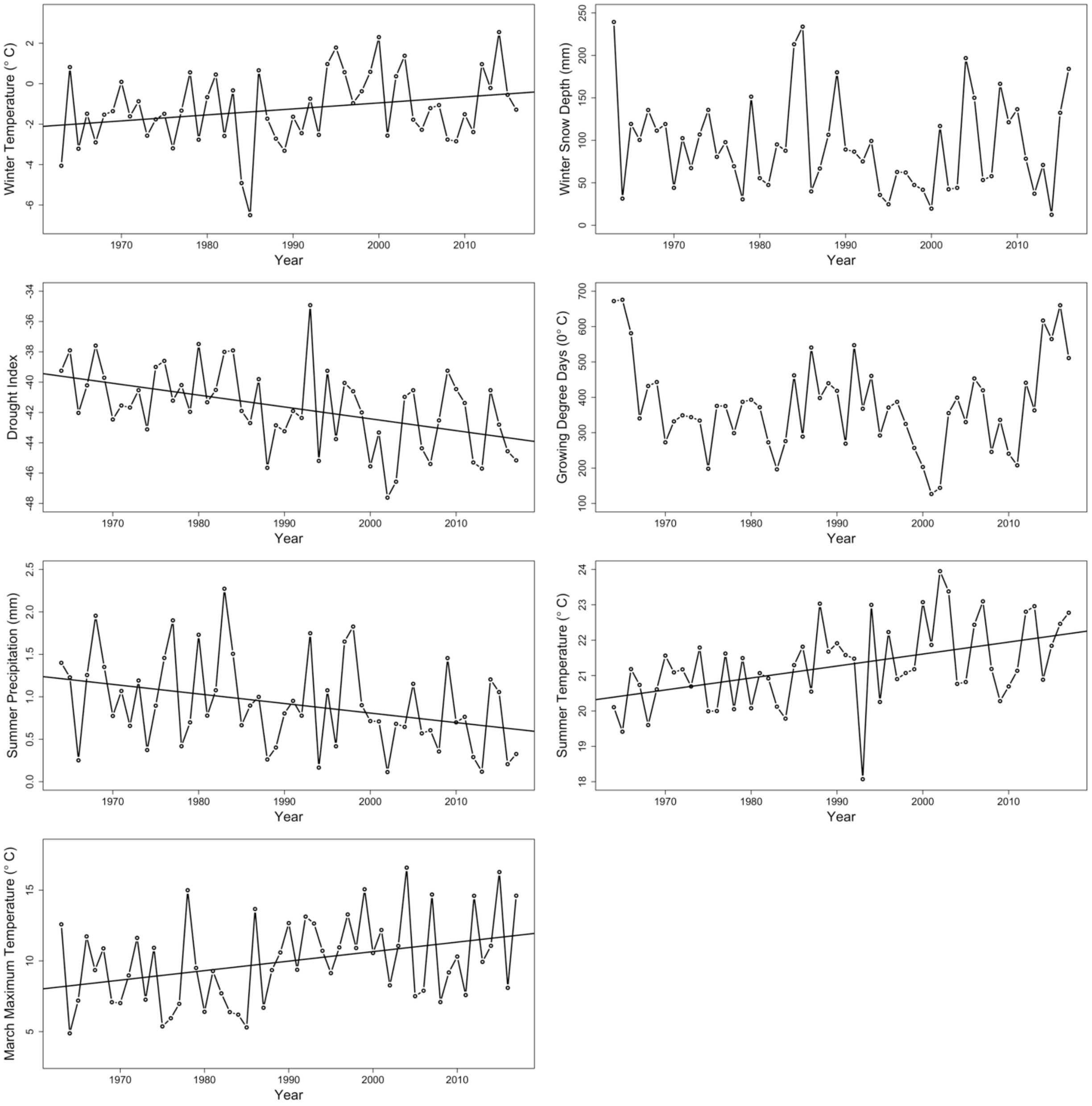

Figure 2.1. Trends in climate variables (winter temperature, winter snow depth, BG drought index, growing degree days, summer precipitation, summer temperature, and maximum March temperature) from 1960-2017 in Logan, UT.

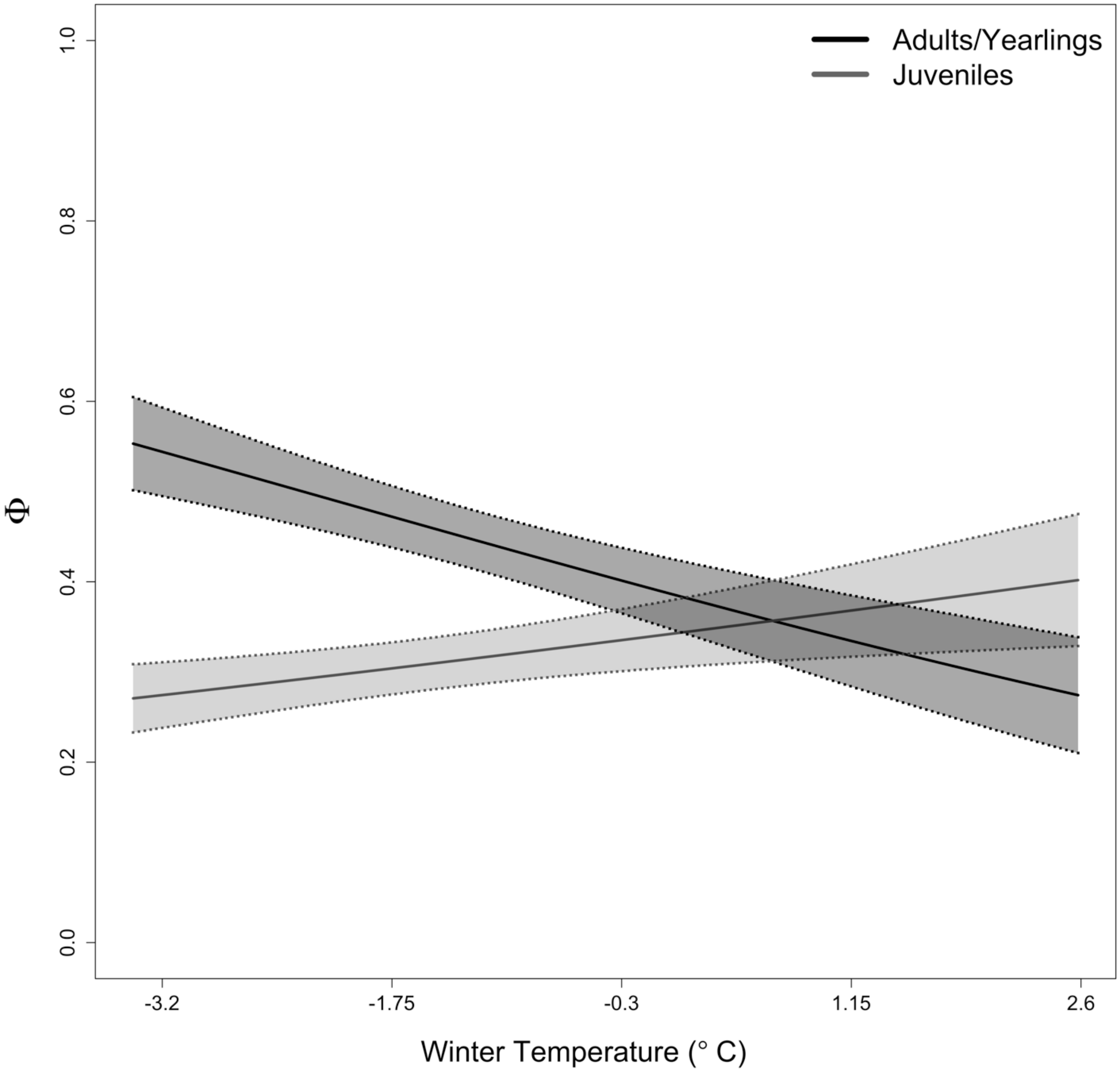

Figure 2.2. The interactive effect of age class (adults and yearlings = black, juveniles = grey) and winter temperature on apparent survival (**Φ**) from the top-ranked model, while holding emergence date constant at its mean value.

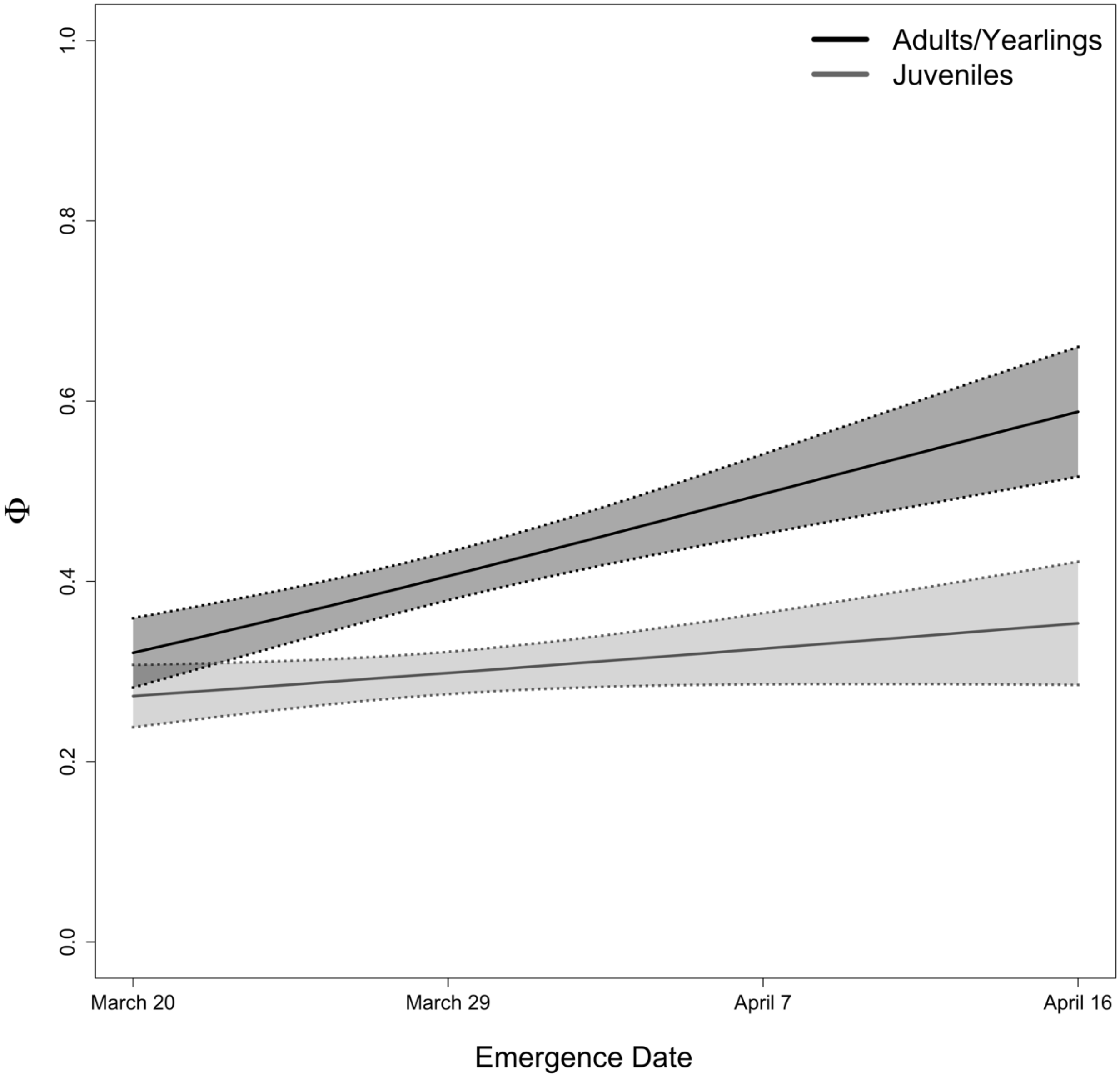

Figure 2.3. The interactive effect of age class (adults and yearlings = black, juveniles = grey) and emergence date on apparent survival (**Φ**) from the top-ranked model, while holding winter temperature constant at its mean value.

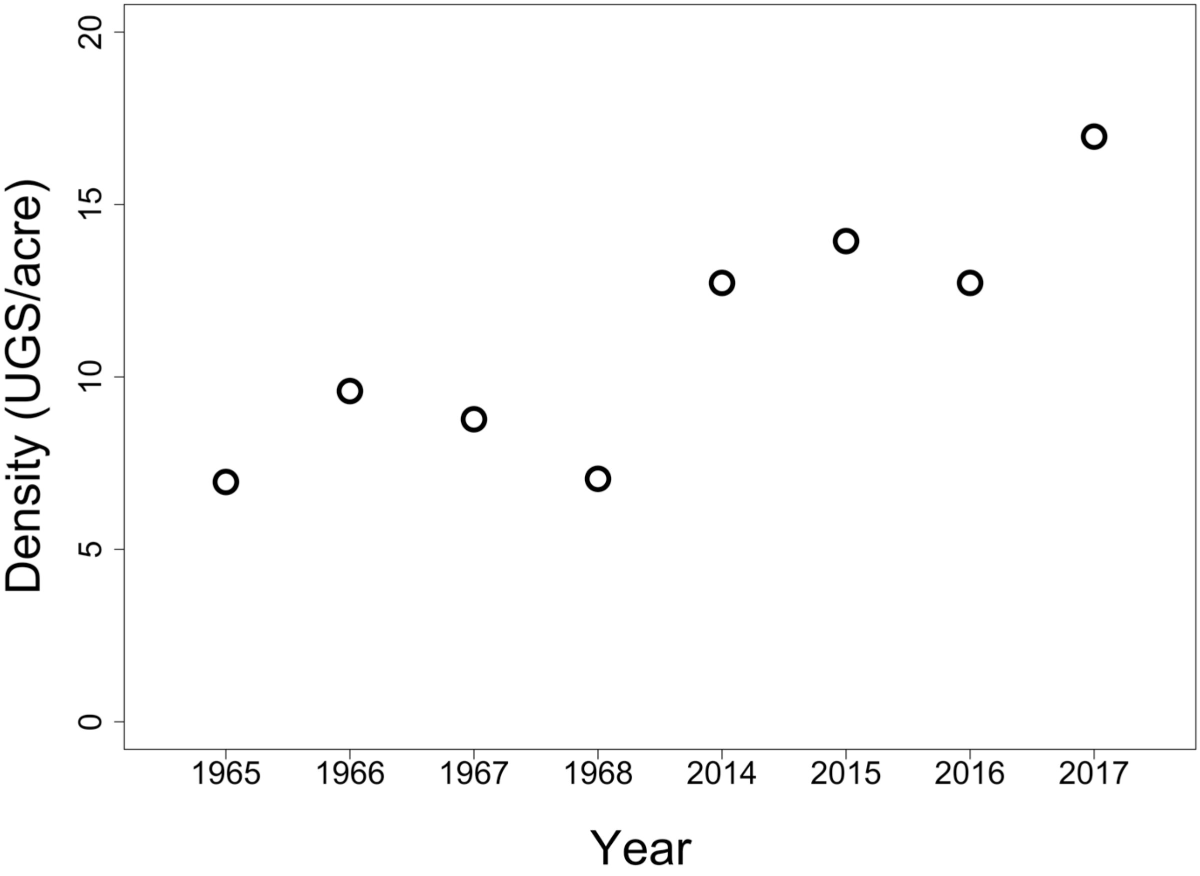

Figure 2.4. Density of UGS per acre over years of the study.

Table 2.1. Final model selection, where "age" refers to the age class (juvenile vs adult and yearling), "adyr" and "juv" are used for models with partial interactions, "wint" refers to winter temperatures, "emer" refers to emergence date, and "gdd" refers to growing degree days. Number of parameters (np), adjusted AIC (QAICc), change in QAICc relative to the top model (ΔQAICc), weight of each model (Wt), and deviance (QDeviance) are also presented.

Model	**Np**	**QAICc**	**ΔQAICc**	**Wt**	**QDeviance**
~-1 + age:wint + age:emer + juv + adyr	7	1778.95	0.00	0.32	780.54
~-1 + age:wint + age:emer + juv:gdd + juv + adyr	8	1779.29	0.35	0.27	778.88
~-1 + age:wint + juv:gdd + juv + adyr	6	1782.74	3.80	0.05	786.34
~-1 + age * wint	5	1782.88	3.94	0.05	788.49
~-1 + juv:wint + juv:gdd + juv + adyr	5	1783.27	4.32	0.04	788.87
~-1 + juv + adyr + juv:wint	4	1783.42	4.47	0.03	791.03
~-1 + juv:wint + age:emer + juv + adyr	6	1784.07	5.12	0.03	787.67
~-1 + age:wint + juv:emer + juv + adyr	6	1784.11	5.17	0.02	787.71
~-1 + age * emer	5	1784.12	5.18	0.02	789.73
~-1 + age:emer + juv:gdd + juv + adyr	6	1784.41	5.47	0.02	788.01
~-1 + juv:wint + age:emer + juv:gdd + juv + adyr	7	1784.42	5.47	0.02	786.01
~-1 + age:wint + juv:emer + juv:gdd + juv + adyr	7	1784.46	5.51	0.02	786.05
~-1 + juv + adyr + juv:gdd	4	1784.62	5.67	0.02	792.23
~-1 + juv:wint + juv:emer + juv + adyr	5	1784.62	5.68	0.02	790.23
~-1 + juv + adyr + juv:emer	4	1784.77	5.83	0.02	792.38
~-1 + juv:wint + juv:emer + juv:gdd + juv + adyr	6	1784.97	6.02	0.02	788.57
~-1 + juv:emer+ juv:gdd + juv + adyr	5	1785.06	6.12	0.02	790.67
~-1 + age	3	1785.54	6.60	0.01	795.16
~1	2	1799.84	20.90	0.00	811.46

Table 2.2. Beta survival estimates from the top model, which includes an interaction between winter temperatures and age class (adult and yearling versus juvenile) and an interaction between emergence date the following year and age class, along with standard error (SE), lower confidence limit (LCL), and upper confidence limit (UCL).

Group	**Beta Estimate**	**SE**	**LCL**	**UCL**
Juv	-0.7896	0.0688	-0.9244	-0.6548
AdYr	-0.1895	0.0674	-0.3216	-0.0574
AdYr:wint	-0.3661	0.0719	-0.5070	-0.2252
Juv:wint	0.1830	0.0644	0.0568	0.3093
AdYr:emer	0.3714	0.0708	0.2326	0.5102
Juv:emer	0.1265	0.0703	-0.0114	0.2643
P (sex)	0.8488	0.5884	-0.3044	2.0021

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CHAPTER 3

The cost of immunity in the wild: elevation shapes life history trade-offs in an endemic hibernator

INTRODUCTION

Identifying the ecological factors that shape the evolution of life histories has been a topic of great interest and research for several decades (Stearns 1992). Many studies of wild vertebrates have observed differences in phenology, reproduction, and survival among populations that live at different elevations (Dunmire 1960; Bronson 1979; Zammuto and Millar 1985; Grant and Dunham 1990; Bears et al. 2009), with higher elevations often characterized by a shorter growing season, lower temperatures, and limited food resources (Bronson 1979; Zammuto and Millar 1985; Ferguson and McLoughlin 2000; Bears et al. 2009; Sheriff et al. 2011). Populations experiencing such conditions tend to achieve lower fecundity, delayed recruitment, and higher adult survival rates, leading to slower life histories (Dunmire 1960; Bronson 1979; Zammuto and Millar 1985; Bears et al. 2009).

Because resources are scarcer at higher elevations, individuals may incur trade-offs between competing functions such as growth, immunity, and reproduction, ultimately shaping different life history strategies than that of lower elevation populations (Dunmire 1960; Stier *et al.* 2014). In the wild, trade-offs and contrasted life history strategies observed across elevations have been documented, with previous investigations supporting the hypothesis that populations adopt elevation-specific life history strategies to maximize fitness at a given elevation (Dunmire 1960; Zammuto and Millar 1985; Bears et al. 2009). Research on how individuals trade limited resources among key functions such as immunity, self-maintenance, growth, and reproduction is needed to advance life history theory (Stearns 1992), especially since the environment is changing at an unprecedented pace (Fuller et al. 2010).

There is a large body of research in laboratory settings that has helped shed light on trade-offs in vertebrates among fitness components such as growth and reproduction with physiological responses involving immunity and stress (Alonso-Alvarez et al. 2004; Wiersma et al. 2004; French et al. 2007; Pike et al. 2007; Cutrera et al. 2014; Neuman-Lee et al. 2015). Indeed, maintaining immune function is energetically costly, and laboratory studies of captive animals have shown that as the host's immune system is stimulated, a proportional decrease in growth occurs as resources are diverted towards immunity (Lochmiller and Deerenberg 2000). Experimental work on wild vertebrates (mostly reptiles and birds) experiencing natural environments, but where aspects of physiology were manipulated, have also provided a better understanding of physiological trade-offs, such as a decline in reproduction (Ilmonen et al. 2000) and parental care (Raberg et al. 2000) as a result of immune stimulation.

Investigations of life history trade-offs between physiology and fitness in wild mammals are less common (but see Boonstra, McColl & Karels 2001; Nussey *et al.* 2009; Dantzer *et al.* 2010). Such studies largely confirm findings from laboratory experiments: for example, an increase in growth rate was positively correlated with an increase in oxidative stress in Soay sheep (*Ovis aries*) (Nussey et al. 2009), and an investment in reproduction was related to increased stress hormones and decreased immune function in male Arctic ground squirrels (*Spermophilus parryii plesius*) (Boonstra et al. 2001). Although chronic increases in stress hormones have been shown to depress the immune system (Dhabhar 2000), and oxidative stress has been proposed as a mechanism responsible for a decline in survival and reproduction with age (Nussey et al. 2009), directly testing the ability of organisms to respond to immune threats would provide a more relevant measure of immunocompetence in the wild. Bacterial killing assays characterize a functionally relevant immune response involving phagocytes (in whole blood), opsonizing proteins (complement and acute phase proteins), and natural antibodies (mainly IgM and IgA) (Demas et al. 2011). This assay measures the ability of an organisms' innate immune system to eliminate a pathogen by incubating plasma samples with a strain of bacteria that could be encountered in the wild, eliciting an immune response that is ecologically relevant (French and Neuman-Lee 2012).

In this study, we examine the relationship between innate immune function (measured by a bacterial killing assay) and changes in body mass during the growing season in two wild populations of Uinta ground squirrels (UGS, *Urocitellus armatus,* Kennicott 1863) that exist 600 meters apart in elevation (but only 5.8 km apart geographically), while accounting for differences in age and sex, to determine whether populations at different elevations exhibit contrasted life history strategies, and how those are mediated by trade-offs between immunity and body mass, which is a key determinant of fitness in UGS (Sauer & Slade 1987).

UGS are sciurids that hibernate 7-9 months out of the year (Eshelman and Sonnemann 2000) and inhabit mountain and subalpine habitats (historically 1200-2400 m., Eshelman & Sonnemann 2000). They must reproduce and amass enough body fat to survive hibernation within a short window of time, with that window being much shorter at higher elevations relative to lower elevations (Knopf 1973). We predict that the UGS population located at the higher elevation will invest more heavily in gaining mass to compensate for a longer hibernation and a shorter active season. We further expect them to exhibit lower levels of bacterial killing ability, as they will have to allocate limited resources towards the accumulation of body mass reserves throughout their active season, at the cost of immunity. Because the strength of life history trade-offs is primarily driven by resource availability and allocation (van Noordwijk and de Jong 1986), accordingly, individuals located at the lower elevation site should experience weaker trade-offs, given an extended active season and access to abundant resources throughout (see Appendix 2.1 for data on plant biomass).

METHODS

Uinta ground squirrel ecology

UGS are a colonial species that emerge from hibernation between April and May and enter estivation in August, depending on elevation (Eshelman and Sonnemann 2000). UGS typically reproduce within a week of emergence, and females produce a single litter each year of four to six juveniles which can live up to seven years in the wild. The factors that determine whether yearlings reproduce vary between sexes; yearling males rarely emerge in breeding condition, while yearling females often emerge too late in the year to obtain quality territories and mates (Walker 1968; Eshelman and Sonnemann 2000). Reproductive activity depends on both population density and spring climatic conditions, which influence emergence date and emergence order of each sex and age class: adult males and females emerge before yearlings in warmer springs, but colder springs cause a more synchronous emergence of sex and age classes (Knopf 1973). Knopf (1973) studied UGS populations in the 1960's located in Logan Canyon (Cache Valley UT) at two elevations: the Forestry Field Station in Logan Canyon (FS, 1830 m.), and near Tony Grove Lake (TG, subalpine, 2400 m.) and observed important differences in phenology between sites (Knopf 1973; Knopf and Balph 1977). UGS were active for 25% longer at FS than TG, and UGS at FS emerged earlier than at TG by approximately a month in each documented year (Knopf 1973). Despite the longer growing season at the lower elevation site, their weights at emergence and immergence were comparable to those of the subalpine population (Knopf 1973). The date at which each site was snow-free determined when food was accessible to them (UGS are mostly herbivorous, feeding on seeds and leaves of graminoids). In years when squirrels emerged long before snowmelt (snow melted late relative to other years), their body mass typically declined before increasing again as food became accessible (Knopf and Balph 1977), which indicates that their ability to gain mass is dependent on snowmelt.

Field sites and data collection

The data were collected in 2016 at two field sites in Logan Canyon, UT. The lower elevation site is located at the Utah State University Forestry Field Station (FS, 1830 m.), and the higher elevation site is found near Tony Grove Lake (TG, 2400 m.). Those sites were chosen so that as part of overall project objectives, we could make direct comparisons to the historically studied populations mentioned above (Knopf 1973). We recognize that the lack of spatial and temporal replicates considerably limits our inference on life history trade-offs across elevations (Quinn and Keough 2002). An optimal design would have included multiple sites at each elevation, followed over multiple years, but limited logistical difficulties pertaining to access to additional high elevation sites precluded us from following such a design. As a first step, this analysis will set the stage for similar studies on life history trade-offs between immunity and fitness components across elevations.

The two sites are not connected by dispersal. The maximum home range of a UGS is about 1080 square meters (Paul 1977), and dispersal distance in sciurids is never more than about 10 times an individual's home range; while FS and TG are about 5.8 km apart from one another and separated in elevation by rugged terrain, dispersal is impossible between those two populations. These sites are similar in terms of the predator community they support (i.e. the American badger *Taxidea taxus*, the red-tailed hawk *Buteo jamaicensis*, the long-tailed weasel *Mustela frenata*, and the coyote *Canis latrans*; Knopf and Balph 1969; Slade 1972; Slade and Balph 1974; personal observations), and in terms of human disturbance (i.e. both sites support recreation activities such as snowmobiling and backcountry skiing in the winter, and camping and hiking in the summer). The sites are located almost 600 m apart in elevation and support slightly different plant communities that experience different climatic conditions and phenologies. The low elevation site includes a central lawn of grass (mainly *Bromus tectorum* and *Poa spp.*) and sagebrush (*Artemisia tridentata*), surrounded by aspen (*Populus tremuloides*). The high elevation site is mostly composed of sagebrush and aspen, and approaches the upper altitudinal limit of Uinta ground squirrel distribution in Utah (Knopf 1973). In 2016, the first date of bare ground (when all snow melted) for the low and high elevation sites, respectively, was April 12th and June 4th, and the first snow of the season was November 27th and September 23rd. Average temperature and precipitation during the UGS active season were 12.86ºC +/- 9.58 and 1.91 mm rain +/- 2.25 (Apr-Aug, +/- 2 standard deviations) at the low elevation site, and 13.65ºC +/- 10.12 and 1.35 mm rain +/- 3.46 (May-Aug, +/- 2 standard deviations) at the high elevation site (Utah Climate Center 2017).

UGS at both sites also experienced different phenologies in response to differences in elevation-specific climatic conditions. Trapping at the low elevation site occurred approximately every two weeks, from emergence post-hibernation on 08-Apr-2016 until entry into estivation around 29-Jul-2016, with 6 trapping sessions (18 total trapping days). Trapping at the high elevation site occurred from 01-Jun-2016 (when snow melted enough that the site was accessible) until 11-August-2016, with 4 trapping sessions (13 total trapping days). Emergence at the high elevation site is estimated around 17-May-2016, confirmed by visual sightings of squirrels on this date. In this particular year, the difference in emergence phenology between both sites was approximately 5 weeks, and the difference in timing of estivation was approximately 2 weeks.

UGS transition from estivation directly into hibernation, without reemerging from their burrows until the following spring.

Squirrels were caught using Tomahawk live-traps (Tomahawk, Wisconsin, USA) that were baited with rolled oats and peanut butter. Traps were set early before squirrels were active, checked within an hour after being set, and squirrels were processed within 2-3 hours of capture. Once captured, individuals were anesthetized using isoflurane to facilitate manipulations and blood draws. At initial capture, the age of the animal was determined and individuals were classified as juveniles (young of the year), 1-year-old (yearling), or ≥ 2-year-old (adult) based primarily on body mass and comparison with individuals of known age from previous years of trapping. Each individual's mass was determined by placing the animal in a cloth bag and using a Pesola scale (+/-2 g.; Pesola Company, Baar, Switzerland). Additional morphometric variables (body, hind foot, and tail length) were measured using a measuring tape or calipers. The reproductive status of males and females were recorded (males as scrotal or non-scrotal; females as pregnant, lactating, or non-reproductive). If individuals were not caught during the reproductive phase of their active season, they were not included in analyses of reproductive versus non-reproductive individuals (as we could not definitively determine reproductive status). Small, numbered, non-corrosive metal ear tags (Monel 1005-1, National Band and Tag Company, Newport, Kentucky) were placed on each squirrel at first capture for identification upon recapture. Passive Integrative Transponders (PIT tags, Biomark) were also injected under the skin to keep track of individuals in case of ear tag loss. All captured individuals were sampled for blood once within each trapping session.

Biological samples and immune assays

Blood samples were collected via retro-orbital bleed using heparinized microhematocrit capillary tubes (in accordance with IACUC protocol # 2220); bacterial killing ability (as measured by bacterial killing assay) has been found to be unaffected by capture stress and handling time (Strobel et al. 2015). After collection, blood samples were stored on ice in a cooler. Upon return to the laboratory (within 2-4 h of collection) blood samples were centrifuged (5 min., 2000 rpm) to separate plasma from red blood cells, then stored for later immune assays at -20°C.

Working under a sterile laminar flow hood, we performed bacterial killing assays on plasma samples, following a modified version of the methods described in French et al. (French et al. 2010) designed to use 96-well plates and spectrophotometry, eliminating some biases associated with counting colonies in the original protocol. Briefly, E. coli (ATCC #8739, Microbiologics, St. Cloud, MN) (1 pellet = 5.6 x 10^7 CFU) was added to 28 mL 1 M sterile PBS warmed to 37 °C, vortexed, and activated by incubation for 30 min at 37 °C to create a bacterial stock solution. The stock bacteria solution (5.6 x 10^7 CFU) was then diluted with sterile 1 M PBS for a working solution of 10^5 CFU. Plasma samples were diluted 1:8 in glutamine enriched CO2-independent media (Invitrogen Corp., Carlsbad, CA). This dilution was validated for serum with a dose response curve prior to the experiment. Diluted plasma and 6 uL bacterial were added to wells (run in duplicate) for a final dilution of 1:3. Positive controls of bacteria and media (1:3) and negative controls of PBS and media (1:3) were included on each plate to estimate bacterial growth. Plates were vortexed, incubated for 30 minutes at 37°, vortexed again, and 125 uL of tryptic soy broth (Sigma Aldrich T8907) was added to each well. A background absorbance reading at 300 nm was taken using a BioRad Xmark spectrophotometer. Following a 12 h incubation at 37°C, each plate was vortexed and absorbance at 300 nm was measured again. Bactericidal capacity was calculated as the mean absorbance for sample duplicates, divided by the mean absorbance for the positive controls (four wells containing only media and bacterial solution), and multiplied by 100 (i.e., % bacteria killed relative to the positive control). Inter-assay CV was 3.89%.

Variables and Statistical Analyses

Change in body mass. For yearlings and adults, we calculated a "within-individual" change in body mass during the active season by dividing the change in body mass (BM) by the number of days between captures when body mass measurements were taken ($BM_{final} – BM_{initial}$ / number of days; FS males n = 8, FS females n = 9, TG males n = 10, TG females n = 8). For each individual, we selected $BM_{initial}$ and BM_{final} by choosing sampling dates that took place before or early in the breeding season, to avoid fluctuations in body mass associated with pregnancy, and late in the season, to approximate body mass upon entrance into estivation as closely as possible (date ranges provided for early and late sample selection, respectively: FS early: 4/9-5/5 and late: 6/29-7/28; TG early: 6/1-6/24 and late: 7/10-8/11). Although other hibernators may reach peak body mass several weeks before estivation/hibernation (e.g. female Arctic ground squirrels, Sheriff et al. 2013), most UGS continued to gain weight until we no longer captured them and estivation was assumed. Note that at FS, all squirrels ceased activity abruptly in 2016 and entrance into hibernation was not staggered as documented historically. We refer to this measurement as the within-individual change in body mass for yearlings and adults (ΔBM_{YA}) which applies to those individuals for which body mass measurements were available both early and late in the season. We pooled yearlings and adults together because preliminary analyses indicated that although they may be structurally different (yearlings may be slightly smaller structurally), they gain mass at the same rate (sample size at FS: 7 yearlings and 10 adults; 9 females and 8 males; at TG: 9 yearlings and 9 adults; 8 females and 10 males). ΔBM_{YA} was used to model the relationship between the change in bacterial killing ability (calculations described below) and body mass throughout the active season for this subset of individuals.

Since juveniles do not reproduce, we utilized all repeated body mass information available for each individual captured on different trapping occasions to calculate a within-individual rate of change in body mass (ΔBM_J) (at FS: 29 individuals; TG: 8 individuals).

Bacterial killing ability (BKA) calculations. A within-individual change in bacterial killing ability, ΔBKA_{YA}, was calculated for yearlings and adults as follows: BKA_{final} - $BKA_{initial}$, where $BKA_{initial}$ and BKA_{final} were calculated from blood samples collected both early and late in the season, to utilize data from the same dates for both ΔBM_{YA} and ΔBKA_{YA}. $BKA_{initial}$ and BKA_{final} were selected using the same criteria as for ΔBM_{YA} calculations (at FS: 8 females, 7 males; at TG: 5 females, 7 males) and we used these values to model the relationship between changes in immune function (ΔBKA_{YA}), body mass (ΔBM_{YA}), sex, and site.

This value was not calculated for juveniles, as they were not above ground for a sufficient period of time to assess a seasonal within-individual change in BKA. To assess changes in immune function in juveniles, we utilized all BKA values collected over the active season to determine overall trends (between-individual; BKA_J) (FS: 73 measurements; TG: 12 measurements).

Statistical framework.

All statistical analyses were performed in R, version 3.2.3 (R Core Team 2013).

1. Factors affecting individual change in body mass. We tested for the effects of sex and site on ΔBM_{YA} and site on ΔBM_J during the active season using a two-way ANOVA to determine if body mass changes differed ("lm" function, Chambers 1992).

2. Factors affecting ΔBKA_{YA} during the active season. We tested for the effects of ΔBM_{YA}, sex, and site on ΔBKA_{YA} during the active season using linear models ("lm" function, Chambers 1992). We fit a global model accounting for all biological variables of interest, as well as biologically relevant interaction terms (i.e., sex by site and ΔBM_{YA}; site by ΔBM_{YA}). When parameter estimates

were not significant, they were removed one by one (starting with the least precise estimate) until AICc values no longer decreased to identify the best performing model based on the lowest AIC_C value and parsimony (i.e., with stepwise selection; Lebreton et al. 1992). We also analyzed the effect of an interaction between sex and site on ΔBKA_{YA} to determine whether there were differences in immune function between these groups.

3. Factors affecting BKA_J during the active season. Since juveniles have a much shorter active season, we could not attain sufficient sample size to test for within-individual differences in BKA over the active season in juveniles (i.e. ΔBKA_J). Instead, we examined the influence of sex, site, and date on overall changes in BKA_J values over the active season using linear mixed effect models to account for non-independence of repeated measurements within individuals (Venables & Ripley 2002). A similar analysis on BKA_{YA} is provided in Appendix 2.1.

4. Model diagnostics. For analyses described above in sections (1), (2), and (3), we conducted statistical diagnostics on the top performing model to test for normality of the residuals (Shapiro-Wilk normality test) and homoscedasticity (Bartlett test of homogeneity of variances). When model residuals were not normally distributed, we considered transformations (i.e. log for body mass, and square-root for BKA) of the response variable to normalize the relationship between the response and explanatory variables. If the assumption of homoscedasticity did not hold, we fit a generalized least squares model to allow variances to differ across the levels of the factor (per Zuur et al. 2009; varIdent function, package 'nlme'; Pinheiro and Bates 2000).

5. Within-individual versus Between-individual analyses. Between-individual analyses of body mass over the season (yearlings and adults: BM_{YA}; juveniles: BM_J) and bacterial killing ability for yearlings and adults (BKA_{YA}) were also performed using a mixed effect ANCOVA to test the effect of an interaction between site and date (BM_J) and the effect of site, date, and sex (BKA_{YA},

BM_{YA}) while accounting for repeated measured ("lme", package nlme). We also statistically assessed the effect of body mass on BKA_{YA} while accounting for sex, site, body mass, and date (Appendix 2.2). The main difference between both sets of analyses is that instead of using two observations per individual (early and late in the active season) to calculate within-individual changes in body mass and BKA, we utilize all of the data that we collected throughout the active season, without keeping track of individuals.

RESULTS

Yearling and adult body mass and immunity

Change in body mass (ΔBM_{YA}). Variances in ΔBM_{YA} between sex and site were unequal (Bartlett's K-squared = 5.728, df = 1, p-value = 0.017), prompting the use of a generalized least square regression model to test for the effects of sex and site on ΔBM_{YA}. Results indicated a significant effect of sex (estimate = 1.182, p-value = 0.004) and a marginal effect of the interaction between site and sex (estimate = 1.669, p-value = 0.051) on ΔBM_{YA}. The effect of site alone was not significant (estimate = 0.132, p-value = 0.858). Average ΔBM_{YA} was not significantly different between FS and TG females (t = -0.181, df = 8.098, p-value = 0.861), but males were significantly different across sites (t = -4.704, df = 12.409, p-value < 0.001). Male and female ΔBM_{YA} within each site were significantly different, with males gaining weight faster at both sites (FS t = -2.139, df = 11.769, p-value = 0.009; TG t = -3.899, df = 8.239, p-value = 0.004) (Table 3.1).

Change in immunity (ΔBKA_{YA}). On average, all groups (sex and site) declined in their bacterial killing ability throughout the season (Mann-Whitney test, mean +/- one standard deviation: FS female = -30.0% +/- 43.0, p-value = 0.055; FS male = -22.7% +/- 41.2, p-value = 0.375; TG female = -11.3% +/- 10.3, p-value = 0.063; TG male = -26.2% +/- 17.9, p-value =

0.016), although the decline was only marginally significant in FS and TG females, and not significant in FS males.

Immunity (ΔBKA_{YA}) and change in body mass (ΔBM_{YA}). The best performing model with lowest AIC_C included an effect of sex, site, and ΔBM_{YA}, as well as an interaction between site and ΔBM_{YA} on ΔBKA_{YA}. However, the effect of sex was only marginally significant (estimate = -24.374, p-value = 0.078), thus inference was based on the second best performing model which was more parsimonious, nested within the top model, and only differed from the top model by 0.556 AICc points (Table 3.2). Estimates from this model indicated that the relationship between ΔBM_{YA} and ΔBKA_{YA} over the growing season was different across sites (Table 3.3). At FS, it seems that ΔBKA_{YA} declined more (was more negative) over the active season for individuals that did not grow much, but remained mostly unchanged for individuals with a greater ΔBM_{YA}. However, at TG a greater ΔBM_{YA} was correlated with a decrease in BKA (a negative ΔBKA_{YA} value) (Table 3.3).

Juvenile body mass and immunity

Change in body mass (ΔBM_J). Variances in ΔBM_J between sex and site were unequal (Bartlett's K-squared = 9.856, df = 1, p-value = 0.002), prompting the use of a generalized least square regression model to test for the effects of sex and site on ΔBM_J. Results indicated no significant effect of site (estimate = 1.500, p-value = 0.223), sex (estimate = 0.001, p-value = 0.999), or an interaction between them (estimate = -1.413, p-value = 0.358) on ΔBM_J. Average ΔBM_J between sites were not significantly different for juveniles (Mann-Whitney test: W = 83.5, p = 0.237) (Table 3.1). Sample size was too small to allow for a comparison of ΔBM_J between sex at TG (TG: 7 females, 1 male; FS: 19 females, 10 males); however, ΔBM_J at FS was identical between sexes (Mann-Whitney test: W = 115, p-value = 0.371; mean female ΔBM_J = 3.53 g/day, male = 3.53 g/day) confirming that there is little difference in growth between sexes in juvenile UGS.

ΔBM_J was significantly higher than ΔBM_{YA} within sites (Mann-Whitney tests: in males at FS, W = 200, p-value = 0.002; in females at FS, W = 243, p-value < 0.001; in females at TG, W = 57, p-value = 0.010) and across sites (FS ΔBM_J vs. TG ΔBM_{YA} females, W = 193, p-value = 0.005; TG ΔBM_J vs. FS ΔBM_{YA} males W = 53, p-value = 0.031; TG ΔBM_J vs. FS ΔBM_{YA} females, W = 72, p-value < 0.001), with the exception of ΔBM_{YA} for males at TG (FS ΔBM_J vs. TG ΔBM_{YA} males, W = 92.5, p-value = 0.094; TG ΔBM_J vs. TG ΔBM_{YA} males, W = 43.5, p-value = 0.787) (Table 3.1).

Bacterial killing ability (BKA_J). The top performing model with lowest AICc included an effect of body mass, with a positive relationship between body mass and bacterial killing ability (estimate = 0.015, p-value = 0.006), indicating that juveniles increase bacterial killing ability as they grow over the season, but there were no significant differences in BKA_J between FS and TG. A model with an interaction between site and date indicated a significant positive effect of calendar date ("lme", package nlme; calendar date estimate = 0.0532, p-value = 0.028), but no significant effect of site (site estimate = -445.37, p = 0.799) or the interaction between site and date (date:site estimate = 0.0261, p = 0.791), confirming that there were no significant differences in BKA_J between FS and TG over the season.

DISCUSSION

As predicted, yearling and adult UGS at the high elevation (TG) gained body mass faster than their counterparts at the low elevation (FS). Despite the shorter growing season, yearlings and adults entered estivation at nearly the same body mass regardless of elevation, resulting from gaining mass faster at TG, the high elevation site. The difference was particularly pronounced in males, as they gained mass nearly twice as fast at TG than at FS. Although females at TG gained mass faster than females at FS, the difference was not significant; differences in reproductive rates at each site may have had an effect on the average increases in body mass. Notably, FS yearling females were more likely to reproduce than TG yearling females (based on captures while reproductively active; at FS, 90% of yearling females were reproductively active while only 55% reproduced at TG) (Falvo C., unpublished data).

We observed a decrease in within-individual immunocompetence (ΔBKA_{YA}) over the season at TG in yearlings and adults. This decrease was negatively correlated with the within-individual change in body mass (ΔBM_{YA}), indicating a possible immune cost of increased growth. Nussey et al (2009) found that in Soay sheep (*Ovis aries*), an increase in growth rate was associated with greater levels of oxidative damage, although this trade-off was only seen in juveniles (Nussey et al. 2009). At FS, the low elevation site, most individuals did not experience the trade-off individuals experienced at the high elevation site. The lack of a trade-off between body mass and immunocompetence throughout the active season might be explained by the increase in forage quantity during spring green-up that is especially pronounced at the lower elevation site (Appendix 2.1). Food resources can mediate changes in the relationship between fitness components and physiology (van Noordwijk and de Jong 1986; Bauer et al. 2013; Neuman-Lee et al. 2015), but when resources are not limiting, trade-offs might not be expressed in that particular time window. Further work will assess the role that forage quality and quantity play in shaping trade-offs, or lack thereof, between body mass and immunity at different elevations and over time.

Brooks and Mateo (2013) found that in another species of squirrel, *Urocitellus beldingi*, chronically-stressed individuals did not show differences in BKA, a measure of innate immunity, with respect to control animals. But when they were exposed to another immune challenge (bacterial lipopolysaccharides, "LPS"), BKA measured after LPS exposure was lower in the group that was exposed to chronic stress (Brooks and Mateo 2013). Stress related to predatory risk or lack of food resources may affect BKA when animals are immune challenged with naturally-occurring pathogens (microbes, parasites, or viruses), which makes it critical to assess if animals were facing other infections at the time of study. The two sites were similar in the predator community they support (see methods), but were chosen because of differential phenology, growing season length, and access to resources for UGS. Overall, differences in snowmelt and graminoid phenologies mostly explained difference in resource availability to UGS between sites, as opposed to biomass per se (Appendix 2.1). We did not detect a significant effect of ectoparasite loads on UGS immunity at either site (Appendix 2.3), although juveniles typically carried fewer ectoparasites than adults and yearlings, which may contribute to their ability to maintain an increase in immune function. We screened a subset of adults and yearlings (males and females) for key diseases that we expected to be present at those sites (14 blood samples were screened using real-time PCR for *Anaplasma phagocytophilum*, *Bartonella spp* (including *henselae*), *Ehrlichia chaffeensis*, *Rickettsia spp* (including Rocky Mountain Spotted Fever)) and none of the pathogens were detected in our samples. However, given the small subset of UGS screened for pathogens, and the potential for the influence of unmeasured sources of stress at the two sites, we cannot definitively rule out the potential influence of disease (including endoparasites) or stress on measures of innate immune response. We did not measure adaptive immune responses, but given the relatively short life span of UGS and the significant contributions of yearling survival and fertility to changes in the growth rate (Oli et al. 2001), we are comfortable associating UGS with a "fast" life history; a life history that is more likely to invest into innate immunity than it is into adaptive immunity. Based on our findings, we hypothesize that differences in food resources are likely the main driver of different innate immune responses across sites, but further data collection, including measurements of cortisol levels across spatial and temporal replicates, may help clarify this relationship.

Interestingly, juveniles did not exhibit trade-offs between body mass and immunocompetence in the same way yearlings and adults did (Fig. 3.1), despite gaining mass faster than older age classes. Although it is expected that juveniles will have a lower immune response than adults because their immune system is still developing (Levy 2007), studies on the ontogeny of immunity in wild animals are scarce. In birds, the evidence is ambiguous (Lozano and Lank 2003; Palacios et al. 2009; Arriero et al. 2013), while only a handful of mammal studies have found that juveniles have stronger physiological responses than adults (oxidative stress: Nussey *et al.* 2009; inflammatory response: Cutrera *et al.* 2014). These limited and conflicting results indicate that there is certainly variation in immune development among vertebrate classes, and possibly between species. Juvenile UGS at each site had similar ectoparasites burdens, but they did differ from adults and yearlings; their lower ectoparasite burden relative to that of older age classes may contribute to their ability to show an improvement in immune function throughout the active season. This is a rare documentation of the fact that UGS juveniles from a wild population are able to maintain an improvement in immune function despite their high investment in mass, while other age classes could not. We further observe that juveniles, unlike yearlings and adults, may not suffer a trade-off between investment in body mass and immunity because they do not have to allocate resources towards reproduction.

Although juveniles at TG emerged from their natal burrows lighter than juveniles at FS, the overall trends in body mass and immunocompetence were similar at both sites (Figs. 2.2B and 2.2D), with TG juveniles entering estivation lighter than FS juveniles. Because the active season at TG is shorter for all age classes, we had anticipated that juveniles, like yearlings and adults, would gain mass faster to ensure that they entered estivation at nearly the same body mass as the FS juveniles. Given that body mass is a key determinant of fitness, and particularly survival in UGS (Sauer and Slade 1987), we suspect juvenile overwinter survival will be much lower at TG where juveniles are overall lighter than at FS. Further data collection will allow for the estimation of over-winter survival rates between sites to determine if lower body mass is a contributing factor to lower juvenile survival, and potentially less recruitment at the higher elevation.

The historical studies on these same populations of UGS provide an interesting basis for comparison. Knopf (1973) found that UGS at FS and the subalpine site (equivalent to TG) both emerged from hibernation and began estivation at the same weight, even though UGS at FS had a longer above-ground season by approximately 25% (Knopf 1973). Comparison to historical data also indicates that existing UGS at FS have approximately the same body mass throughout the season now as they did in the past; while existing UGS at both sites entered estivation at approximately the same body mass, they emerged at TG lighter than at FS (Falvo C., unpublished data). Based on the historical data, the higher elevation UGS populations already gained mass faster than the UGS at FS (Knopf 1973); however, this disparity in the rate at which body mass accrues during the active season between elevations appears to have increased over the last 50 years.

Model predictions for climate change in the western US suggest milder winters and decreased snow insulation (Leung et al. 2004) along with more frequent extreme weather events (Carr 2013) such as spring snowstorms. Winter snowfall in this region has already decreased by nearly 10 percent in the last half century, with an increasing proportion of precipitation falling as rain instead of snow (Gillies et al. 2012). Since the date of snowmelt determines when UGS are able to access food (Knopf 1973) and winter snowpack can provide insulation that is crucial for over-winter survival (Tafani *et al.* 2013 Appendix D), climatic changes that have occurred in Logan Canyon over the last 50 years could be responsible for the differences in body mass dynamics at TG that we observed.

The results of this study add to existing knowledge on life history trade-offs and strategies across elevations in a number of ways: yearlings and adults adjusted their growth to compensate for different active season lengths and hibernation phenologies between elevations, with higher elevation individuals gaining mass faster within their shorter active season, at the expense of immune function. Increased growth, in conjunction with the energetic burden of reproduction, led to greater declines in immune function in higher elevation yearlings and adults, but this trade-off was not observed in juveniles, who gained mass faster but actually improved in immunocompetence throughout the season. Juveniles also weighed less at the higher elevation sites when weaned but grew at the same rate as their lower elevation counterparts, demonstrating no increased growth mechanism, despite the importance of body mass to overwinter survival (Sauer and Slade 1987).

Further research will help determine whether changes in body mass and immunocompetence vary between years at each site, if the observed relationship between mass and immunocompetence will hold over time, and whether disease burdens or stress play a role in these differences. Studying populations of the same species that experience different environmental conditions (in this case variable active season length and resource levels) is comparable to a natural experiment where climatic conditions and resources have been naturally manipulated. Elevation gradients provide a more accessible substitute for climatic gradients, and we hope to extend this study temporally in an effort to tackle challenging questions such as the impact of climate on life history strategies and trade-offs. We further hope to establish whether the decline in immunocompetence associated with higher investment in growth in yearlings and adults has consequences for other fitness components. By using continuous individually-based monitoring and sampling, we will be able to estimate the role phenotypic plasticity plays in shaping these different life history strategies across elevations, since plasticity in such trade-offs could ultimately affect survival and population growth, and thereby dictate the ability of UGS populations to adapt to an increasingly variable climate.

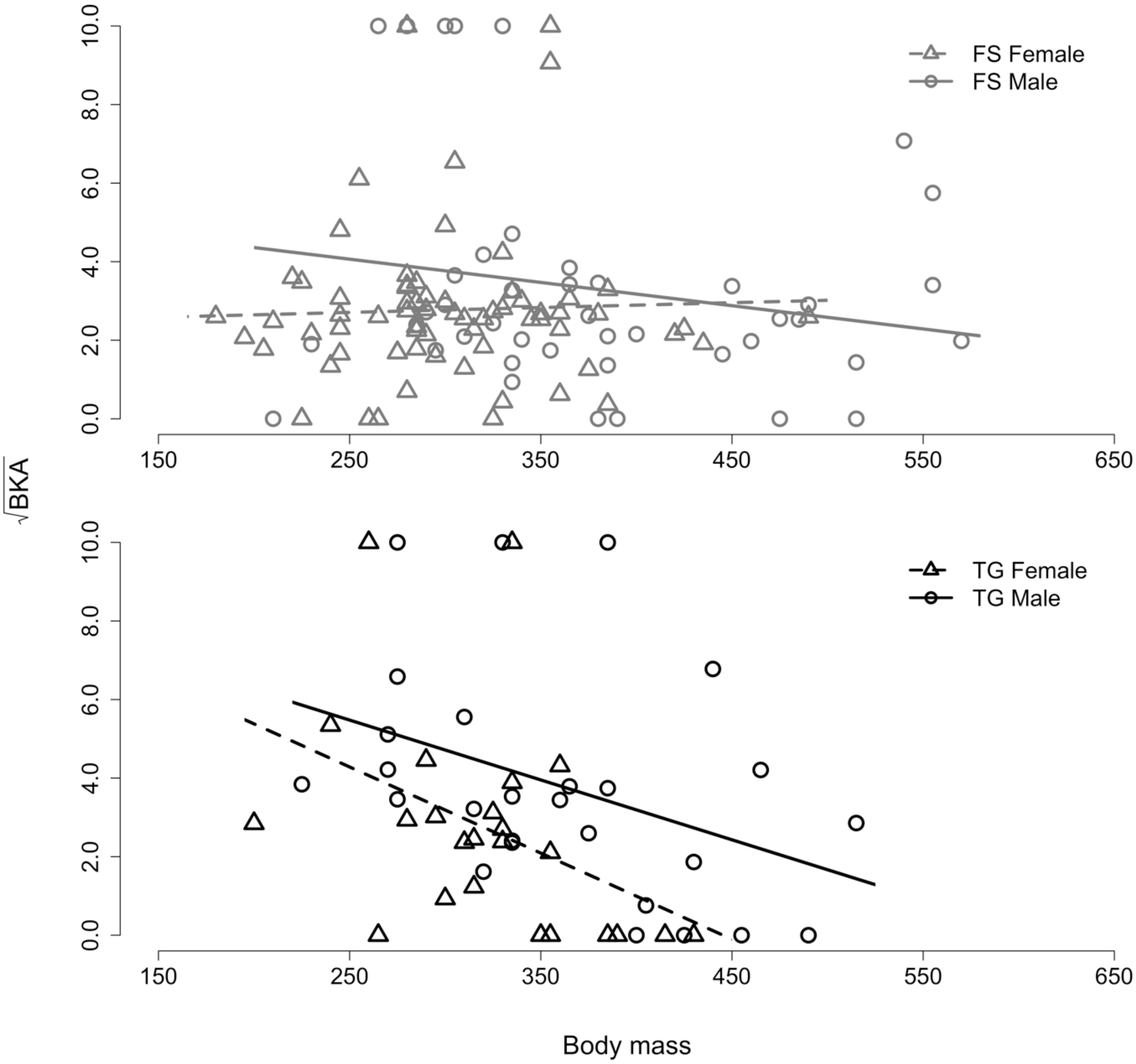

Figure 3.1. Relationship between body mass (in grams) and BKA_{YA} (bacterial killing ability, square-root transformed) for adult and yearling males (circles) and females (triangles) at the Forestry Field Station (upper panel; FS, grey) and Tony Grove (lower panel; TG, black) field sites, respectively.

Table 3.1. Average change in body mass (grams/day) throughout the active season for yearlings/adults and juveniles (ΔBM_{YA}) and (ΔBM_{J}), along with standard deviations, upper (UCI), and lower confidence intervals (LCI) are reported below.

Group	Average Change in Body Mass (g/day)	Std. Deviation	LCI	UCI
ΔBM_{YA} FS Female	1.21	0.59	0.06	2.37
ΔBM_{YA} FS Male	2.40	0.91	0.62	4.18
ΔBM_{YA} TG Female	1.35	1.98	-2.54	5.23
ΔBM_{YA} TG Male	4.20	0.65	2.90	5.49
ΔBM_{J} FS	3.53	1.26	1.06	5.99
ΔBM_{J} TG	4.85	2.97	2.38	7.31

Table 3.2. Model selection results on the effect of sex, site, ΔBM_{YA}, and biologically meaningful interactions on ΔBKA_{YA}. Model selection was based on AIC_C weights (w_i), reported along AIC_C, ΔAIC_C, deviance, and the number of parameters associated with each model (NP). Interaction terms are denoted by a colon.

Models	**AIC_C**	**ΔAIC_C**	**Deviance**	**NP**	**w_i**
Site:ΔBM_{YA} + Site + ΔBM_{YA} + Sex	264.960	0.000	248.759	4	0.423
Site:ΔBM_{YA} + Site + ΔBM_{YA}	265.516	0.556	252.658	3	0.320
~ 1 (intercept only model)	267.260	2.300	247.790	1	0.133
Sex:ΔBM_{YA} + Site:ΔBM_{YA} + Sex + Site + ΔBM_{YA}	267.685	2.725	247.790	5	0.108
Sex:Site+Sex:ΔBM_{YA}+Site:ΔBM_{YA}+Sex+Site +ΔBM_{YA}	271.682	6.722	247.682	6	0.015

Table 3.3. Model selection results on the effect of sex, site, ΔBM_{YA}, and biologically meaningful interactions on ΔBKA_{YA}. Estimates from the selected top performing and most parsimonious model includes site, ΔBM_{YA}, and an interaction between site and ΔBM_{YA}. Mean parameters estimates, along with standard errors, upper (UCI) and lower confidence intervals (LCI), and p-values are reported below. Interaction terms are denoted by a colon.

Parameter	**Estimate**	**Std. Error**	**LCI**	**UCI**	**p-value**
Intercept	-74.728	16.971	-107.991	-41.4645	< 0.001
Site (TG)	66.198	31.295	4.860	127.536	0.045
ΔBM_{YA}	26.761	8.523	10.056	43.466	0.005
Site(TG):ΔBM_{YA}	-30.113	11.234	-52.132	-8.094	0.013

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CHAPTER 4

Conclusion

In Chapter 1, we found that UGS survival has not changed significantly over time, but annual survival appeared to vary strongly with climate and with emergence phenology, especially for juveniles. Increasing winter temperatures had a negative effect on adults and yearlings, but a positive effect on juvenile survival. Emergence phenology also affected over-winter survival, with a later emergence associated with greater survival for all ages, likely because this led to a greater match between the timing of emergence from hibernation and the timing of plant green-up. Despite these variations in yearly survival, the density of this population increased over time. Juvenile survival was higher in years with warmer winters; since winters are significantly warmer than they were 50 years ago, and this trend is predicted to continue, juvenile survival may continue to increase. In addition to the potential for climate warming to bolster juvenile survival, the invasion of cheatgrass in this area may provide an additional food source that is roughly timed with juvenile emergence from their burrows. Although we had predicted that warmer climate would detrimentally effect survival, this population appears to be benefiting from it.

In Chapter 2, we found that a population of UGS at a higher elevation was more constrained by food and climate, as expected. Interestingly, the physiological mechanism at play designed to compensate for a short (high elevation) versus a long growing season (low elevation) were different. Yearlings and adults adjusted their growth to compensate for different active season lengths and hibernation phenologies between elevations, with higher elevation individuals gaining mass faster within their shorter active season, at the expense of immune function. Increased growth, along with the energetic burden of reproduction, led to greater declines in immune function in higher elevation yearlings and adults, but this trade-off was not observed in juveniles, who gained mass faster but actually improved in immune function throughout the season. Juveniles also weighed less at the high elevation site when weaned but grew at the same rate as their lower elevation counterparts, demonstrating no compensatory growth mechanism, despite the importance of body mass to overwinter survival (Sauer and Slade 1987). These results indicate that two populations of UGS adopt different life history strategies that may be driven by trade-offs between immunocompetence and body mass; these trade-offs are only expressed in reproductively active animals at the high elevation where resources are more limited. The high elevation females also seem to delay reproduction until they are adults, while the low elevation is less likely to delay reproduction. Given these strategies, the high elevation population may be less buffered against changes in their environment and more susceptible to climate variability.

Since predictions indicate that worldwide temperatures will continue increasing (IPCC 2013), its possible that warming could positively influence the survival of juvenile UGS via the encroachment and invasion of cheatgrass as a novel food source at higher elevations. However, summers are also predicted to get longer and drier in the western US, and an increase in drought frequency is thought to lead to population crashes in similar species (Ozgul et al. 2010). A continued effort in measuring the longitudinal and elevational responses of hibernating populations within and across species will help us between understand and predict wildlife responses to climate change, especially within ecosystems that are most sensitive to warming (i.e. alpine ecosystems).

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APPENDIX 1.1

Climate Correlations

We present correlation coefficients between two weather stations climatic indices relevant to Uinta ground squirrel life history: the Tony Grove Road weather station, located <1 mile from the study site, for which only climatic data from 2009 onwards is available, and the Utah State University weather station located 17 miles from the study area, for which climatic data is available since 1893.

WEATHER STATION INFORMATION

Tony Grove Snowtel (TGRS): located in Logan Canyon, Cache County, Utah, 0.9 miles from our field site. Elevation: 6,332 feet; latitude 41.89; Longitude: -111.57. Reporting since 2009-10-01 (Source: Utah State University Climate Center; https://climate.usurf.usu.edu/mapGUI; site accessed: Sept. 9th 2017).

Utah State University (USU): located in Logan, Cache County, Utah. Elevation: 4,778 feet; latitude: 41.75; longitude: -111.8, located 15.96 mi (25.69 km) from our field site. Reporting since 1893-01-01 (Source: Utah State University Climate Center; https://climate.usurf.usu.edu/mapGUI; site accessed: Sept. 9th 2017).

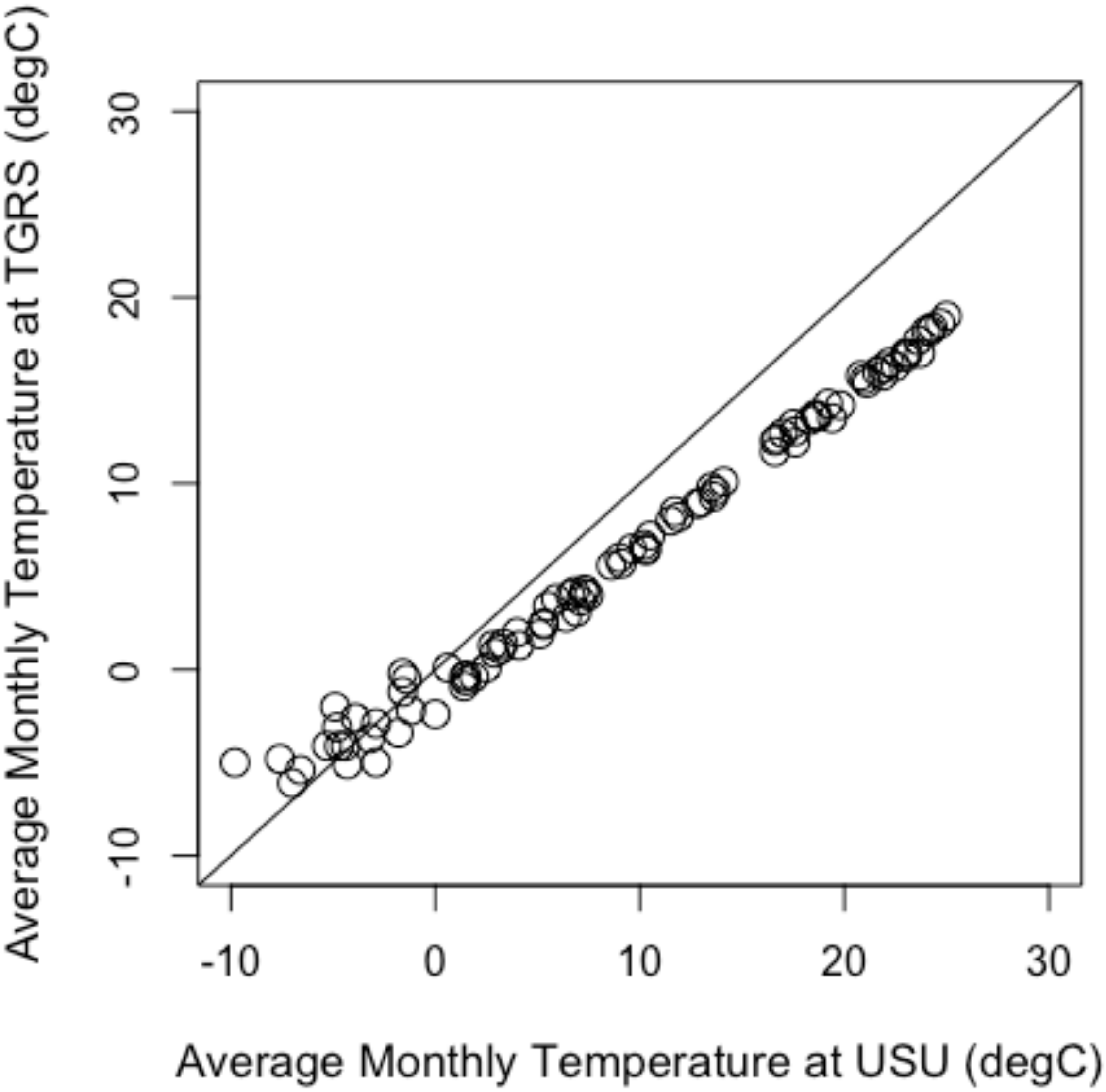

Figure 1.1A. Relationship between TGRS and USU average monthly temperatures from 2009 to 2016.

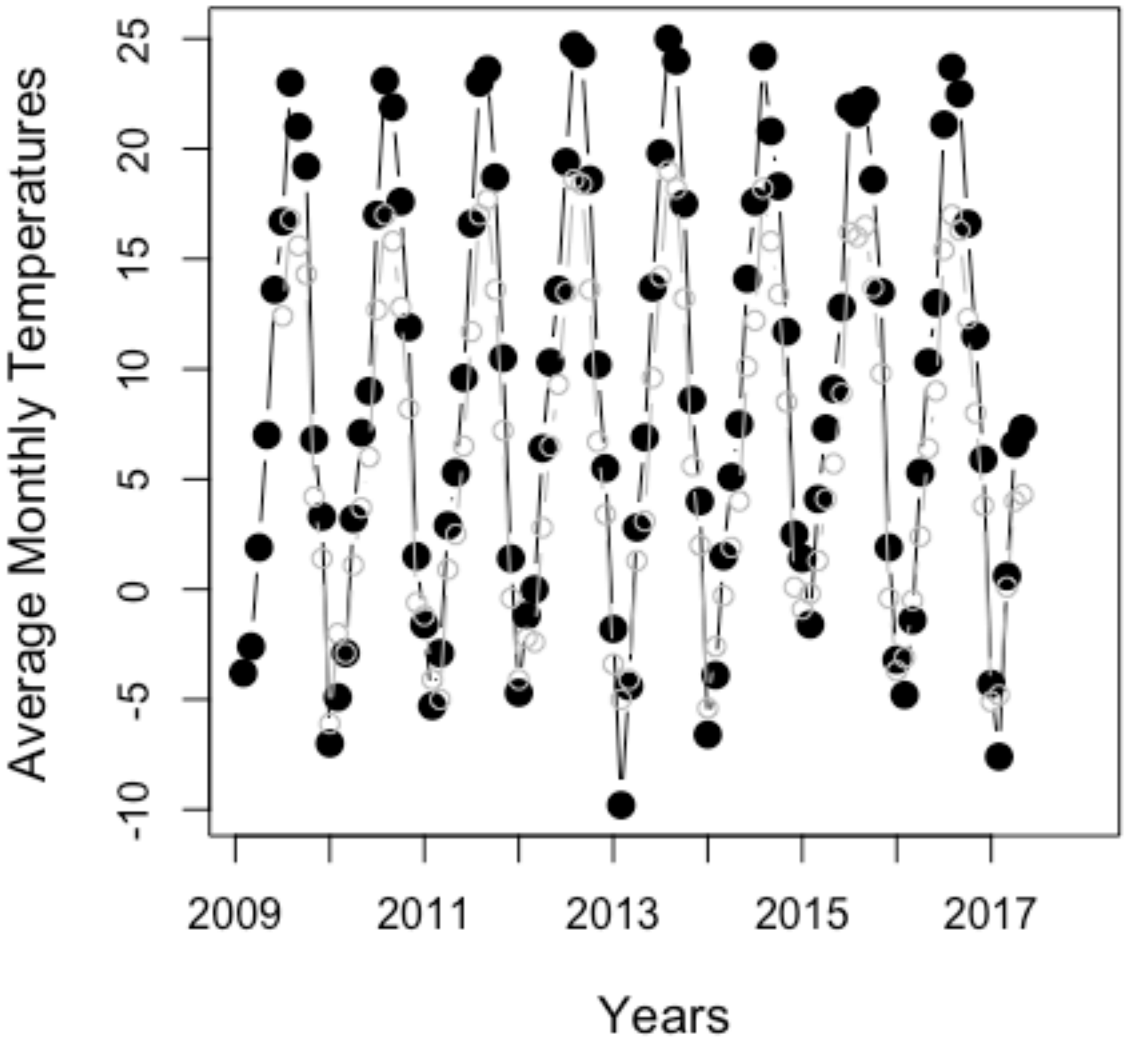

Figure 1.1B. Mean monthly spring and summer temperature fluctuations from 2009-2016 at TGRS (open grey dots) and USU (solid black dots), respectively.

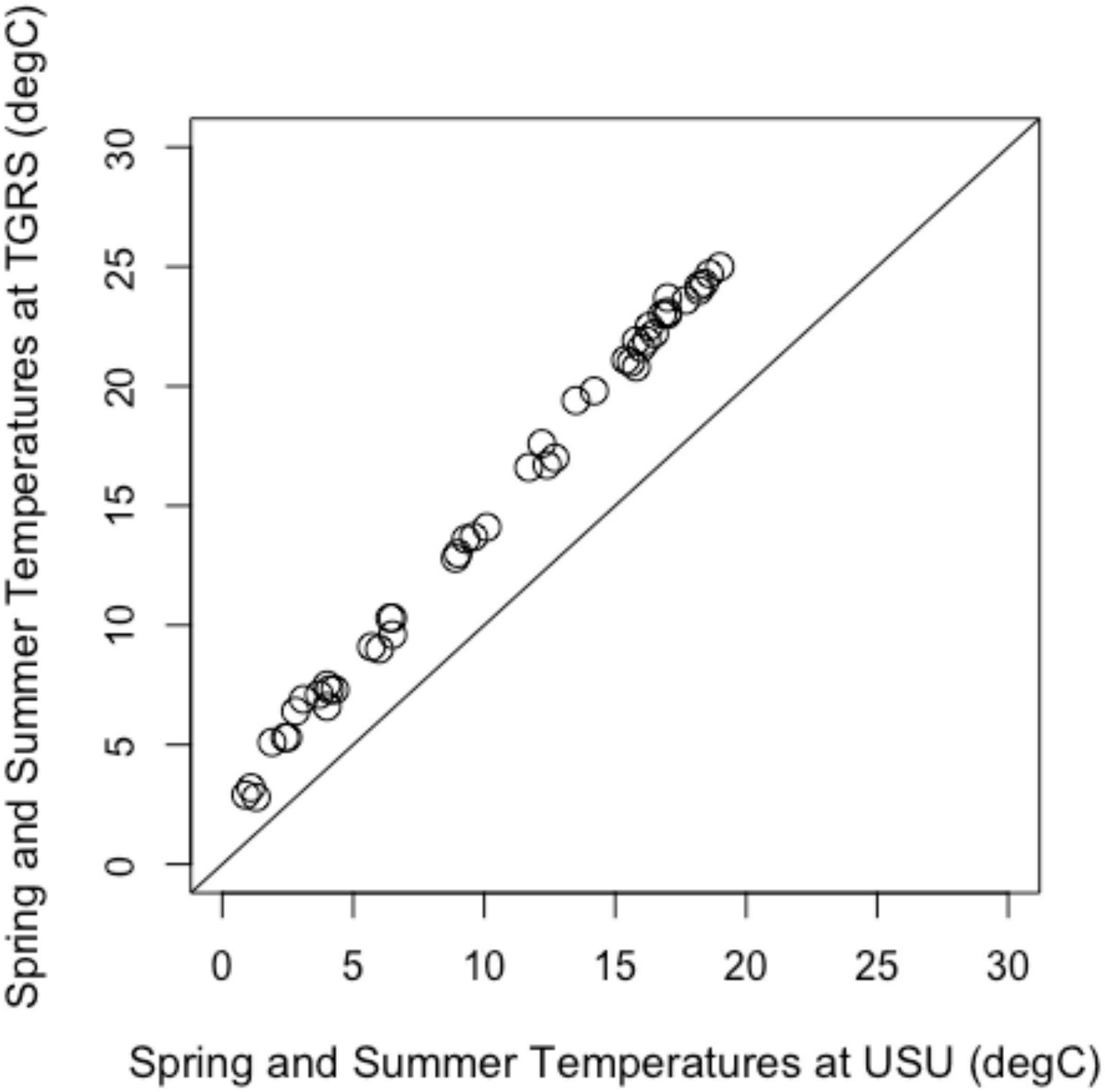

Figure 1.1C. Relationship between TGRS and USU mean monthly spring and summer temperatures (March-August) from 2009-2016.

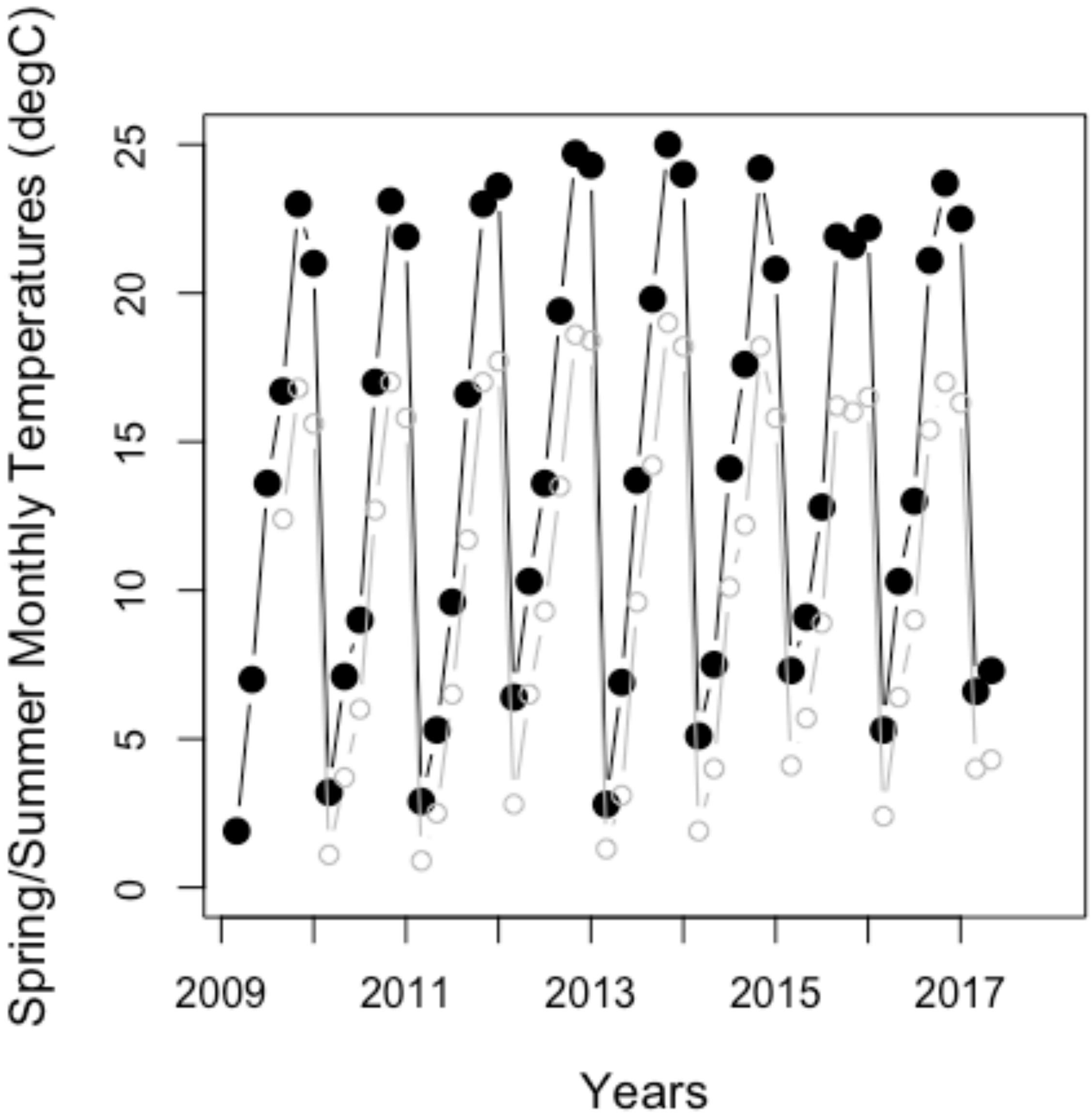

Figure 1.1D. Mean monthly spring and summer temperature fluctuations from 2009-2016 (March-August) at TGRS (open grey dots) and USU (solid black dots), respectively.

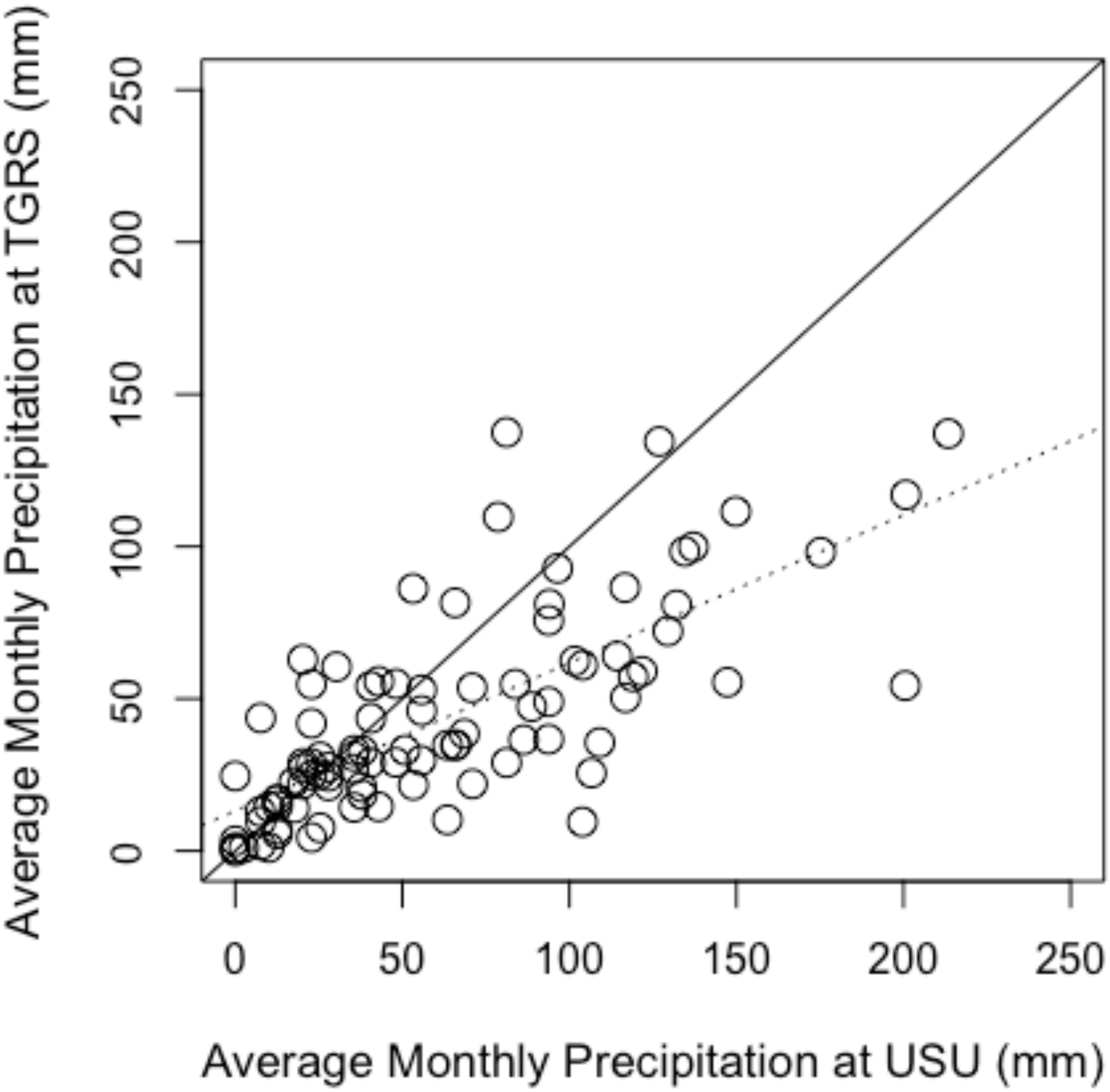

Figure 1.1E. Relationship between TGRS and USU average monthly precipitation from 2009 to 2016. Dotted line indicates the strength of the association between both weather stations, USU and TGRS, respectively.

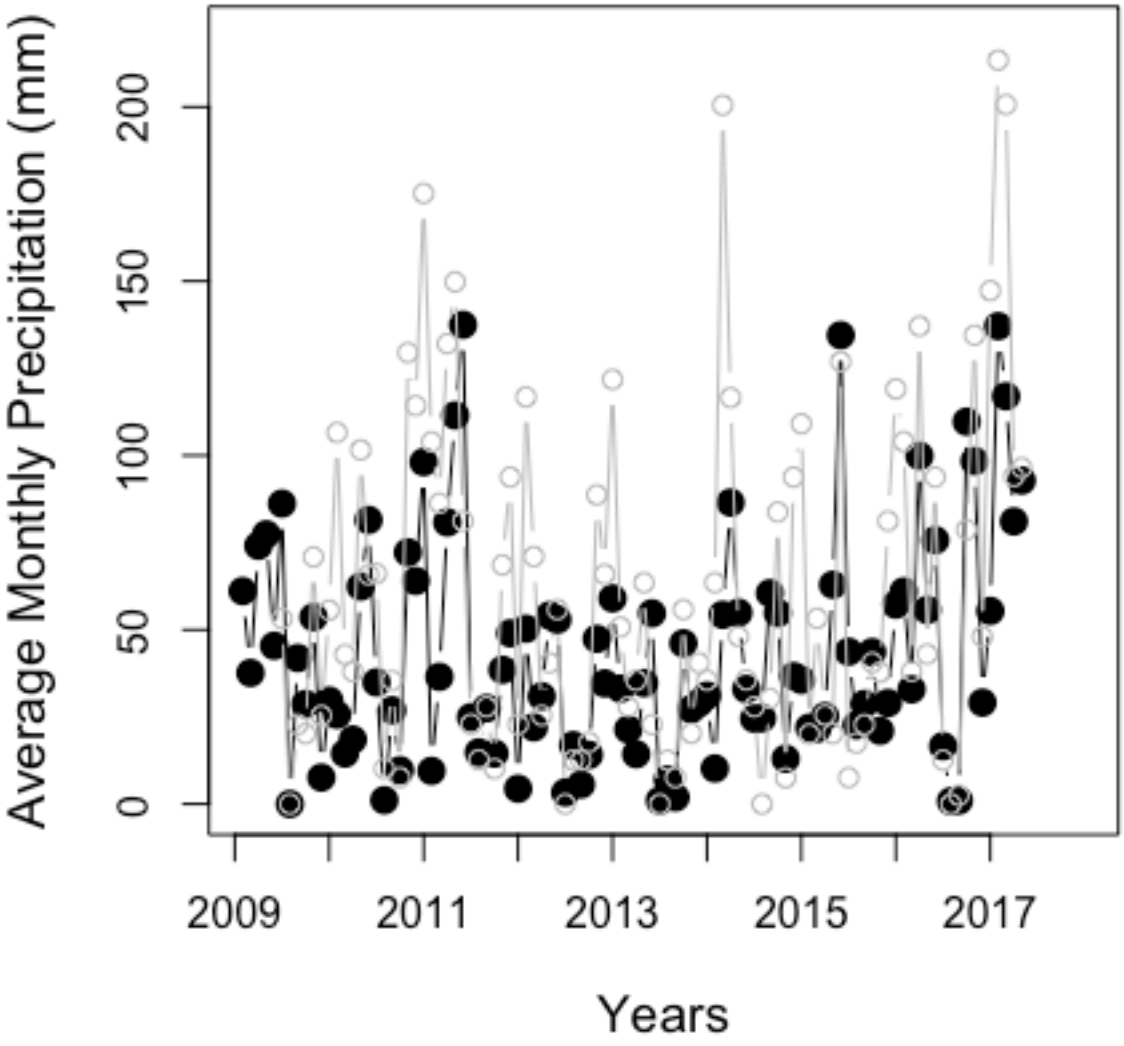

Figure 1.1F. Average monthly precipitation fluctuations from 2009-2016 at TGRS (open grey dots) and USU (solid black dots), respectively.

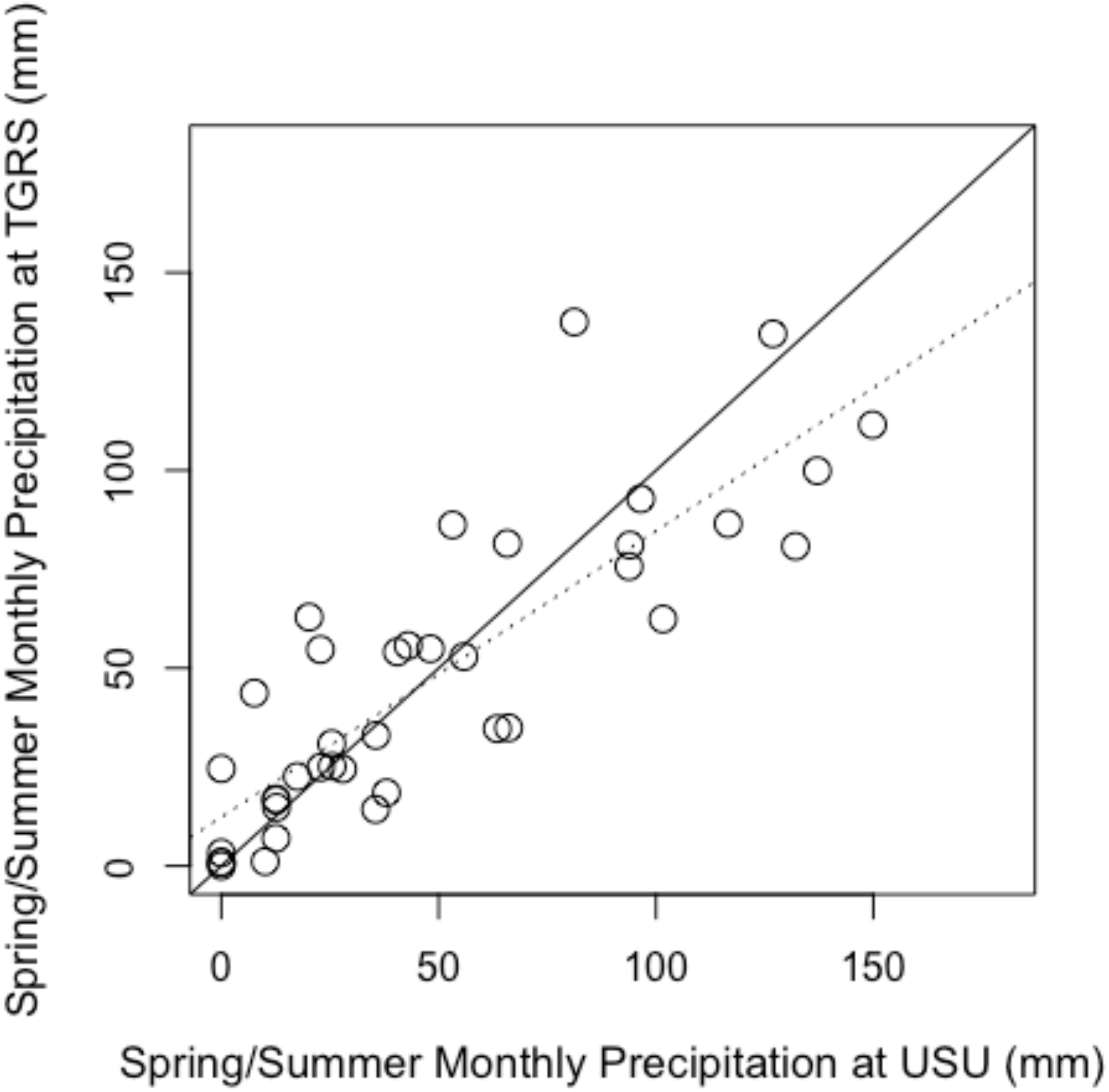

Figure 1.1G. Relationship between TGRS and USU mean monthly spring and summer precipitation (March-July) from 2009-2016.

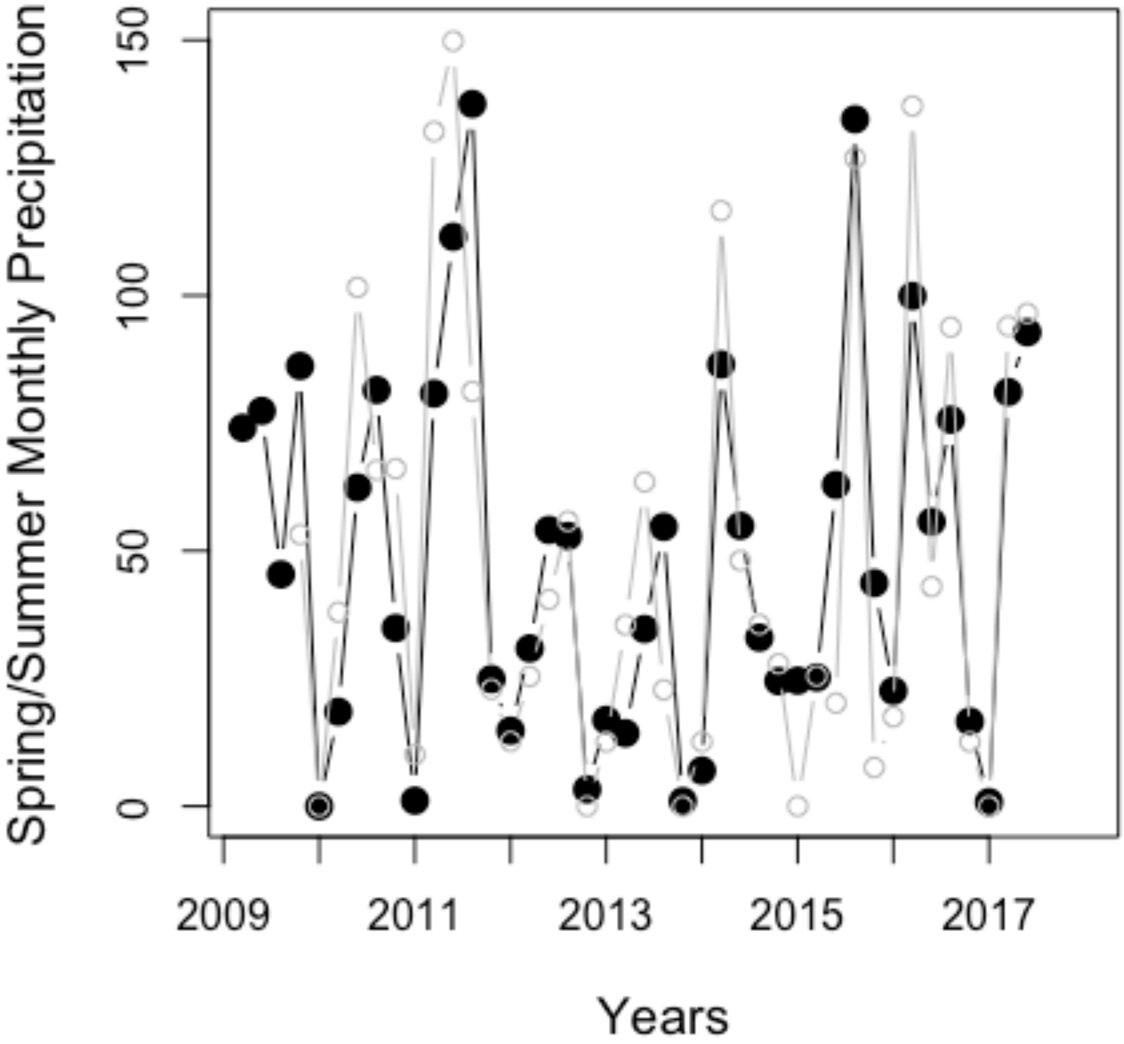

Figure 1.1H. Mean monthly spring and summer precipitation fluctuations (i.e. March-July) from 2009-2016 at TGRS (open grey dots) and USU (solid black dots), respectively.

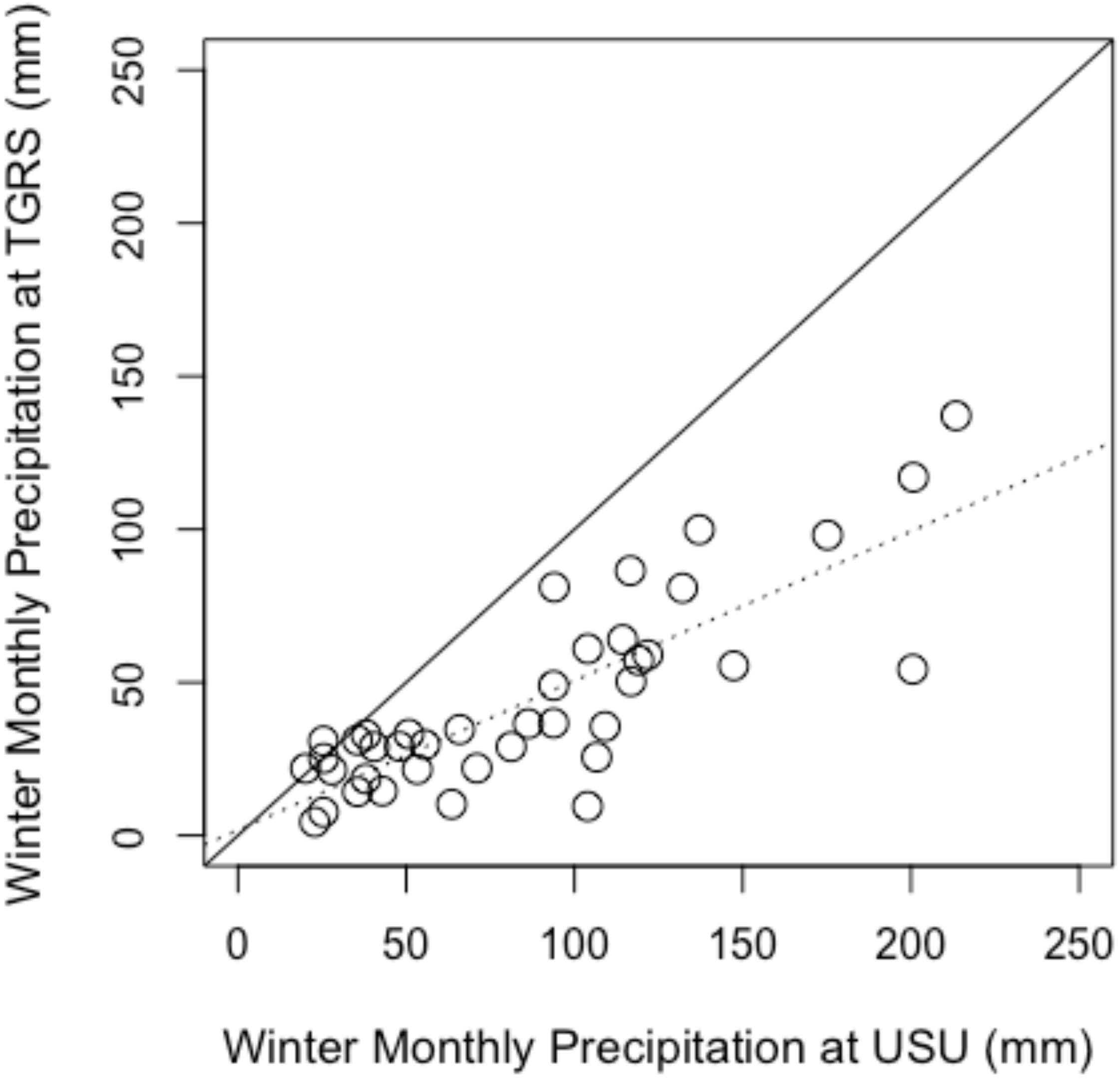

Figure 1.1I. Relationship between TGRS and USU average winter monthly precipitation (November – March) from 2009 to 2016. Dotted line indicates the strength of the association between both weather stations, USU and TGRS, respectively.

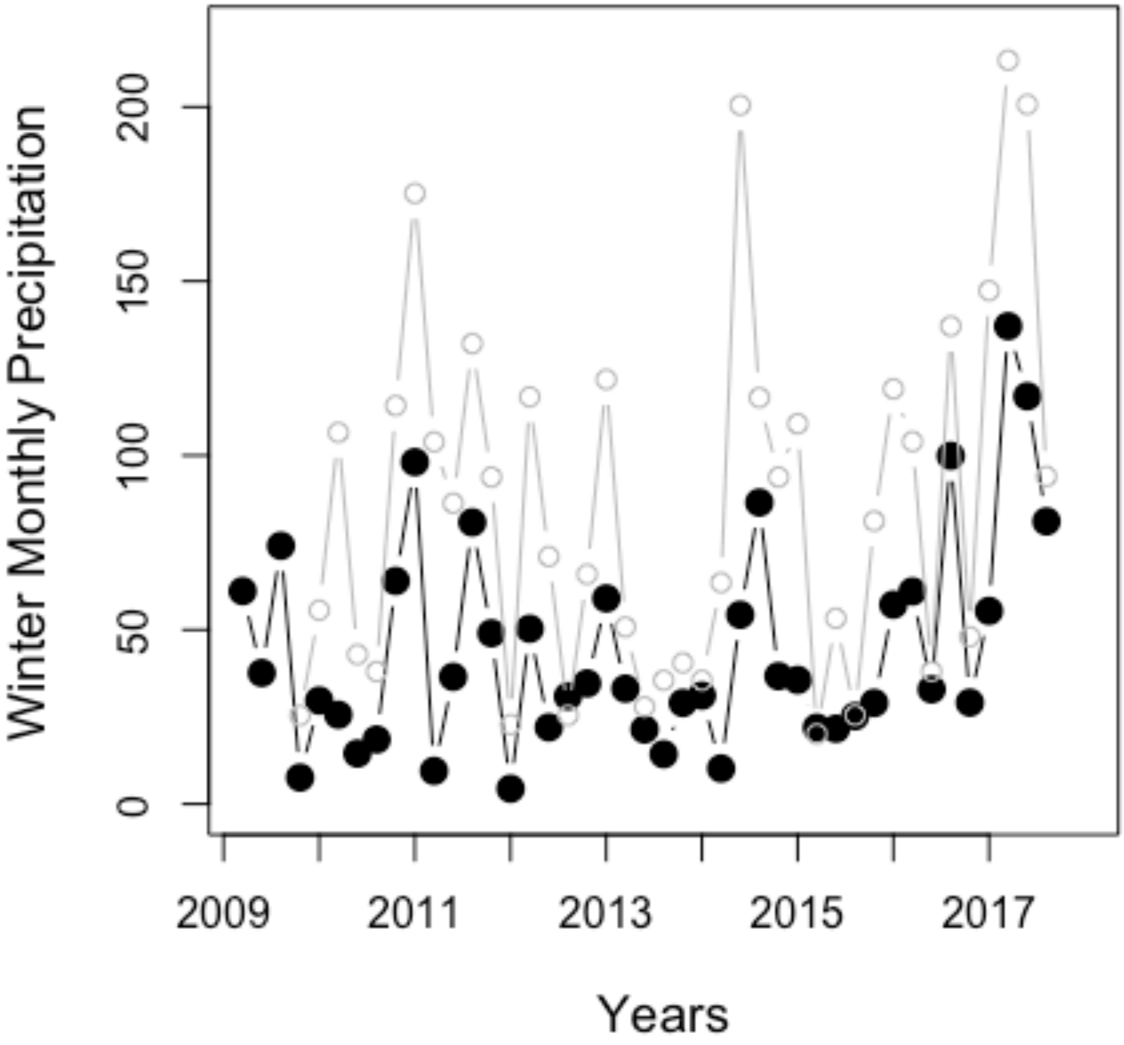

Figure 1.1J. Mean monthly spring and summer precipitation fluctuations (i.e. March-July) from 2009-2016 at TGRS (open grey dots) and USU (solid black dots), respectively.

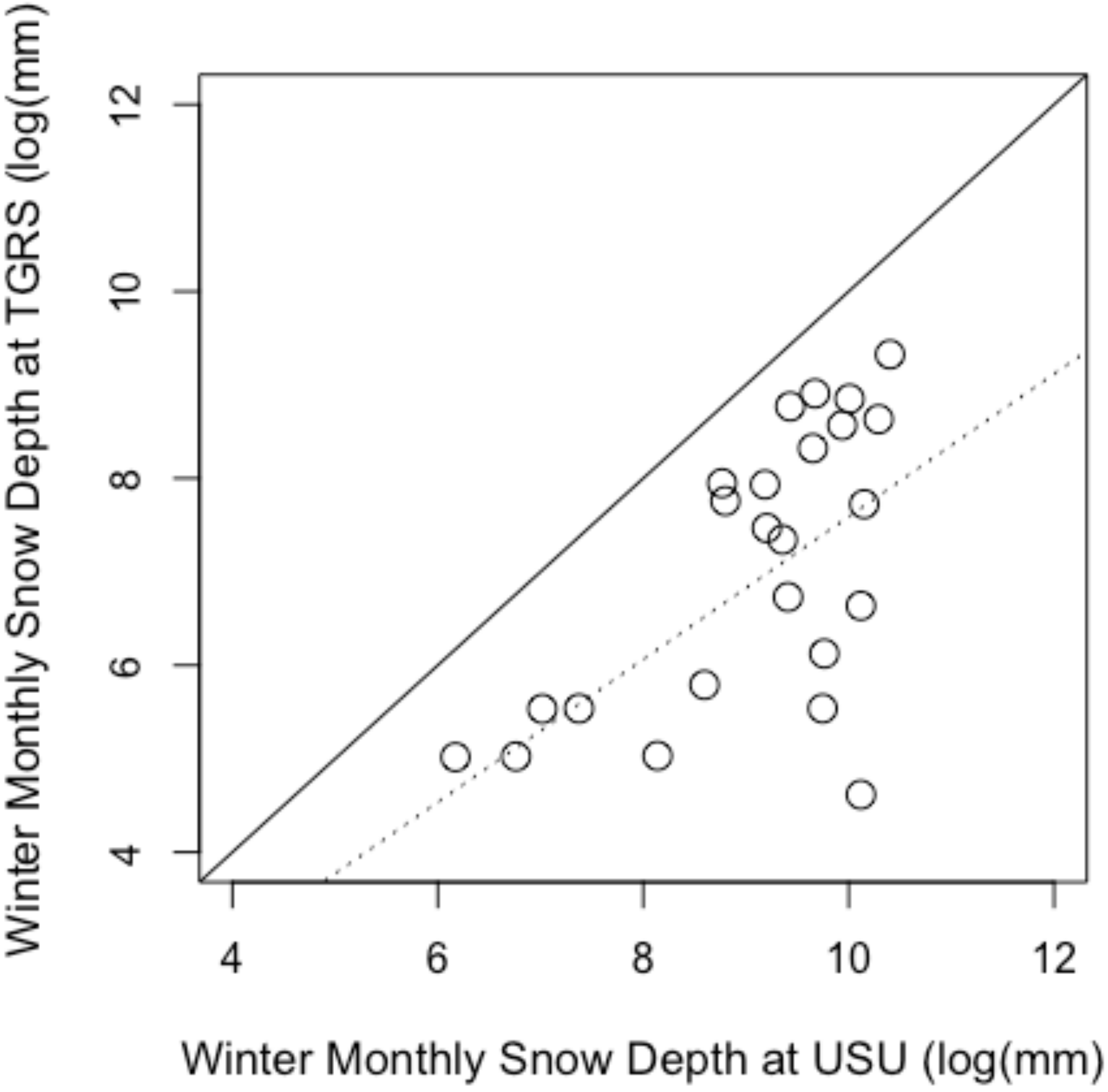

Figure 1.1K. Relationship between TGRS and USU average winter monthly snow depth (November – March) from 2009 to 2016. Dotted line indicates the strength of the association between both weather stations, USU and TGRS, respectively.

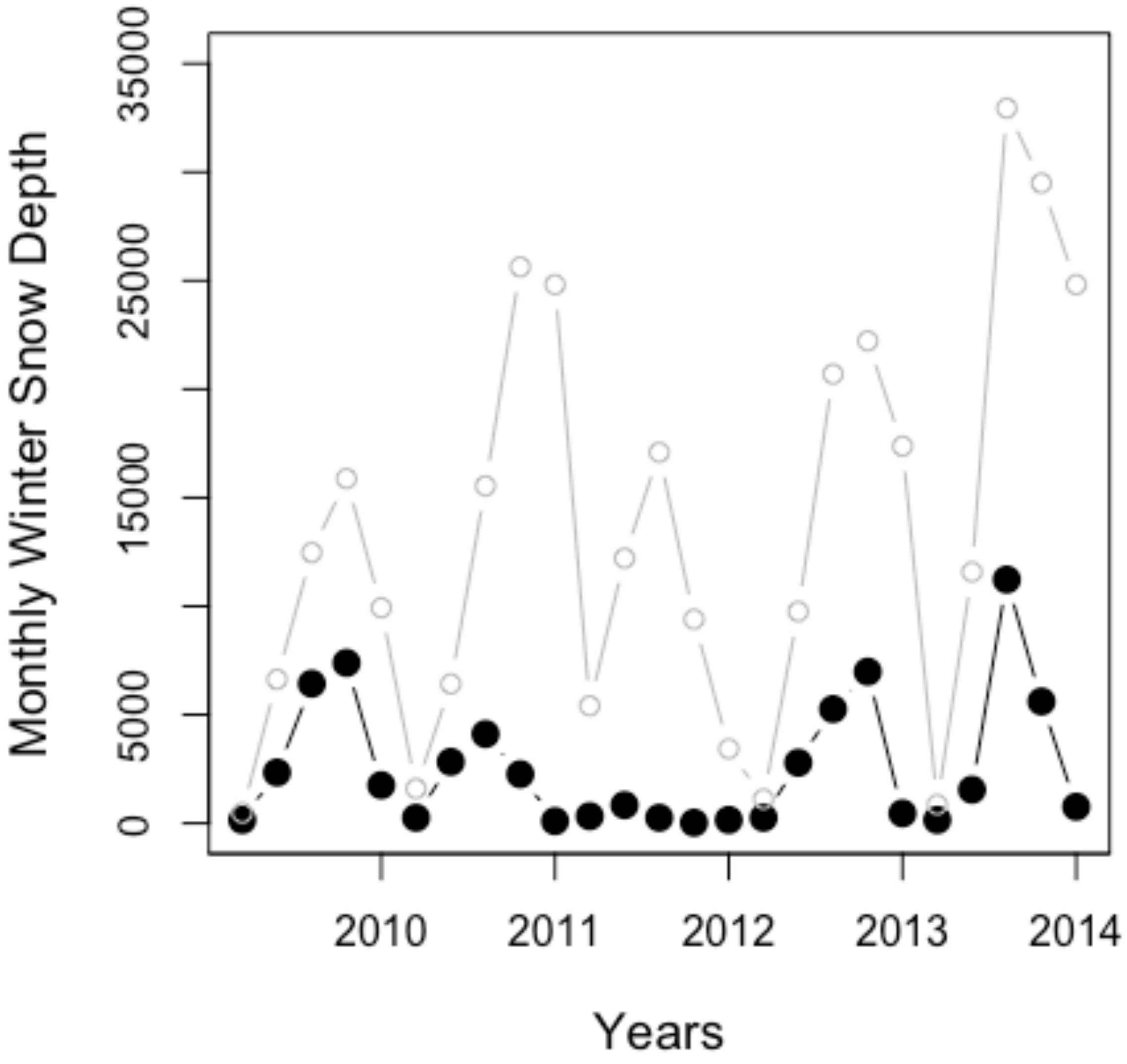

Figure 1.1L. Mean monthly spring and summer snow depth fluctuations (i.e. March-July) from 2009-2016 at TGRS (open grey dots) and USU (solid black dots), respectively.

APPENDIX 1.2

Goodness-of-fit tests, estimating median c-hat, and model selection tables

GOODNESS OF FIT

We used goodness-of-fit tests computed in the RELEASE software implemented in program MARK, which tests the assumptions of the CJS model that every marked animal present at Time *t* has the same probability of recapture, and that every marked animal immediately after *t* has the same probability of surviving until *t* + 1 (More 2013). We considered a global model where survival was allowed to vary by age, sex, and time period (historical versus contemporary) to test these assumptions; results are presented in Table 1.2A. The null hypothesis is that the assumptions of the CJS model are true so rejecting the null indicates a violation of assumptions. The GOF test conducted on this model indicated that juveniles in 1967 violated the assumptions of Test 3 (χ^2 = 74.1728, df = 19, p = 0). However, the addition of cohort effects for these two groups resolved the issue (Table 1.2B; χ^2 = 15.6503, df = 16, p = 0.4776). Test 3 determines whether individuals tagged that particular year are seen again at the same rate as individuals tagged in previous years, and the survival interval that violated assumptions was the last for our study period, so we are not concerned about this violation which simply results from the fact that our study ended.

MEDIAN C-HAT

We estimated the median c-hat to adjust model likelihoods for over-dispersion in the data by dividing the overall χ^2 values (74.173) and dividing by the total degrees of freedom (19) to obtain a c-hat of 3.90 (Laake 2013).

Model Selection Tables

Model selection results from every tier of model selection (QAICc, ΔQAICc, weight, and QDeviance) are presented in Table 1.2C. Beta estimates from the top model of each tier are presented in Table 1.2D.

Table 1.2A. Goodness of fit results from less parameterized model, Phi ~ age + sex + time period.

Group	χ^2	df	p-value
1	0.000	0	1.000
2	0.000	0	1.000
3	0.000	0	1.000
4	0.000	0	1.000
5	1.366	1	0.243
6	0.000	0	1.000
7	0.000	0	1.000
8	1.669	2	0.434
9	1.932	2	0.381
10	2.532	2	0.282
11	0.579	2	0.749
12	0.897	2	0.639
13	3.694	4	0.449
14	26.010	2	0.000
15	0.000	0	1.000
16	35.494	2	0.000
17	0.000	0	1.000
18	0.000	0	1.000
19	0.000	0	1.000
20	0.000	0	1.000
Total	74.173	19	0.000

Table 1.2B. Goodness of fit results from model that also included cohort effects for juveniles in 1967.

Group	χ^2	df	p-value
1	0.000	0	1.000
2	0.000	0	1.000
3	0.000	0	1.000
4	0.000	0	1.000
5	1.366	1	0.243
6	0.000	0	1.000
7	0.000	0	1.000
8	1.669	1	0.196
9	1.932	2	0.381
10	2.532	2	0.282
11	0.579	2	0.749
12	0.897	2	0.639
13	3.694	4	0.449
14	0.000	1	1.000
15	0.000	0	1.000
16	2.982	1	0.084
17	0.000	0	1.000
18	0.000	0	1.000
19	0.000	0	1.000
20	0.000	0	1.000
21	0.000	0	1.000
22	0.000	0	1.000
23	0.000	0	1.000
24	0.000	0	1.000
Total	15.650	16	0.478

Table 1.2C. Model selection results from each tier of model selection. In demography section, “age group” refers to three age groups (adult, yearling, juvenile), “age” refers to the age class (juvenile vs adult and yearling), and “Juv” or “AY” refer to each age class for partial interaction. In preliminary climate section, “Emer” refers to emergence date, “SL” to season length, “WT” refers to winter temperatures, “SD” is snow depth, “MT” is March temperatures, “ST” is summer temperatures, “SP” is summer precipitation high versus low, “GDD” is growing degree days, “BG” is Bagnoul-Gaussen drought index, and “Freeze” is number of below freezing days Variables with “lag” indicate a cross seasonal effect.

		npar	**QAICc**	**ΔQAICc**	**weight**	**QDeviance**
Demography	~-1 + Age + Age	4	1784.99	0.00	0.12	792.60
	~-1 + Time + Age + Age	10	1785.33	0.34	0.10	780.89
	~-1 + Age	3	1785.54	0.55	0.09	795.16
	~-1 + Time * Age	15	1785.58	0.59	0.09	771.07
	~-1 + Time + Age	9	1785.82	0.83	0.08	783.39
	~-1 + Time * Age + Age	16	1785.86	0.87	0.08	769.34
	~-1 + Age * Age	5	1786.75	1.76	0.05	792.36
	~-1 + Age + Age group	5	1786.90	1.91	0.05	792.51
	~-1 + Sex * Age + Time	11	1787.10	2.11	0.04	780.65
	~-1 + Time + Sex + Age group	11	1787.27	2.28	0.04	780.82
	~-1 + Age group	4	1787.42	2.43	0.04	795.03
	~-1 + Time * Juv + AY	16	1787.60	2.61	0.03	771.07
	~-1 + Time * AY + Juv	16	1787.60	2.61	0.03	771.07
	~-1 + Time + Age group	10	1787.73	2.74	0.03	783.29
	~-1 + Time * Juv + AY + Sex	17	1787.88	2.89	0.03	769.34
	~-1 + Time * AY + Juv + Sex	17	1787.88	2.89	0.03	769.34
	~-1 + Sex * Juv + AY	6	1788.76	3.77	0.02	792.36
	~-1 + Sex * AY + Juv	6	1788.76	3.77	0.02	792.36
	~-1 + Sex * Juv + AY + Time	12	1789.11	4.12	0.02	780.65
	~-1 + Sex * AY + Juv + Time	12	1789.11	4.12	0.02	780.65
	~-1 + Sex * Age group	7	1790.64	5.65	0.01	792.23
	~-1 + Sex * Age group + Time	13	1790.95	5.96	0.01	780.48
	~-1 + Sex	3	1794.98	9.99	0.00	804.60
	~-1 + Time + Sex	9	1795.09	10.10	0.00	792.66
	~-1 + Time * Age group	22	1797.07	12.08	0.00	768.41
	~-1 + Time * Age group + Sex	23	1797.36	12.37	0.00	766.69
	~1)	2	1799.84	14.85	0.00	811.46
	~-1 + Time * Sex	15	1805.21	20.22	0.00	790.70

Preliminary Climate	~-1 + Age * WT	5	1782.88	0.00	0.11	788.49
	~-1 + Juv + AY + Juv:WT	4	1783.42	0.53	0.09	791.03
	~-1 + Age * Emer	5	1784.12	1.24	0.06	789.73
	~-1 + Juv + AY + Juv:GDD	4	1784.62	1.73	0.05	792.23
	~-1 + Juv + AY + Juv:Emer	4	1784.77	1.89	0.04	792.38
	~-1 + Juv + AY + AY:Emer	4	1784.89	2.01	0.04	792.50
	~-1 + Juv + AY + AY:WT	4	1785.09	2.20	0.04	792.70
	~-1 + Juv + AY + AY:MT	4	1785.32	2.44	0.03	792.93
	~-1 + Juv + AY + AY:SP	4	1785.35	2.46	0.03	792.96
	~-1 + Age	3	1785.54	2.66	0.03	795.16
	~-1 + Juv + AY + AY:SD	4	1785.60	2.72	0.03	793.21
	~-1 + Juv + AY + Juv:BG	4	1785.82	2.94	0.03	793.43
	~-1 + Juv + AY + AY:WT_lag	4	1785.89	3.00	0.03	793.50
	~-1 + Juv + AY + AY:BG	4	1786.09	3.20	0.02	793.70
	~-1 + Juv + AY + Juv:ST	4	1786.14	3.26	0.02	793.75
	~-1 + Juv + AY + AY:ST	4	1786.20	3.32	0.02	793.81
	~-1 + Age * SL_lag	5	1786.31	3.43	0.02	791.92
	~-1 + Age * BG	5	1786.35	3.47	0.02	791.95
	~-1 + Juv + AY + AY:MT_lag	4	1786.60	3.71	0.02	794.21
	~-1 + Age * GDD	5	1786.62	3.74	0.02	792.23
	~-1 + Age * SP	5	1786.62	3.74	0.02	792.23
	~-1 + Juv + AY + Juv:Emer_lag	4	1786.66	3.77	0.02	794.27
	~-1 + Juv + AY + AY:Emer_lag	4	1786.69	3.81	0.02	794.30
	~-1 + Age * ST	5	1786.78	3.90	0.02	792.39
	~-1 + Juv + AY + Juv:SD	4	1786.79	3.90	0.02	794.40
	~-1 + Juv + AY + Juv:SP	4	1786.82	3.93	0.02	794.43
	~-1 + Age * SD	5	1786.86	3.98	0.02	792.46
	~-1 + Juv + AY + AY:Freeze	4	1786.88	4.00	0.02	794.49
	~-1 + Juv + AY + AY:SL_lag	4	1787.04	4.15	0.01	794.65
	~-1 + Age * MT	5	1787.07	4.18	0.01	792.67
	~-1 + Juv + AY + Juv:MT	4	1787.31	4.43	0.01	794.92
	~-1 + Juv + AY + AY:SD_lag	4	1787.35	4.46	0.01	794.96
	~-1 + Juv + AY + Juv:Freeze	4	1787.43	4.54	0.01	795.04
	~-1 + Juv + AY + AY:GDD	4	1787.55	4.66	0.01	795.16
	~-1 + Age * Emer_lag	5	1787.81	4.92	0.01	793.41
	~-1 + Age * WT_lag	5	1787.86	4.97	0.01	793.46
	~-1 + Age * MT_lag	5	1788.58	5.69	0.01	794.18
	~-1 + Age * Freeze	5	1788.77	5.88	0.01	794.37
	~-1 + Age * SD_lag	5	1789.06	6.18	0.01	794.67
	~1	2	1799.84	16.96	0.00	811.46

Table 1.2D. Real estimates from the top model, which includes an interaction between winter temperatures and age class (adult and yearling versus juvenile) and an interaction between emergence date the following year and age class, along with standard error (SE), lower confidence limit (LCL), and upper confidence limit (UCL).

Age	AdYr:wint	Juv:wint	AdYr:emer	Juv:emer	Real Estimate	SE	LCL	UCL
Adult	1.17	-	1.47	-	0.4820	0.0358	0.4127	0.5520
Adult	-1.1	-	-0.71	-	0.4874	0.0196	0.4491	0.5258
Adult	-0.13	-	-1.51	-	0.3312	0.0189	0.2953	0.3693
Adult	-0.93	-	-0.94	-	0.4506	0.0166	0.4183	0.4834
Adult	2.14	-	-0.82	-	0.2180	0.0320	0.1617	0.2872
Adult	0.4	-	0.44	-	0.4570	0.0215	0.4153	0.4993
Adult	-0.01	-	-0.25	-	0.4308	0.0146	0.4024	0.4596
Juvenile	-	1.17	-	1.47	0.4038	0.0361	0.3356	0.4760
Juvenile	-	-1.1	-	-0.71	0.2534	0.0136	0.2276	0.2810
Juvenile	-	-0.13	-	-1.51	0.2681	0.0166	0.2367	0.3019
Juvenile	-	-0.93	-	-0.94	0.2537	0.0121	0.2308	0.2781
Juvenile	-	2.14	-	-0.82	0.3772	0.0406	0.3014	0.4595
Juvenile	-	0.4	-	0.44	0.3406	0.0202	0.3022	0.3812
Juvenile	-	-0.01	-	-0.25	0.3051	0.0129	0.2805	0.3309

Table 1.2E. Beta and real survival estimates from the 2nd model, which includes an interaction between winter temperatures and age class (adult and yearling versus juvenile), an interaction between emergence date the following year and age class, and a partial interaction between juveniles and growing degree days (GDD). Also presented with standard error (SE), lower confidence limit (LCL), and upper confidence limit (UCL).

	Beta Estimate	**SE**	**LCL**	**UCL**
Phi:juv	-1.0792	0.1361	-1.3458	-0.8125
Phi:adyr	-0.1896	0.0674	-0.3217	-0.0575
Phi:adyr:wint	-0.3661	0.0719	-0.5070	-0.2252
Phi:wint:juv	0.1794	0.0641	0.0538	0.3050
Phi:adyr:em	0.3713	0.0708	0.2325	0.5101
Phi:juv:em	0.0812	0.0724	-0.0607	0.2231
Phi:juv:gdd	0.1585	0.0634	0.0342	0.2828
p:sexm	0.8810	0.5975	-0.2901	2.0521

Age	**Adyr: wint**	**Juv: wint**	**Adyr: emer**	**Juv: emer**	**Juv: gdd**	**Real estimate**	**SE**	**LCL**	**UCL**
Adult	1.17	-	1.47	-	-	0.4820	0.0358	0.4126	0.5520
Adult	-1.1	-	-0.71	-	-	0.4874	0.0196	0.4491	0.5258
Adult	-0.13	-	-1.51	-	-	0.3312	0.0189	0.2953	0.3693
Adult	-0.93	-	-0.94	-	-	0.4506	0.0166	0.4183	0.4834
Adult	2.14	-	-0.82	-	-	0.2180	0.0320	0.1617	0.2872
Adult	0.4	-	0.44	-	-	0.4569	0.0215	0.4152	0.4993
Adult	-0.01	-	-0.25	-	-	0.4308	0.0146	0.4024	0.4596
Juvenile	-	1.17	-	1.47	2.3	0.4049	0.0361	0.3365	0.4771
Juvenile	-	-1.1	-	-0.71	2.32	0.2756	0.0167	0.2441	0.3095
Juvenile	-	-0.13	-	-1.51	1.59	0.2743	0.0170	0.2422	0.3088
Juvenile	-	-0.93	-	-0.94	-0.29	0.2029	0.0218	0.1635	0.2489
Juvenile	-	2.14	-	-0.82	1.87	0.3857	0.0410	0.3091	0.4684
Juvenile	-	0.4	-	0.44	1.46	0.3230	0.0209	0.2833	0.3653
Juvenile	-	-0.01	-	-0.25	1.04	0.2816	0.0155	0.2522	0.3130

APPENDIX 1.3

Correlation of Climate Variables

Table 1.3. Correlation coefficients of variables used in model selection process. Variables are: snow depth (avg. snow depth from Dec *t*-1 until emergence *t*), winter temperature (avg. temp. from Dec *t*-1 until emergence *t*), Bagnoul-Gaussen drought index (daily rainfall (mm) minus twice avg. temp. (°C) from Jun-Aug), growing degree days (with 0°C as base temp.; from emergence until 42 days later), summer precipitation (avg. rainfall (mm) from Jun-Aug), summer temperature (avg. temp from Jun-Aug), March maximum temperature (Mar 15-31), below-freezing days (after estivation and before first snowfall), emergence date, and season length.

	Snow Depth	**BG**	**GDD**	**Summer Precipitation**	**Summer Temperature**	**March Temperature**	**Below-freezing days**	**Emergence Dates**	**Season**
Winter Temperature	**-0.895** t = -5.322, df = 7, p = 0.001	**-0.423** t = -1.235, df = 7, p = 0.257	**-0.041** t = -0.108, df = 7, p = 0.917	**-0.251** t = -0.687, df = 7, p = 0.514	**0.445** t = 1.316, df = 7, p = 0.230	**0.3902** t = 1.121, df = 7, p = 0.299	**-0.465** t = -1.388, df = 7, p = 0.208	**0.239** t = 0.650, df = 7, p = 0.536	**-0.459** t = -1.365, df = 7, p = 0.214
Snow Depth		**0.287** t = 0.793, df = 7, p = 0.4537	**0.224** t = 0.608, df = 7, p = 0.563	**0.221** t = 0.598, df = 7, p = 0.569	**-0.302** t = -0.839, df = 7, p = 0.429	**-0.5644** t = -1.809, df = 7, p = 0.113	**0.599** t = 1.977, df = 7, p = 0.089	**-0.024** t = -0.063, df= 7, p = 0.9516	**0.285** t = 0.788, df = 7, p = 0.457
BG			**0.1623** t = 0.435, df = 7, p = 0.677	**0.879** t = 4.866, df = 7, p = 0.002	**-0.994** t = -23.221, df=7, p< 0.001	**-0.3369** t = -0.947, df = 7, p = 0.375	**-0.316** t = -0.880, df = 7, p = 0.408	**0.072** t = 0.190, df = 7, p = 0.8545	**0.491** t = 1.493, df = 7, p = 0.179
GDD				**-0.032** t = -0.086, df = 7, p = 0.934	**-0.216** t = -0.586, df = 7, p = 0.576	**-0.2375** t = -0.647, df = 7, p = 0.538	**-0.283** t = -1.046, df= 7, p = 0.330	**0.577** t = 1.867, df = 7, p = 0.1042	**-0.187** t = -0.502, df= 7, p = 0.6308
Summer Precipitation					**-0.825** t = -3.863, df = 7, p = 0.006	**-0.3258** t = -0.912, df = 7, p = 0.392	**-0.14** t = -0.374, df = 7, p = 0.720	**-0.016** t = -0.042, df= 7, p = 0.9678	**0.481** t = 1.450, df = 7, p = 0.190
Summer Temperature						**0.3689** t = 1.050, df = 7, p = 0.329	**0.357** t = 1.011, df = 7, p = 0.346	**-0.134** t = -0.356, df = 7, p = 0.732	**-0.441** t = -1.299, df = 7, p = 0.235
March Temperature							**-0.0177** t = -0.047, df = 7, p = 0.964	**-0.6145** t = -2.061, df = 7, p = 0.0783	**0.3871** t = 1.111, df = 7, p = 0.303
Below-freezing days								**-0.557** t = -1.775, df= 7, p = 0.119	**0.364** t = 1.035, df= 7, p = 0.3353
Emergence									**-0.743** t = -2.935, df = 7, p = 0.022

APPENDIX 2.1

Differences in biomass dynamics during the active season between sites

INTRODUCTION

The strength of life history trade-offs is primarily driven by resource availability and allocation (van Noordwijk and de Jong 1986). Accordingly, individuals located at the lower elevation site should experience weaker trade-offs, given an extended active season and access to abundant resources throughout. Herein, we document differences in graminoid phenology and biomass from samples collected at each site throughout the Uinta ground squirrel (UGS) active season.

METHODS

Biomass was collected every two weeks at each site as soon as snow melted until the squirrels began estivation and were no longer active. Six quadrats at each site were sampled each time and 100 cm^2 of vegetation were clipped, dried, and weighed. Sampling dates where there was still snow on the ground have zero biomass and are not plotted (early April at FS; through early June at TG) although squirrels were active before these dates. Total biomass was weighed, as well as edible biomass (removing unpalatable plants such as thistle and thick woody twigs). Uinta ground squirrels (UGS) at this location are known to consume almost exclusively grass leaves for the first month after emergence (later including forb leaves and seeds as they become available) (Walker 1968), so the measures of edible biomass should accurately reflect the amount of food available to UGS that they would consume.

RESULTS

Although the low elevation site (Forestry Field Station, 'FS') had higher levels of biomass throughout the season, differences between biomass at the sites were not significant (confidence intervals overlap; Figures 2.1A and 2.1B). Additionally, the high elevation site (Tony Grove, 'TG') had a much shorter period of time when biomass was available, with emergence of UGS estimated around 17-May-2016 and snow melt (with associated green-up of vegetation) not occurring until 07-June-2016 (a difference of approximately 3 weeks), while emergence at FS was 08-Apr-2016 and green-up at that site began around 12-Apr-2016 (less than a week after emergence). Overall, differences in snowmelt and graminoid phenologies mostly explained difference in resource availability to UGS between site, as opposed to biomass *per se*.

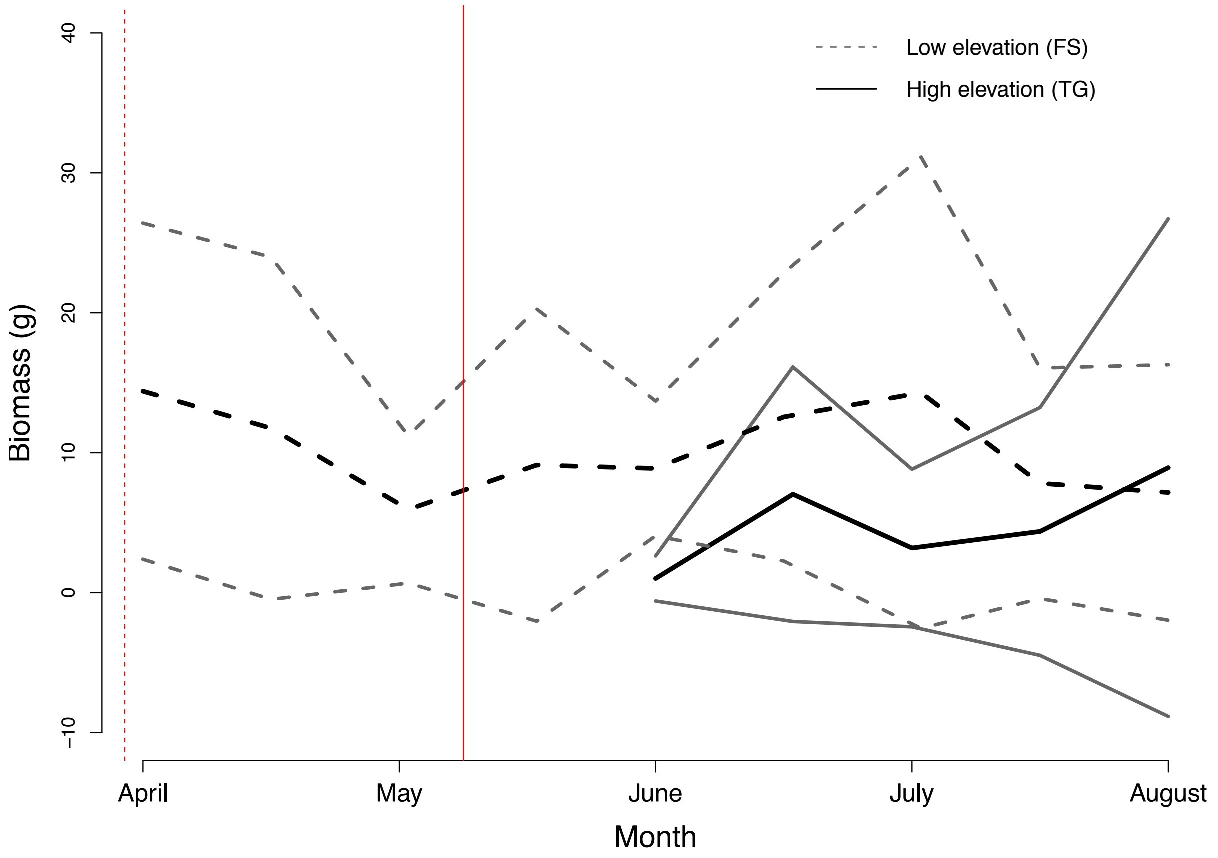

Figure 2.1A. Total biomass throughout the season (averaged by sampling date) at the Forestry Field Station (FS, dashed lines) and Tony Grove (TG, solid lines) field sites, respectively. Lighter lines indicate 95% confidence intervals; red vertical lines indicate dates of emergence at FS (08-Apr-2016, dashed line) and TG (17-May-2016, solid line).

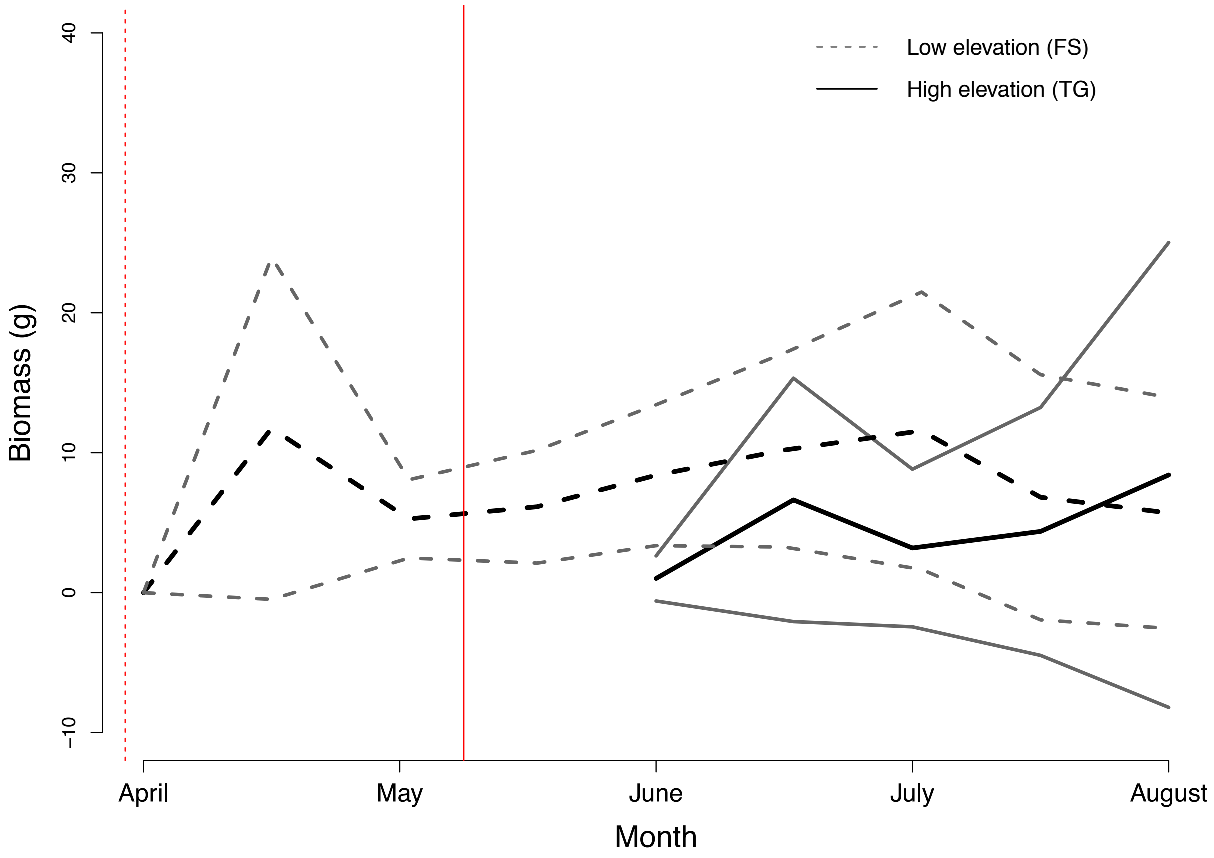

Figure 2.1B. Edible biomass throughout the season (averaged by sampling date) at the Forestry Field Station (FS, dashed lines) and Tony Grove (TG, solid lines) field sites, respectively. Lighter lines indicate 95% confidence intervals; vertical lines indicate dates of emergence at FS (08-Apr-2016) and TG (17-May-2016).

REFERENCES

Walker RE (1968) Local Distribution in a Population of Uinta Ground Squirrels. PhD dissertation, Utah State University, Logan.

APPENDIX 2.2

Between-individual changes in BKA and body mass across sites and age classes

INTRODUCTION

The purpose of inspecting trends in BKA, body mass, and the relationship between BKA and body mass between individuals is to assess whether such trends corroborate findings within individuals (see within-individual analysis presented in the manuscript), while maximizing sample size. The main difference between both sets of analyses is that instead of using two observations per individual (early and late in the active season) to calculate within-individual changes in body mass and BKA, we herein utilize all of the data that we collected throughout the active season, without keeping track of individuals (i.e. between-individual).

METHODS

All statistical analyses were performed in R, version 3.2.3 (R Core Team 2013).

We were interested in accounting for all weight fluctuations during the growing season, even the ones associated with reproduction, and thus utilized all the data collected across all trapping occasions to assess between-individual trends in body mass over the active season (BM_J and BM_{YA} for juveniles and yearlings/adults, respectively; 371 observations at FS: 79 juvenile female, 41 juvenile male, 151 adult and yearling female, 100 adult and yearling male; and 153 observations at TG: 21 juvenile female, 7 juvenile male, 61 adult and yearling female, 64 adult and yearling male). We tested for differences in BM over the active season across sex and site using linear mixed effects models ("lme" function, nlme package).

To assess between-individual trends in immune function, we examined the relationship between BKA (all measures of BKA; the square root was taken to improve homoscedasticity) and date, while accounting for sex and site (185 observations at FS: 50 juvenile female, 23 juvenile male, 69 adult and yearling female, 43 adult and yearling male; 63 observations at TG: 8 juvenile female, 4 juvenile male, 24 adult and yearling female, 27 adult and yearling male). These values were utilized for both juveniles (BKA_J), and yearlings and adults (BKA_{YA}), but results for juveniles are presented in the manuscript. We tested for differences in BKA over the active season across sex and site using a mixed effect ANCOVA.

We also statistically assessed the effect of body mass on BKA_{YA} (repeated measures throughout the season) while accounting for sex, site, body mass, and date, using linear mixed effect models (Venables & Ripley 2002). We fit an individual random effect to account for non-independence of repeated BKA_{YA} measurements. We initiated the model selection process with a global model accounting for all variables of interest (i.e. sex, site, body mass, and date, as well as relevant interactions). We removed parameter estimates that were not significant, starting with the least precise estimate, to identify the best performing model based on lowest AIC_C value and parsimony (Lebreton et al. 1992).

RESULTS

Yearling and adult body mass and immunity.

Changes in BM_{YA} were significantly influenced by calendar date, site, and sex (“lme”; $BM_{YA} \sim site*date + sex$). All groups increased in mass throughout the season (date estimate = 0.005, p-value < 0.001), but UGS at TG started out the season weighing less (FS estimate = -86.8, p-value < 0.001, TG estimate = -140.35, p-value < 0.001) and gained mass faster (TG:date estimate = 0.003, p-value = 0.037). Males also gained mass slightly faster than females at each site (male estimate = 0.13, p-value < 0.001).

Changes in BKA_{YA} over the active season were significantly influenced by calendar date, site, the interaction between date and site, and marginally influenced by sex ("lme"; BKA_{YA} ~ site*date + sex). This model indicated that the effect of site was significant, with TG starting higher (FS estimate = 353.08, p-value = 0.004; TG estimate = 765.372, p-value = 0.022) and UGS at both FS and TG declined throughout the season (lme; calendar date estimate = -0.0297, p-value = 0.004), but the interaction between site and date had a greater negative effect at TG (estimate = -0.045, p-value = 0.020). Males had a marginally significantly higher BKA than females (estimate = 0.789, p-value = 0.064).

Trade-off model. The best performing model testing for the potential trade-off between BKA_{YA} and BM_{YA} retained the effects of site, body mass, as well as an interaction between site and body mass (AIC_C = 785.547, w_i = 0.999, Table 2.2A). Estimates from this model indicate that the relationship between body mass and the observed change in BKA over the growing season was different across sites (Table 2.2B). At FS, the relationship between and BKA and body mass was weak and non-significant (p-value = 0.698). However, an increase in body mass was correlated with a significant decrease in BKA at TG (p-value = 0.021).

Within-individual versus between-individual analyses

Between-individual changes in body mass for yearlings and adults, BM_{YA}, agreed with within-individual changes in body mass, ΔBM_{YA}, in both direction and significance. Between-individual trends in bacterial killing ability, BKA_{YA}, agreed with the direction of average ΔBKA_{YA}, although differences that were observed among sex and site when using all data were not detected at the within-individual level. The best performing model for BKA_{YA} in terms of AIC_C included an effect of site, body mass, as well as an interaction between site and body mass on BKA_{YA} (AIC_C = 785.547, w_i = 0.999, Table 2.2A). The within-individual positive relationship between ΔBM_{YA} and

ΔBKA_{YA} at FS dissipated at the between-individual level (BM_{YA} and BKA_{YA}), as sample size increased. However, the negative relationship we found between ΔBKA_{YA} and ΔBM_{YA} at TG was accentuated when looking at BKA_{YA} and BM_{YA}, with site ($p = 0.029$) and an interaction between site and body mass ($p = 0.035$) both significant (Table 2.2B; linear mixed effects models), further indicating a possible trade-off between immunity and body mass (Fig. 2.2C).

For juveniles, between-individual trends in BM_J were congruent with findings based on within-individual change in body mass, ΔBM_J. Although there was no significant effect of site on BM_J (lme, estimate = -2.68, SE = 37.99, p-value = 0.9441), juveniles at TG emerged from their natal burrows lighter than those at FS and remained lighter throughout the season until estivation.

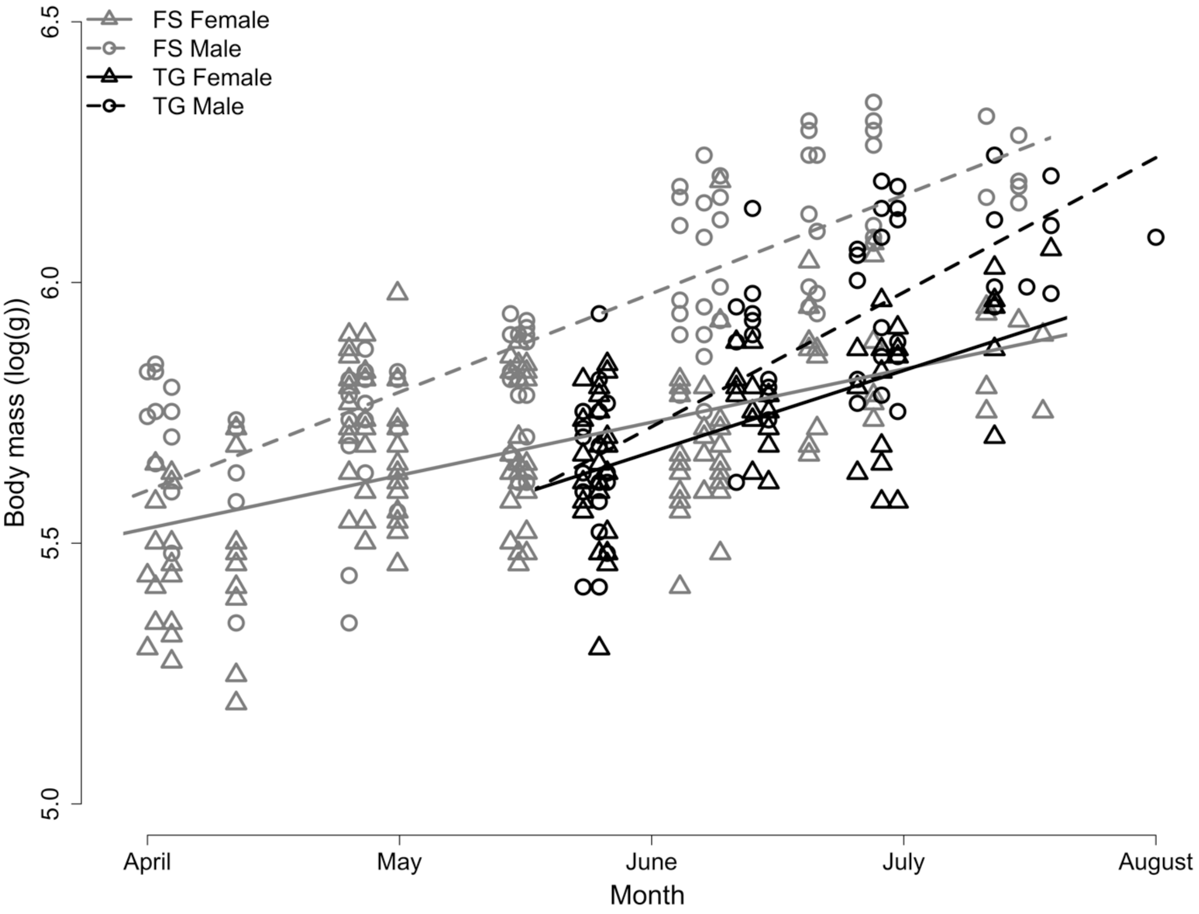

Figure 2.2A. Body mass (log transformed) throughout the active season for adult and yearling males (circles) and females (triangles) at the Forestry Field Station (FS, grey) and Tony Grove (TG, black) field sites, respectively.

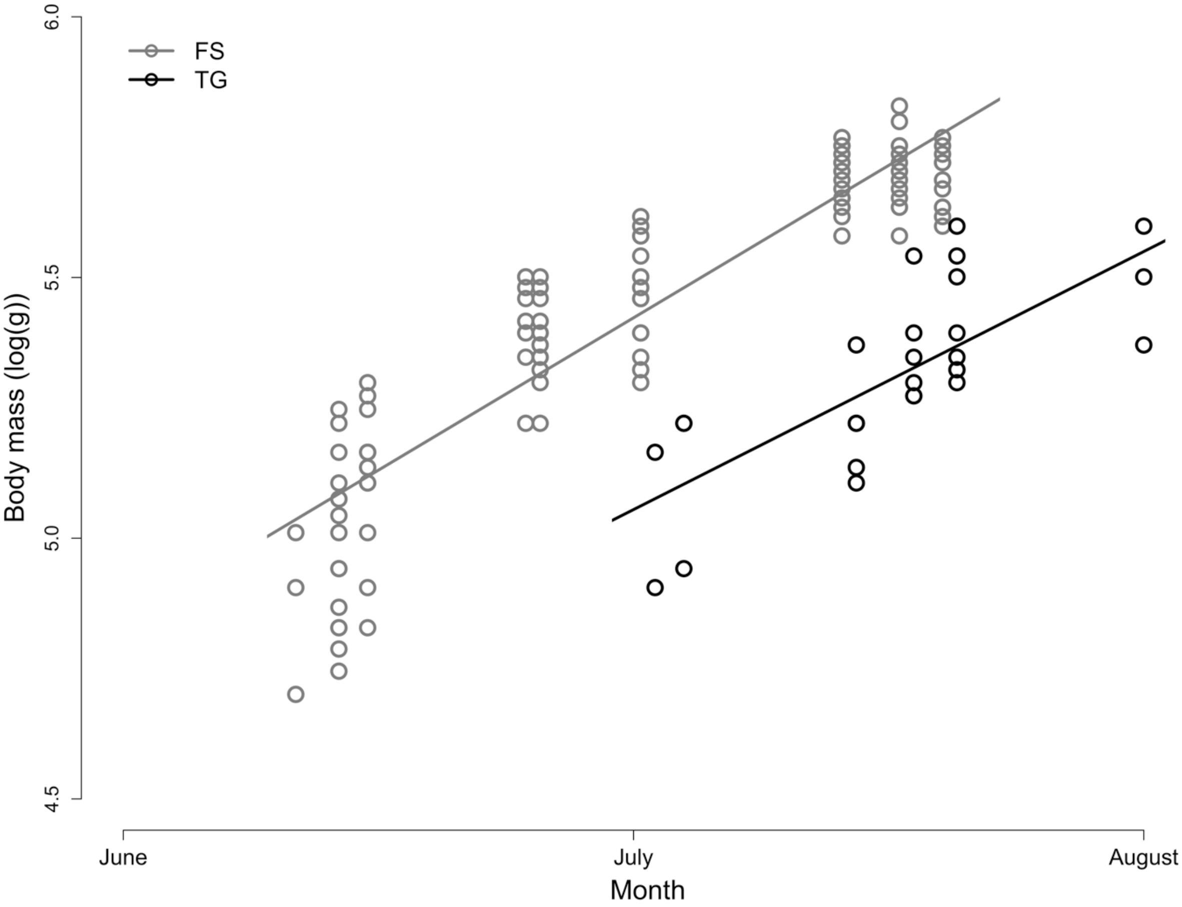

Figure 2.2B. Body mass (log transformed) throughout the active season for juveniles at the Forestry Field Station (FS, grey) and Tony Grove (TG, black) field sites, respectively.

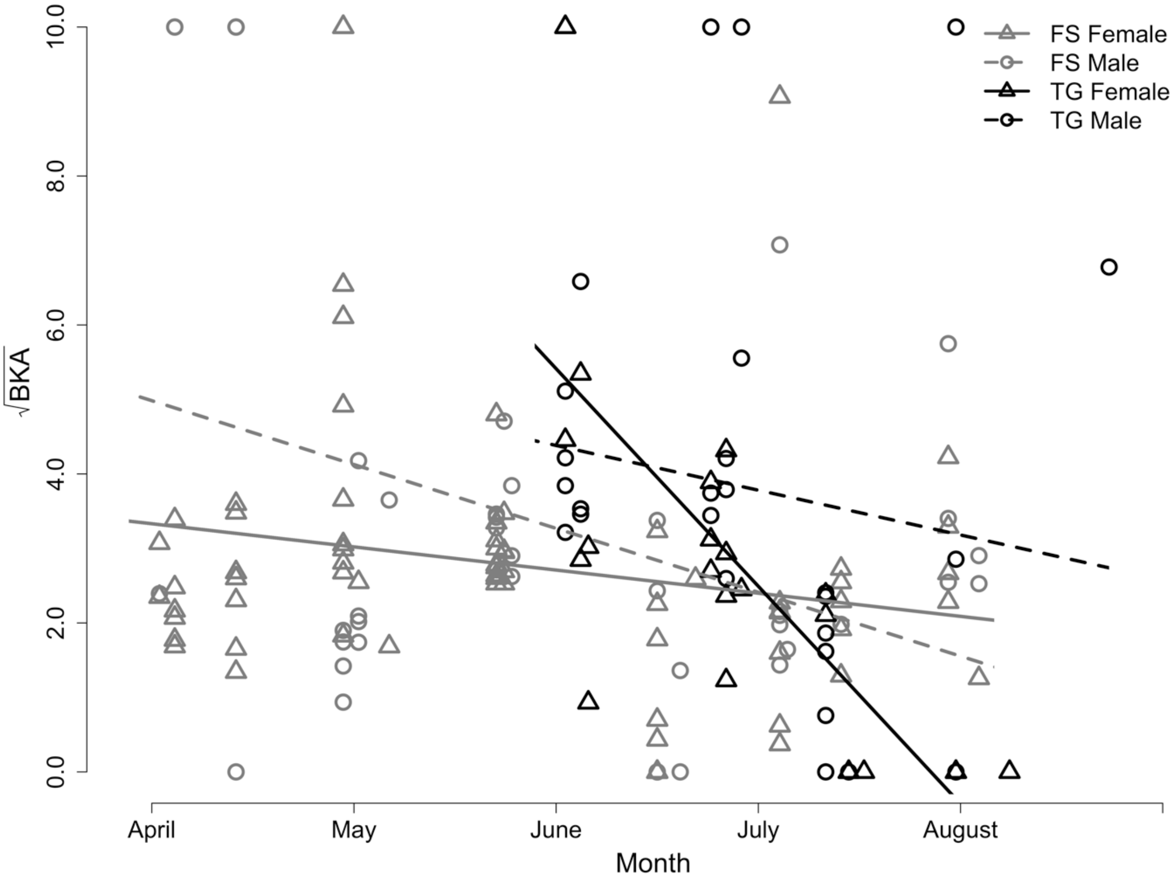

Figure 2.2C. Repeated measures of $\mathrm{BKA}_{\mathrm{YA}}$ (bacterial killing ability, square-root transformed) throughout the active season for adult and yearling males (circles) and females (triangles) at the Forestry Field Station (FS, grey) and Tony Grove (TG, black) field sites, respectively.

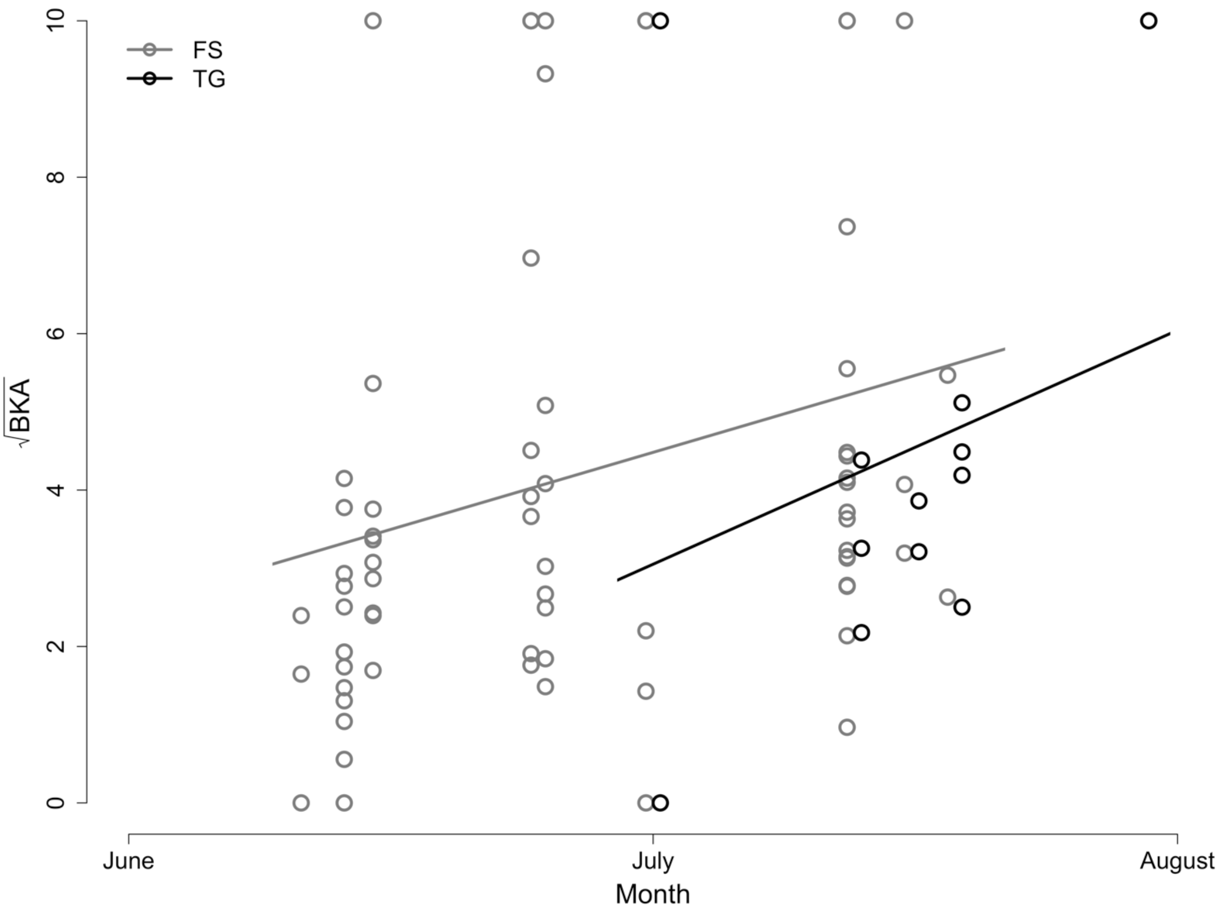

Figure 2.2D. Repeated measures of BKA_J (bacterial killing ability, square-root transformed) throughout the active season for juveniles at the Forestry Field Station (FS, grey) and Tony Grove (TG, black) field sites, respectively.

Table 2.2A. Model selection results testing for the effects of sex, site, body mass, date, and interactions on BKA_{YA}. Model selection was based on AIC_C weights (w_i), reported along AIC_C, ΔAIC_C, deviance, and the number of parameters associated with each model (NP). Interaction terms are denoted by a colon.

Models	**AIC_C**	**ΔAIC_C**	**Deviance**	**NP**	**w_i**
site+sex+bm+date+bm:date+sex:bm+ site:bm+sex:site	785.547	0	784.0123	8	0.999
site+bm+date+bm:date+site:bm	800.201	14.654	783.266	5	0.001
site+bm+site:bm	807.761	22.214	773.008	3	0

Table 2.2B. Estimates from the selected top performing and most parsimonious model based on AICc. The top model includes site, body mass, and an interaction between site and body mass. Mean parameters estimates, along with standard errors, upper (UCI), lower confidence bounds (LCI), and p-values are reported below. Interaction terms are denoted by a colon.

Parameter	**Estimate**	**Std. Error**	**LCI**	**UCI**	**p-value**
site (FS)	3.535	0.985	1.554	5.517	<0.001
site (TG)	8.156	1.791	4.552	11.760	<0.001
bm	-0.002	0.003	-0.007	0.004	0.547
Site (TG):bm	-0.013	0.006	-0.024	-0.001	0.035

Table 2.2C. Average BKA values for adults and yearlings at the beginning and end of the season, used to calculate ΔBKA_{YA}.

	Beginning		End	
	Mean	**Std Dev**	**Mean**	**Std Dev**
FS male	32.43	46.33	9.75	10.81
FS female	34.83	42.10	4.80	3.29
TG male	34.75	30.83	8.55	16.82
TG female	11.28	10.31	0.00	0.00

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APPENDIX 2.3

Ectoparasite loads in UGS

Ectoparasite loads were not a good predictor of variability in immunity (as measure by BKA) at either site (Fig. 2.3A). The weak trend was similar across sites, indicating that differential access to resources does not seem the affect immune response to ectoparasite loads.

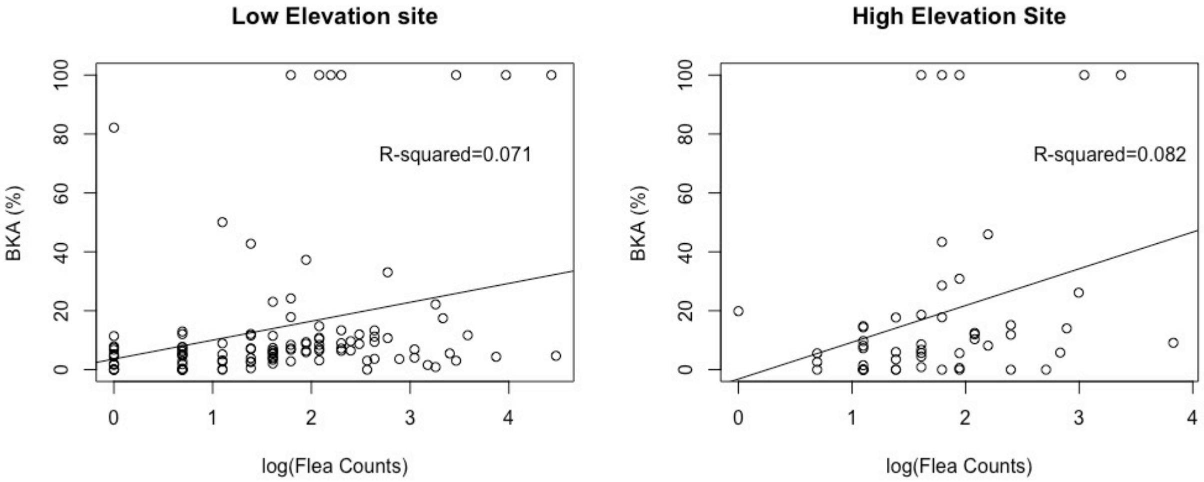

Figure 2.3A. Relationship between bacterial killing ability and ectoparasite load in adults and yearlings at FS (low elevation site) and TG (high elevation site).