To the Faculty of Washington State University:

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Animals face multiple risks while foraging such as the risk of acquiring inadequate energy from food, the risk of predation, and the risk of thermal stress. My first objective was to predict the overall perceived risk in foraging patches with multiple types of risks using a novel modeling approach that quantifies tradeoffs among competing risks by foraging animals. I evaluated how two sympatric rabbits (pygmy rabbits, *Brachylagus idahoensis*, and mountain cottontail rabbits, *Sylvilagus nuttallii*) that differ in size, use of burrows, and habitat specialization in the sagebrush-steppe of western North America respond to different levels of perceived risks, including fiber and toxins (1, 8 cineole) in food, exposure to predation (inverse of concealment cover), and distance from a burrow refuge. Exposure to predation risk and distance from a burrow refuge were riskier for pygmy rabbits than cottontails, but the dietary toxin was riskier for cottontails than for pygmy rabbits. Pygmy rabbits consumed lower quality food, containing higher fiber or toxins, to avoid feeding in exposed patches or traveling far from their burrow to forage. In contrast, cottontails fed in exposed patches and traveled farther from the burrow to obtain higher quality food with lower fiber and toxins.

My second objective was to evaluate how the interactions between ambient temperature and food quality influence selection of food patches and diets by pygmy rabbits and cottontails. I
examined preferences for temperature in food patches, and the effect of temperature on diet selection, intake, digestion, passage rate, and metabolism in both species of rabbits. Both species generally chose to feed in patches that were relatively colder, and this effect was greater for the larger cottontails. Both species also chose to eat more total food and a greater proportion of high fiber food when the ambient temperature was colder, passing food more quickly through their digestive system. Temperature did not affect how much 1,8 cineole they consumed nor how thoroughly they digested food. Food quality affected dry matter digestibility, but not resting metabolic rate of the rabbits.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>iii</td>
</tr>
<tr>
<td>Abstract</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xi</td>
</tr>
<tr>
<td>CHAPTER ONE</td>
<td>1</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>METHODS</td>
<td>6</td>
</tr>
<tr>
<td>Capture and maintenance of study animals</td>
<td>6</td>
</tr>
<tr>
<td>Preference trials</td>
<td>7</td>
</tr>
<tr>
<td>Equivalence point trials</td>
<td>8</td>
</tr>
<tr>
<td>Relative risk model</td>
<td>9</td>
</tr>
<tr>
<td>RESULTS</td>
<td>11</td>
</tr>
<tr>
<td>Preference trials</td>
<td>11</td>
</tr>
<tr>
<td>Equivalence point trials</td>
<td>11</td>
</tr>
<tr>
<td>Relative risk model</td>
<td>12</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>13</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>20</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>21</td>
</tr>
<tr>
<td>CHAPTER TWO</td>
<td>35</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>35</td>
</tr>
<tr>
<td>METHODS</td>
<td>39</td>
</tr>
</tbody>
</table>
Table 1.1 The proportion of the total food eaten that was consumed from the patch containing the lower level of fiber concentration (indicated in the row heading) when simultaneously offered a second patch of food containing the higher fiber concentration (indicated in the column heading) by pygmy rabbits (Brachylagus idahoensis) and mountain cottontail rabbits (Sylvilagus nuttallii). Asterisks indicate proportions > 0.5 (α = 0.05). ................................................................. 30

Table 1.2. The proportion of the total food eaten that was consumed from the patch containing the lower level of 1,8-cineole concentration (indicated in the row heading) when simultaneously offered a second patch of food containing the higher 1,8-cineole concentration (indicated in the column heading) by pygmy rabbits (Brachylagus idahoensis) and mountain cottontail rabbits (Sylvilagus nuttallii). Asterisks indicate proportions > 0.5 (α = 0.05). ................................................................. 31

Figure 2.3 The predicted relative perceived risk values at patches with a range of values of 1,8 cineole (%), fiber (% NDF), exposure (% openness) and distance from a burrow (m) for pygmy rabbits (Brachylagus idahoensis) and mountain cottontail rabbits (Sylvilagus nuttallii). The risk values were predicted using the model $E(R) = 0.064F + 0.076T + 0.509E + 0.098D$ for pygmy rabbits and $E(R) = 0.042F + 0.282T + 0.145E - 0.011D$ for cottontails, where $E(R)$ was the expected relative risk level, $F$ was fiber, $T$ was toxin, $E$ was exposure, and $D$ was distance from the burrow. .................................................................................. 62

Figure 2.4. Relative risks of different types of food patches in a simulated sagebrush landscape in winter and summer for pygmy rabbits (Brachylagus idahoensis) and mountain cottontail rabbits (Sylvilagus nuttallii). Food patches include Wyoming big sagebrush (Artemisia tridentata wyomingensis) on burrow systems (On-WBS) and off burrow systems (off-WBS), black sagebrush (A.nov., BLS), and bluebunch wheatgrass (Pseudoroegneria spicata, BBWG). Values for plant size, fiber, toxins, and exposure were collected during the summer and winter in Washington, Idaho, and Montana (Table 2). The risk values were predicted using the model $E(R) = 0.064F + 0.076T + 0.509E + 0.098D$ for pygmy rabbits and $E(R) = 0.042F + 0.282T + 0.145E - 0.011D$ for cottontails, where $E(R)$ was the expected relative risk level, $F$ was fiber, $T$ was toxin, $E$ was exposure, and $D$ was distance from the burrow. White areas are non-food patches. .................. 63

Table 2.1. Numbers of animals and trials used in paired preference trials (Patch 1 and Patch 2) with different levels of two types of dietary risks (fiber and toxin) and two types of predation risks (exposure to predation and distance from burrow) conducted with pygmy rabbits (Brachylagus idahoensis) and mountain cottontail rabbits (Sylvilagus nuttallii) between August 2013 and 2015 at the Small Mammal Research Facility at Washington State University, Pullman, WA. Fiber is % neutral detergent fiber, Toxin is % 1,8 cineole, Exposure is % openness and Distance is distance (m) from the burrow entrance. .................................................................................. 65
Table 2.2. Values for risk variables for four types of plant patches, including Wyoming big sagebrush (Artemisia tridentata wyomingensis) on and off pygmy rabbit (Brachylagus idahoensis) burrow systems, black sagebrush (A. nova) and bluebunch wheatgrass (Pseudoroegneria spicata), in simulated sagebrush landscape. Variables included plant size (area, cm², J. Nobler unpublished data, Frye et al. 2013), neutral detergent fiber (% Crowell 2016, Wagoner et al. 2012, N. Wiggins, unpublished data), total monoterpenes expressed in cineole equivalents (Crowell 2016, Ulappa et al. 2014, Nobler 2016), and exposure (J. Nobler unpublished data, Frye et al. 2013, M. Camp unpublished data). We assumed bluebunch wheatgrass would be covered in snow and unavailable during winter.

Table 2.3. Mean estimated βs from the relative risk model (equation 2) and 95% confidence intervals (CI) for pygmy rabbits (Brachylagus idahoensis) and mountain cottontail rabbits (Sylvilagus nuttallii). Fiber is % neutral detergent fiber, Toxin is % 1, 8 cineole, Exposure to predation is % openness, and Distance is distance (m) from the burrow entrance. An asterisk next to a β indicates that the 95% confidence interval of the mean does not overlap 0, and differences between βs between species were determined from non-overlapping confidence intervals.

Table 2.4. Mean estimated marginal rates of substitution (MRS) and 95% confidence intervals (CI) between pairs of risk variables for pygmy rabbits (Brachylagus idahoensis) and mountain cottontail rabbits (Sylvilagus nuttallii). Fiber is % neutral detergent fiber, Toxin is % 1, 8 cineole, Exposure is % openness, and Distance is distance (m) from the burrow entrance. MRS is the ratio of a pair of βs from the relative risk model (equation 2) developed for each animal. In the MRS, the denominator represents the risk the animal is willing to accept more of to reduce the risk in the numerator. Differences between risk coefficients between species were determined from non-overlapping confidence intervals.
LIST OF FIGURES

Figure 1.1 The equivalence points (equal dry matter intake) between 1,8-cineole and fiber levels in paired food patches for 9 pygmy rabbits (Brachylagus idahoensis, filled circles, solid lines) and 9 mountain cottontail rabbits (Sylvilagus nuttallii, open circles, dashed lines) and the associated equivalence curves. Increasing axes from the origin indicate increasing risk, and the slope of the curves indicate the marginal rate of substitution of cineole for fiber. ........................................ 32

Figure 1.2 The predicted relative perceived risk values at patches with a range of 1,8-cineole values for pygmy rabbits (Brachylagus idahoensis, A) and mountain cottontail rabbits (Sylvilagus nuttallii, B), and the associated indifference curves for pygmy rabbits, C) and cottontails, D). The risk values were predicted using the model $E(R) = (0.07F) + (0.10T)$ for pygmy rabbits and $E(R) = (0.05F) + (0.33T)$ for cottontails, where $E(R)$ was the expected relative risk level, $F$ was fiber and $T$ was toxin. The indifference curves were predicted using the equation $T_1 = T_2 - \beta_1 \beta_2 (F_1 - F_2)$. Each isoline depicts the combinations of fiber and toxin concentrations in food that the rabbits perceive as equally risky. The predicted relative risk levels of sagebrush (Artemisia tridentata wyomingensis) stems and leaves and mature bluebunch wheatgrass (Pseudoregneria spicata) are shown on the indifference curves................................................................. 33

Figure 3.1. The predicted relative risk values at food patches with a range of temperatures for pygmy rabbits (Brachylagus idahoensis) and mountain cottontail rabbits (Sylvilagus nuttallii). The risk values were predicted using the model $E(R) = -0.19T + 0.00024T^2$ for pygmy rabbits and $E(R) = 0.054T - 0.00033T^2$ for cottontails, where $E(R)$ was the expected relative risk level and $T$ was the temperature at a foraging patch. Risk coefficients for $T$ and $T^2$ were lower for pygmy rabbits than cottontails................................................................. 104

Figure 3.2. Proportion ± SE consumed of the diet containing cineole (5% for pygmy rabbits and 3% for cottontails) relative to the control diet (0% cineole) by pygmy rabbits (Brachylagus idahoensis) and mountain cottontail rabbits (Sylvilagus nuttallii) housed at three different temperatures (10.0 °C, 18.3 °C, and 26.7 °C). Pygmy rabbits ate a greater proportion of the cineole diet than did cottontails, but temperature did not influence diet composition. Significant differences in the proportion of the cineole diet consumed between species and temperatures are indicated by different lowercase letters above the bars......................... 105

Figure 3.3. Proportion consumed of the high fiber diet (50% neutral detergent fiber) relative to the control diet (36% neutral detergent fiber) ± SE by pygmy rabbits (Brachylagus idahoensis) and mountain cottontail rabbits (Sylvilagus nuttallii) housed at three different temperatures (10.0 °C, 18.3 °C, and 26.7 °C). Both species consumed a greater proportion of the high fiber diet with decreasing temperature. Significant differences in the proportion of the high fiber diet consumed among temperatures are indicated by different letters above the bars............. 106
Figure 3.4. Mass-adjusted daily dry matter intake ± SE for control (36% neutral detergent fiber) and high fiber (50% neutral detergent fiber) diets for pygmy rabbits (Brachylagus idahoensis) and mountain cottontail rabbits (Sylvilagus nuttallii) housed at two different temperatures (10.0 °C and 26.7 °C). Both pygmy rabbits and cottontails ate more dry mass of food at 10.0 °C than 26.7 °C, and ate more when consuming high fiber food than low fiber control diet. Pygmy rabbits had a higher mass-adjusted dry matter intake than cottontails. Significant differences in dry matter intake between temperatures and diets are indicated by lowercase letters.

Figure 3.5. Dry matter digestibility ± SE for control (36% neutral detergent fiber) and high fiber (50% neutral detergent fiber) diets for pygmy rabbits (Brachylagus idahoensis) and mountain cottontail rabbits (Sylvilagus nuttallii) housed at two different temperatures (10.0 °C and 26.7 °C). When consuming the high fiber diet, both pygmy rabbits and cottontails had a lower dry matter digestibility than when consuming the control diet, but dry matter digestibility did not vary with temperature or species. Significant differences in dry matter digestibility between diets are indicated by different lowercase letters above the bars.

Figure 3.6. Mass-adjusted daily digestible dry matter intake ± SE for control (36% neutral detergent fiber) and high fiber (50% neutral detergent fiber) diets for pygmy rabbits (Brachylagus idahoensis) and mountain cottontail rabbits (Sylvilagus nuttallii) housed at two different temperatures (10.0 °C and 26.7 °C). Both pygmy rabbits and cottontails had higher digestible dry matter intakes when housed in 10.0 °C than in 26.7 °C, and digestible dry matter intake differed between species but not between diets. Significant differences in digestible dry matter intake between temperatures and species are indicated by different lowercase letters above the bars.

Figure 3.7. Mean retention times (h) ± SE of food particles in the digestive system for control (36% neutral detergent fiber) and high fiber (50% neutral detergent fiber) diets for four pygmy rabbits (Brachylagus idahoensis) housed at two different temperatures (10.0 °C and 26.7 °C). Mean retention times differed by temperature, but not diet. Significant differences in mean retention time between temperatures are indicated by different lowercase letters above the bars.

Figure 3.8. Mass-adjusted resting metabolic rate as measured by oxygen consumption ± SE for control (36% neutral detergent fiber), high fiber (50% neutral detergent fiber), and cineole diets (3% 1,8 cineole) consumed by pygmy rabbits (Brachylagus idahoensis) and mountain cottontail rabbits (Sylvilagus nuttallii). Oxygen consumption did not differ among diets for either species, but oxygen consumption by pygmy rabbits when fed the cineole diet was higher than oxygen consumption of cottontails fed the cineole and high fiber diets. Significant differences in oxygen consumption between species and diets are indicated by different uppercase letters above the bars.
Dedication

For Reid and Teddy,

but not Donald Trump
MANUSCRIPT ATTRIBUTION

My dissertation advisor, Dr. Lisa A. Shipley, and I were responsible for all levels of work for this dissertation, including study design, data collection, lab work, analysis, and manuscript writing. Lisa Shipley, Janet Rachlow, and Jennifer Forbey collaborated on funding acquisition for this project and will be involved in editing the final manuscripts prior to publication.

Chapter one, “Modeling tradeoffs between plant fiber and toxins: a framework for quantifying risks perceived by foraging herbivores,” of this dissertation is formatted for the journal *Ecology* and was published in December 2015. The co-authors are:

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CHAPTER ONE
MODELING TRADEOFFS BETWEEN PLANT FIBER AND TOXINS: A FRAMEWORK FOR QUANTIFYING RISKS PERCEIVED BY FORAGING HERBIVORES

INTRODUCTION

Understanding factors that shape habitat use and selection of resources by animals is a central focus of ecology. When selecting habitat patches, animals must acquire adequate energy and nutrients from food while avoiding risks such as toxic plant secondary metabolites, predation and thermal stress (Werner and Hall 1988, Bakker et al. 2005, Dearing et al. 2008, Hebblewhite and Merrill 2009). Habitats vary in levels of resources and risks, forcing animals to make fitness tradeoffs when selecting patches (e.g., Sih 1980, Lima et al. 1985, Houston et al. 1993, Rachlow and Bowyer 1998, Grand 2002). Optimization approaches have been used to predict decisions animals make about which foods to eat (MacArthur and Pianka 1966, Estabrook and Dunham 1976, Mittelbach 1981, Shipley et al. 1999, Simpson and Raubenheimer 1999), which patches to feed in (e.g., Charnov 1976, Brown 1988), or how much time to spend searching for food (e.g., (Naef-Daenzer 2000) as a function of characteristics of animals and available resources by weighing costs and benefits of alternative choices in terms of a common currency related to animal fitness (Brown 1988, Pyke et al. 1977).

The economic concept of the marginal rate of substitution (MRS), the rate at which a consumer is willing to give up one good in exchange for another while maintaining the same level of utility, has been used by behavioral ecologists to convert costs and benefits of foraging decisions into a single currency or fitness function (Caraco 1979). For example, Brown (1988) used the MRS in models predicting “giving up densities” (GUD), the amount of food left in a depletable food patch after the forager has quit harvesting the patch, to determine how much
additional energy gain it would take at a patch to get a forager to accept a higher risk of predation. Subsequent models have incorporated MRS when foragers face a variety of additional costs such as toxins (Schmidt et al. 1998, Schmidt 2000), distance to water (Shrader et al. 2008), and the time lost to alternative fitness-enhancing activities (Brown 1999, Hochman and Kotler 2006, Fedriani and Boulay 2006). Although the concept of MRS has been central to optimal foraging theory, few studies have quantified the MRS between competing costs and benefits and compared them among animal species, except Schmidt (2000), who quantified MRS using the ratio of quitting harvest rate or the marginal value of two patches exploited by the same forager. However, most studies have relied on qualitative predictions about fitness or animal decisions, or indirect measures of MRS (Brown 1988, Jacob and Brown 2000, Druce et al. 2006, Bedoya-Pérez et al. 2014, Wheeler and Hik 2014).

Choice experiments provide a means for quantifying an animal’s MRS between the benefits and costs associated with foraging or between competing risks. Researchers have used behavioral titration experiments in which the energy reward or predation risk was continuously altered until a point of equivalence (i.e., indifference point) was identified (Todd and Cowie 1990, Kotler and Blaustein 1995, Nersesian et al. 2011, Kralik and Sampson 2012). In economic theory, the MRS corresponds to the slope of the indifference curve passing through bundles of goods that consumers are willing to exchange to obtain the same level of utility (Mankiw 2011). Therefore, the slope of a model fit to a series of equivalence points predicts the rate at which the forager will trade off one resource or risk for another (i.e., MRS). We use the term equivalence point rather than indifference point because the equivalence point is where the animal perceives the low reward/low risk option and the high reward/high risk option or two competing risks as equal. Under some circumstances, risk titration from a foraging animal can be a more useful
behavioral indicator of an animal’s perceptions of risk than actual measures of fitness (Brown and Kotler 2004), which are difficult to accurately measure, especially in long-lived species. Previous research has demonstrated that animals titrate marginal costs and marginal benefits between the risk of predation and potential energy gain by increasing the marginal value of resource patches with high predation risk until the high predation risk and low predation risk microhabitats were of identical value to foragers (i.e., animals harvested the same amount out of the each patch; Kotler and Blaustein 1995). Other studies have measured individual equivalence points for the risk of food toxicity and predation for foraging herbivores (Nersesian et al. 2011, McArthur et al. 2012). However, most titration studies have estimated only one equivalence point for a particular tradeoff (but see Kralik and Sampson 2012), which limits their usefulness for understanding how animals make tradeoffs in natural landscapes where risks exist over a range of values, and precludes measuring the MRS of one risk for the other.

In this study, we sought to measure the MRS between competing risks (commonly referred to as “costs” in optimal foraging theory) and used it to quantify tradeoffs between risks using behavioral titration experiments and concepts of economic decision theory. To do this, we used the method of paired comparisons (PC) to estimate utility, and the inverse, relative risk, to create a model predicting relative risk from combinations of two variables. The method of PC elicits binary choices from a subject for any number of paired items in a series of discrete choice sets (David 1988). The method determines the subject’s preference order among the various items by asking it to choose the item in each pair that best meets a given criterion (Kendall and Smith 1940, Kingsley and Brown 2013). If the subject’s preferences obey the principles of utility theory (transitivity and comparability), the result will be a relative ranking of the items in the choice set (Kendall and Smith 1940), similar to the utility model that Caraco et al. (1980)
applied to foraging theory. In contrast to foraging models that assume an animal has preferences among deterministic values of resources, Caraco et al. (1980) demonstrated that utility theory allows preferences to be extended over probability distributions. As in previous foraging models, Caraco (1980) assumed that animals should act in a way that maximizes their fitness, but utility replaces time and energy as the surrogates of fitness and the optimal strategy maximizes expected utility. Because utility, as a function of a random variable, ranks the variable on a relative scale (Keeney and Raiffa 1993), it allows animals to reveal habitat preferences on a single scale representing an integrated response to a variety of risks and rewards in their environment (Rapport 1971, Caraco 1980, Caraco et al. 1980, Lima et al. 1985, Real and Caraco 1986). In our modeling approach, we used the inverse of utility, which we refer to as “risk”, with the expectation that animals should minimize risk.

The PC method makes tradeoffs and indifferences between items explicit by forcing subjects to make choices between two items or conditions, thus the ratio of the coefficients of two variables estimates the MRS. This method can be used to determine how animals evaluate and trade off habitat attributes where the benefits and risks faced are difficult to quantify or reduce to a common currency, and to integrate diverse risks that influence fitness and habitat selection into a single index of habitat value. The outcome is a relative risk model that allows us to predict the overall perceived risk at habitat patches with multiple types of risks.

Specifically, we evaluated how two species of herbivores – the pygmy rabbit (*Brachylagus idahoensis*) and the mountain cottontail rabbit (*Sylvilagus nuttallii*, hereafter, cottontail) – that differ in their tolerance for fiber and toxins in food, make tradeoffs when selecting food patches. Plant fiber is risky to herbivores because it reduces the extent and rate at which nutrients can be extracted from food (i.e., digestibility reducer, Hoover and Heitmann
1972, Van Soest 1994, Iason and Van Wieren 1999). Defensive plant toxins (i.e., plant secondary metabolites such as terpenes, alkaloids, and phenolics) are risky because they impose a physiological cost as they are absorbed, metabolized and excreted by the animal (Freeland and Janzen 1974, Sorensen et al. 2005a, Torregrossa and Dearing 2009, Shipley et al. 2012). These costs range from small reductions in the nutritional benefits of food (Robbins et al. 1991, DeGabriel et al. 2009) to serious consequences where over-ingestion of a toxin or even small doses of some types could result in toxicosis or death (Freeland and Janzen 1974, Fowler 1983).

Although pygmy rabbits and cottontails inhabit the same sagebrush (Artemisia spp.) ecosystems in the intermountain western United States, they vary in their reliance on sagebrush for food. The pygmy rabbit is a dietary specialist, consuming primarily sagebrush, especially during the winter (Thines et al. 2004), whereas the mountain cottontail is considered a generalist and consumes < 4% sagebrush in sagebrush communities (MacCracken and Hansen 1984). Although sagebrush leaves are relatively high in digestible protein and low in fiber compared to grasses and forbs in the community (Thines et al. 2004), they contain high levels of toxins (e.g., monoterpenes; (Kelsey et al. 2006). Moreover, pygmy rabbits are more tolerant of (e.g., consume higher concentrations of toxins) sagebrush than cottontails, and cottontails can tolerate (e.g., digest) higher fiber concentrations than pygmy rabbits (Demment and Soest 1985, Shipley et al. 2012). Therefore, we predicted that 1.) pygmy rabbits would have a higher MRS of a toxin (i.e., 1,8-cineole, a major monoterpene in sagebrush; Kelsey et al. 2006) for fiber than cottontails, 2.) high-toxin patches would have a higher relative risk for cottontails than pygmy rabbits, and 3.) high-fiber patches would have higher relative risk for pygmy rabbits than cottontails.
We tested these hypotheses using a series of paired choice experiments to titrate the equivalence curve. We then used the method of PC to quantify the relative risk at food patches based on concentrations of fiber and toxins, calculate the MRS of toxin for fiber, and model the indifference curves for each species, which depicted combinations of fiber and toxins in food that the rabbits would perceive as equally risky. Finally, we used our model to predict and compare the relative risk of consuming high-toxin, low-fiber sagebrush leaves and low- or no-toxin, high-fiber sagebrush stems and grasses for each rabbit species. We predicted that consuming stems and grasses will be riskier for pygmy rabbits than cottontails and consuming leaves will be riskier for cottontails than pygmy rabbits.

METHODS

Capture and maintenance of study animals

We captured pygmy rabbits in Camas and Lemhi Counties in Idaho and Beaverhead County in Montana and mountain cottontail rabbits in Whitman County, Washington (Idaho Wildlife Collection Permits #010813 and 100310, Washington Scientific Collection Permit #13-102, Montana Scientific Collection Permit #2014-062, Washington State University Institutional Animal Care and Use Committee Protocol #4398). While they were not participating in experiments, rabbits were housed individually on pine shavings in 1.2 m × 1.8 m mesh cages located in a barn at the Small Mammal Research Facility at Washington State University, Pullman, Washington. Rabbits were fed ad libitum a basal diet of water, commercial rabbit pellets (Purina Professional Rabbit Chow, Purina Mills, LLC, St. Louis, MO, 36% NDF), greenhouse-grown or commercially-produced greens, and wild-grown sagebrush. Rabbits were
provided with a nest box and a 3-m long, 4-cm diameter tube or a wooden hutch for security cover.

Preference trials

First, to establish that pygmy rabbits and cottontails respond to differences in fiber and toxin concentrations in food, we conducted a series of preliminary preference trials in which 5–7 pygmy rabbits (both sexes, $\bar{X} = 412$ g, SD = 67 g) and cottontail rabbits (both sexes, $\bar{X} = 1127$ g, SD = 65 g) were offered 2 food patches containing different levels of fiber or toxin. To create diets that varied in fiber concentrations from 36% – 50% (dry mass, DM), we added rice hulls (77% Neutral Detergent Fiber, NDF) to a basal diet consisting of a completely-balanced herbivore pellet (Purina Professional Rabbit Chow, Purina Mills, LLC, St. Louis, MO, 36% NDF) using a pellet mill (PM605, Buskirk Engineering, Ossian, IN, USA). Grasses, forbs and sagebrush consumed by free-ranging pygmy rabbits and mountain cottontails range from about 30% – 78% NDF (Thines et al. 2004). We measured fiber content of each diet using NDF analysis (Goering and Soest 1970) with filter bags, sodium sulfite, and alpha amylase (Ankom Fiber Analyzer 200/220®, Ankom Technology, Fairport NY). We created toxin diets by adding 1,8-cineole (a major monoterpane in sagebrush, hereafter, cineole) in levels ranging from 0 – 5% DM to the basal diet. Monoterpenes in sagebrush leaves typically range from 1 – 4% by dry weight (Kelsey et al. 2006). We mixed the cineole with the basal diet daily by misting the pellets with an olive oil sprayer (Misto™, Lifetime Brands, Garden City, NY, USA) to achieve consistent distribution of the cineole on the pellets. The non-toxic diets were not misted.

The preference trials were conducted in 3.8 × 3.6 m covered, outdoor pens constructed of chain-link fence with a 2-cm mesh during May - June 2013. During each 24-hr trial, each animal was offered a choice of two food patches (food bowls) that each contained more than the
animals’ normal daily intake of food. First, we examined the rabbits’ preference for fiber by offering two food patches, each with a different level of fiber (36%, 39%, 42%, 44%, 47%). During a series of 10 trials, rabbits were offered all combinations of fiber levels. Next, we conducted a series of trials in which rabbits were offered two food patches with different concentrations of cineole (0%, 1%, 2%, 3%, 4%, 5%) mixed with the basal diet (36% NDF). During a series of 10 trials, rabbits were offered all combinations of cineole concentrations. The order of each combination of fiber or toxin concentration offered was determined using a Latin squares design, and the patch location for each diet within the pen was determined from a coin flip. To control for variable weather and moon phase, pygmy rabbit and cottontail trials were conducted simultaneously within an array of nine pens.

We allowed the rabbits three days of acclimation in the trial pens before beginning the series of trials. To minimize their perception of predation risk, the food bowls were placed on rubber mats, 3 m apart, under 0.46- m³ dark acrylic boxes with 10-cm diameter holes as entryways. We corrected fresh mass of food pellets offered by the dry matter content once for each level of fiber and cineole by drying 10-g a sample at 100° C for ≥ 24 h. We weighed the food remaining each morning, dried the refusals at 100° C ≥ 24 h, and calculated intake as the difference between DM offered and refused. We compared the proportion of total intake between the two food patches to a μ = 0.50 (equal consumption from each patch) using a one sample t-test.

**Equivalence point trials**

To evaluate tradeoffs that individuals make between the risk of fiber and toxins, we conducted a series of titration trials to obtain the equivalence points for combinations of fiber and toxin concentrations using nine pygmy rabbits and nine cottontail rabbits during June –
November 2013. For 5 levels of fiber content (36%, 39%, 44%, 47%, 50%), we conducted 3 – 6 trials per animal in which a patch with the food of the specified fiber concentration without cineole (i.e., toxin-free patch) was paired with a second patch of food containing the basal diet (the lowest fiber content, 36%) and varying amounts of cineole (i.e., toxin patch, 0% – 15%). Our goal was to find the concentration of cineole that resulted in equal intake between the two food patches (toxin-free and toxin) for each level of fiber concentration (i.e., equivalence points). To do this, we titrated the amount of cineole in the paired bowl over 3 – 6 trials per level of fiber to produce an adequate linear fit ($R^2 > 0.60$, $P < 0.05$) between the proportion of the toxin diet eaten and the concentration of cineole for each level of fiber for each animal. We estimated the equivalence points by fitting a linear model in which the dependent variable was the proportion of the toxin diet eaten and the independent variable was the concentration of cineole. We then solved for the concentration of cineole where the proportion of the toxin diet consumed by each animal was 0.5, indicating equal preference for the toxin-free and toxin diet.

To estimate the marginal rate of substitution of cineole for fiber, and compare it between pygmy rabbits and cottontails, we used analysis of covariance (ANCOVA; PROC GLM, SAS Version 9.3. SAS institute 2011). The dependent variable was the concentration of cineole in the toxin food patch and the independent variables were fiber concentration in the toxin-free patch, rabbit species, and the fiber × species interaction.

Relative risk model

Using the method of PC and data collected from eight pygmy rabbits and eight cottontails during the preference and equivalence trials, we created a model predicting the relative risk of patches containing different levels of cineole and fiber. We assumed that

$$E(R_{ijk}) = \beta_{0i} + \beta_{1i}F_{ijk} + \beta_{2i}T_{ijk}, \quad (1)$$
where \(E(R_{ijk})\) denoted the expected “risk level” of the \(k\)th food patch for the \(i\)th animal on the \(j\)th trial and \(F_{ijk}\) and \(T_{ijk}\) were the concentration of fiber, \(F\), or toxin, \(T\), in the food in the \(k\)th food patch presented to the \(i\)th animal in the \(j\)th trial. \(\beta_{1i}\) is the rate at which an increase in fiber increases relative risk and \(\beta_{2i}\) is the rate at which an increase in toxin increases relative risk. Because we offered two food patches \((k = 1,2)\), let \(Y_{ij} = R_{ij1} - R_{ij2}\) denote the difference in the risk of each patch. Then, from Equation (1), we have that

\[
E(Y_{ij}) = \beta_{1i}(F_{ij1} - F_{ij2}) + \beta_{2i}(T_{ij1} - T_{ij2}).
\] (2)

We quantified \(Y_{ij}\) as the difference in intake between the two bowls divided by the total intake during each trial. We used a regression analysis to provide estimates of \(\beta_{1i}\) and \(\beta_{2i}\) for each animal (R Development Core Team 2008). We tested if the mean values of \(\beta_{1i}\) and \(\beta_{2i}\) were significantly different than zero using a one sample t-test. We compared \(\beta_{1i}\) and \(\beta_{2i}\) within species and between species using a two sample t-test. We then created indifference curves for each species that depicted food patches in terms of \(F\) and \(T\) in food that the rabbits would perceive equally risky. These are defined by the expression

\[
T_1 = T_2 - \frac{\bar{\beta}_1}{\bar{\beta}_2}(F_1 - F_2),
\] (3)

where \(\bar{\beta}_1\) and \(\bar{\beta}_2\) are the average values of the estimate coefficients for each species. This equation defines, for each species, all pairs of potential food patches between which an average animal would be indifferent (i.e., equal risk). We then calculated the MRS of toxin for fiber as \(\beta_{1i}/\beta_{2i}\) and compared the MRS between species using a two-sample t-test.

Finally, we used the modeled indifference curves to predict the relative risk level of sagebrush leaves and stems and native grasses in the winter perceived by pygmy rabbits and cottontails. We used the values reported by Crowell (2015) for NDF and total monoterpene content of leaves (30% NDF, 2.48% monoterpenes) and stems cut at \(\leq 3\) mm diameter (77%
NDF, 0.39% monoterpenes) from Wyoming big sagebrush (A. tridentata wyomingensis) collected in January near Leadore, ID, where a portion of our rabbits were caught. We used the NDF value reported by Thines et al. (2004) for monoterpane-free bluebunch wheatgrass (Pseudoregneria spicata, 76% NDF) collected in winter in habitat supporting both pygmy rabbits and cottontails near Ephrata, WA. We converted the total monoterpane values of sagebrush into 1,8-cineole equivalents (1.18 for pygmy rabbits and 1.28 for pygmy rabbits, J. Nobler, Boise State University, unpublished), estimating a cineole equivalent of 2.80% for pygmy rabbits and 3.20% for cottontails for sagebrush leaves, and a cineole equivalent of 0.43% for pygmy rabbits and 0.49% for cottontails for sagebrush stems. We calculated the relative risk for the sagebrush leaf and stem patches using equation 1, where F was the fiber concentration in the stems or leaves and T was the cineole concentration in the stems or leaves and then plotted the value in relation to the indifference curves.

RESULTS

Preference trials

Both pygmy rabbits and cottontail rabbits avoided risks of fiber and toxins by typically consuming a greater proportion of diets with lower levels of fiber (Table 1) and cineole (Table 2). However, cottontails were less discriminant among higher levels of fiber than were pygmy rabbits.

Equivalence point trials

The concentration of cineole that the rabbits perceived as equally risky to the concentration of fiber in food patches increased with fiber content of the toxin-free patch (F_{3,71} = 44.71, P < 0.0001), and varied with rabbit species (F_{3, 71} = 69.99, P < 0.0001). Moreover, there was an interaction between fiber and rabbit species (F_{3, 71} = 28.80, P < 0.0001). These results
indicate that animals were making a tradeoff between the risk of fiber and toxins, but that the rate of the tradeoffs (i.e., MRS) differed between pygmy rabbits and cottontails (Fig. 1). As fiber in the toxin-free diet increased, the amount of cineole in the toxin diet that both species perceived as equivalent also increased. The slope of the regression line (i.e., MRS) was steeper for pygmy rabbits ($\bar{X} = 0.68$, SE = 0.13) than cottontails ($\bar{X} = 0.12$, SE = 0.01, Fig. 1), indicating that they traded off the risk of toxicity to avoid the risk of starvation at a faster rate than did cottontails.

**Relative risk model**

Using data from 905 paired choice trials ($\bar{X} = 54$ per animal), the model predicting relative risk value at a food patch for pygmy rabbits was $E(R) = (0.07F) + (0.10T)$ (mean $R^2 = 0.55$), and for cottontails was $E(R) = (0.05F) + (0.33T)$ (mean $R^2 = 0.55$), where $E(R)$ was the expected relative risk level, $F$ was fiber (% NDF), $T$ was toxin (% cineole, Fig. 2A, B), and the coefficients were the average estimated values within each species. Relative risk increased with both fiber (pygmy rabbit: $t_8 = -11.14$, $P < 0.0001$, cottontail: $t_7 = -8.14$, $P < 0.0001$) and cineole (pygmy rabbit: $t_8 = -14.51$, $P < 0.0001$, cottontail: $t_7 = -8.79$, $P < 0.0001$) for both species. However, risk increased faster with fiber for pygmy rabbits ($t_{14} = 2.35$, $P = 0.03$, Fig. 2A), and with cineole for cottontail rabbits ($t_{14} = -5.67$, $P < 0.001$, Fig. 2B). For pygmy rabbits, the slope for fiber ($\bar{X} = 0.07$, SE = 0.007) was not significantly different than that for cineole ($\bar{X} = 0.10$, SE = 0.008, $t_{16} = 2.07$, $P = 0.06$). However, for cottontails the slope for fiber ($\bar{X} = 0.05$, SE = 0.006) was shallower than that for cineole ($\bar{X} = 0.33$, SE = 0.04), indicating that cineole was riskier than fiber for cottontails ($t_{14} = 6.9$, $P > 0.001$). The estimated MRS of cineole for fiber (i.e., $\beta_1/\beta_2$) was greater for pygmy rabbits ($\bar{X} = 0.86$, SE = 0.17) than cottontails ($\bar{X} = 0.15$, SE = 0.02, $t_{15} = 3.81$, $P = 002$), and similar to that estimated from our equivalence curves. Using
equation 1, sagebrush leaves were 2.3 times less risky than stems and mature grass for pygmy rabbits, but only 1.5 times less risky for cottontails (Fig. 2B, C).

DISCUSSION

We developed a novel framework for quantifying and comparing the tradeoffs (i.e., MRS) and relative risks faced by animals when selecting food patches based on two risks that operate in different ways – an incremental decrease in nutrient digestibility for plant fiber and a more acute toxicity from monoterpenes above a certain threshold value (Sorenson et al. 2005, Wiggins et al. 2006). We did this by finding where two species of rabbits perceived the risks of fiber and toxins as equivalent across a range of risk values (i.e., the equivalence curve, Fig. 1) using behavior (amount of food eaten in the patch) as the common currency. We then used the PC method to model the relative risk posed by fiber and toxins and construct indifference curves for these risks. Pygmy rabbits were willing to consume nearly five times more cineole in their diets to avoid consuming higher levels of fiber than were mountain cottontails. Fiber posed a greater relative risk for pygmy rabbits than cottontails and cineole a greater risk for cottontails than pygmy rabbits. For cottontails, the risk of incremental increases in the concentration of cineole was higher than the risk of incremental increases in the concentration of fiber, whereas for pygmy rabbits, fiber and cineole were equally risky. These differences in risk tradeoffs likely reflect differences in body size and physiology between the rabbit species.

First, pygmy rabbits are less than half the size of mountain cottontails, thus are expected to have a higher mass specific metabolic rate (Kleiber 1975) and a higher energy requirement to gut size ratio (Demment and Van Soest 1985). In fact, pygmy rabbits required about 36% greater daily digestible energy intake in relation to their metabolic body mass (mass$^{0.75}$) to maintain their body mass than did cottontails (Shipley et al. 2006). In addition, pygmy rabbits
have a smaller gut volume, which scales in proportion to body mass, than cottontails, and therefore would be expected to have a lower capacity to digest the cellulose in plant fiber (Demment and Van Soest 1985), which requires mechanical breakdown of cell wall and fermentation by microorganisms (Iason and Van Wieren 1999). When consuming the same diets ranging from 29 – 55% NDF, pygmy rabbits only digested 50 – 90% of the amount of NDF as did cottontails (Shipley et al. 2006). Fiber reduces the amount of nutrients animals can acquire from plants, while increasing the time needed to digest food and reducing the rate at which animals can consume food (Iason and Van Wieren 1999). For example, domestic rabbits that were fed a high fiber diet had a larger mass-specific cecal volume and a lower body mass compared to rabbits on a low fiber diet (Hoover and Heitmann 1972). Therefore, the risk of starvation is higher for the smaller pygmy rabbit consuming high fiber diets than for the larger cottontail.

On the other hand, the risk of toxicity was likely lower for pygmy rabbits than cottontails because pygmy rabbits are better able to minimize systemic exposure to toxins found in sagebrush (Shipley et al. 2012). Exposure to consumed toxins can be reduced by minimizing absorption into the bloodstream and maximizing the rate of detoxification (Sorensen et al. 2006). Several mechanisms, such as efflux transporters, allow excretion of toxins in the feces, thereby decreasing concentrations of toxins in the blood (Hunter and Hirst 1997). For example, P-glycoproteins in the gut epithelial cells actively transport toxic compounds out of the gut cell and reduce delivery to circulation (Sparreboom et al. 1997). Plant toxins such as monoterpenes can also be detoxified in the liver through alternative metabolic pathways such as conjugation or oxidation (Dearing and Cork 1999, Marsh et al. 2006). In a comparative study in which pygmy rabbits and cottontails were fed 1,8-cineole, pygmy rabbits excreted two times more conjugated
cineole metabolites in urine than did cottontails (Shipley et al. 2012). An average of 28% of the ingested cineole was recovered as cineole metabolites in the urine and 3.8% as metabolites in feces of pygmy rabbits, whereas no metabolites were found in the feces of cottontails (Shipley et al. 2012). Similarly, Stephen’s woodrats (*Neotoma stephensi*) that specialize on juniper (*Juniperus monosperma*) had a greater capacity to limit exposure to plant toxins by excreting more alpha-pinene, a monoterpene found in juniper, in their feces than did the generalist white-footed woodrat (*Neotoma albigena*) (Sorensen and Dearing 2003, Sorensen et al. 2004).

The rate and extent of detoxification, in turn, determines the amount of toxic food an animal can consume (Marsh et al. 2005). Herbivores maintain systemic toxin levels behaviorally by regulating total food intake, meal size, timing of consumption, and mixing diets (Sorensen et al. 2005, Wiggins et al. 2005). Pygmy rabbits, with their higher capacity to detoxify monoterpenes, were able to maintain their daily intake of rabbit pellets as the concentration of cineole increased over 5%, whereas mountain cottontails began to reduce intake when cineole concentration was only 1% (Shipley et al. 2012). Likewise, the specialist Stephen’s woodrat maintained its intake on diets containing up to 4% phenolic resin found in creosote bush (*Larrea tridentata*), whereas the generalist white-throated woodrat reduced their intake at concentrations of 2% (Torregrossa et al. 2012). Therefore the risks of toxins in food, including increased energy expenditure (Sorensen et al. 2005b), reduced nutrient digestibility (Robbins et al. 1987, DeGabriel et al. 2009), and toxicosis or death from over exposure (Freeland and Janzen 1974) can be especially high for generalist herbivores like cottontails that lack specific adaptations for eliminating and detoxifying plant toxins. Furthermore, variability in how individual animals perceive, tolerate, and respond to risk of toxicity in specialist herbivores might explain the larger variance in the equivalence curve for pygmy rabbits than cottontails (Fig. 1). Individuals may
differ physiologically (e.g., detoxification capacity, stress hormones, metabolism; (Maltby 1999, Montooth et al. 2006) or behaviorally (e.g., boldness; Mella et al. 2014). For example, individual brushtail possums (*Trichosurus Vulpecula*) varied in measures of boldness, and boldness influenced foraging at patches with high predation risk and patches with low predation risk only when food toxins were low (Mella et al. 2014). However, Simpson and Raubenheimer (1999) found that locusts that were dietary specialists (*Locusta gregaria*) were more likely to minimize the error of overeating one nutrient and undereating another than were generalist locusts (*Schistocerca gregaria*), presumably because the generalists would be more likely to later encounter and consume a plant with a complementary imbalance to the present food, allowing the ingested excess from the current food to balance the deficit in subsequent one.

In this study, we also demonstrated how our model could be applied to understanding diet choices of pygmy rabbits and cottontails observed in the field. Our relative risk model predicted that consuming sagebrush leaves, with low fiber and high monoterpenes, is relatively less risky than consuming sagebrush stems and mature grasses, and that stems and grasses were relatively riskier for pygmy rabbits than for cottontails. These predictions are supported by observations from both field and captive studies that pygmy rabbits consume a greater proportion of sagebrush relative to grasses and a greater leaf to stem ratio of sagebrush than do cottontails (Crowell 2015). In fact, cottontails often discard the leafy tips of sagebrush branches uneaten. Most herbivores, especially browsers, are highly selective foragers within habitats (Hofmann 1989). For example, snowshoe hares (*Lepus americanus*) selectively fed on the mature internodes over the juvenile internodes when feeding on Alaska green alder (*Alnus crispa*, Clausen et al. 1986). This preference was correlated with the higher levels of toxins in the juvenile internodes (Clausen et al. 1986). Using the relative risk levels of fiber and monoterpenes in leaves and
stems, our model predicted that pygmy rabbits and cottontails should select different forages and different parts of sagebrush plants when sharing sagebrush landscapes. The difference in the foraging strategies between these two species provides insight into a possible mechanism for coexistence in sagebrush habitats.

Clearly fiber and toxin concentrations are not the only risks faced by herbivores when selecting food patches or habitats. Like GUD models (Brown 1999, Schmidt 2000, Hochman and Kotler 2006, Fedriani and Boulay 2006, Shrader et al. 2008), our model framework easily allows addition of any number of additional risks (e.g., predation, thermal stress) or even benefits (e.g., nutrient content, food density). For each risk (or benefit) included in the model, its β depicts its contribution to overall risk of that patch or habitat, and the ratio of the β’s for any two risks estimates the MRS for one risk over the other. Exploring three-, four and n-way interactions can elucidate complex foraging behavior of herbivores. For example, Schmidt (2000) found that the MRS for predation risk, as measured by the ratio of GUDs in safe and risky habitats, did not vary with the level of oxalates (defensive compounds), whereas MRS for tannins (digestibility reducers) increased with missed opportunity cost and decreased with metabolic cost. He concluded that foods containing plant defenses that act as digestibility reducers will be relatively less depleted under higher predation risk that will foods without defenses, whereas foods containing defensive toxins that affect fitness directly and not through diminished physiological uptake of energy will be depleted without bias to predation risk or foraging costs.

Unlike classic patch theory (e.g., marginal value theorem, Charnov 1976) and most GUD models (Schmidt 2000, Price and Correll 2001, Brown and Kotler 2004, Searle et al. 2005), our model framework does not assume a decelerating gain function at a food patch caused by
monotonically decreasing harvesting rates as the patch is depleted. In our experiments, we offered food patches with equal harvesting rates and linear gain functions over 24-hour trials (M. Camp, unpublished data) by providing food patches that exceeded the rabbits’ daily intake packaged in uniformly small bites of pellets in a bowl. However, our model framework could be used to assess relative risks associated with time spent harvesting food (e.g., increased exposure to predation and thermal stress, reduced time for other fitness-enhancing activities) and MRS of harvesting rate for other perceived risks altering bite size, cropping, chewing or searching time in one patch (MacArthur and Pianka 1966, Brown 1988, Gross et al. 1993). In addition, in our model we assumed a linear relationship for fiber and toxins with relative risk, and that fiber and toxins acted independently on perceived risk. Nevertheless, the model could be modified to include a nonlinear relationship between the response and explanatory variables by adding polynomial functions and interactions between risks. For example, risks of tannins depend on protein content of food (Simpson and Raubenheimer 1999), risks of monoterpenes depend on the environmental temperature (Dearing et al. 2008), and risks of feeding away from a burrow depend on the amount of concealment cover (Crowell 2015).

Like Raubenheimer and Simpson (1993), we did not make any a priori judgments about the relative importance of different food properties or nutritional requirements of animals and allowed the animal to indicate how it prioritized their ingestion. Because our model uses the animal’s behavior (i.e., its choice between two patches) as the currency, this choice can be measured in a variety of ways. Although we used dry matter intake at a feeding patch as our response variable, other measures of choice could be used. For example, the time spent in a non-food patch can be used to quantify the perceived risk of non-food patches such as nesting or loafing areas, and determine which perceived risks most strongly influence selection of different
types of habitat patches. Finally, our model differs from many resource selection models (Manly et al. 1993) that are based on discrete choices (i.e., presence or absence) because it produces a continuous response variable (i.e., the relative amount of food consumed) that is a quantitative proxy to relative risk. Although we used a series of controlled titration experiments with captive animals to provide data for our relative risk model, the method of PC could be applied in field experiments where animals could be presented with two food patches at a time and the habitat properties at each food location measured.

In summary, animals face multiple risks in their environments that vary in time and space. Many previous approaches to understanding habitat use have not fully incorporated or quantified tradeoff strategies that animals with different tolerances to habitat features use to reduce risk, or are limited by inflexible assumptions and statistical techniques. Our application of the method of PC to foraging behavior is useful for quantifying tradeoffs that animals make among risks. Furthermore, it allows animals to reveal habitat preferences on a single scale representing an integrated response to a variety of risks in their environment. This approach could be useful for identifying and mapping relative risk levels at habitat patches across landscapes by creating a spatially-explicit “landscape of risk” and the predicted relative risk of habitat patches across the landscape could be compared with measures of intensity of use by free-ranging animals (Searle et al. 2008, Willems and Hill 2009). The application of the method of PC to ecological questions in both controlled captive experiments and in field studies provides a more realistic approach to understanding how animals balance competing risks through tradeoffs.
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REFERENCES


Table 1.1 The proportion of the total food eaten that was consumed from the patch containing the lower level of fiber concentration (indicated in the row heading) when simultaneously offered a second patch of food containing the higher fiber concentration (indicated in the column heading) by pygmy rabbits (*Brachylagus idahoensis*) and mountain cottontail rabbits (*Sylvilagus nuttallii*). Asterisks indicate proportions > 0.5 (\(\alpha = 0.05\)).

<table>
<thead>
<tr>
<th>Rabbit Species</th>
<th>% Fiber in lower fiber patch</th>
<th>% Fiber in higher fiber patch</th>
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<tr>
<td></td>
<td></td>
<td>39</td>
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<tr>
<td>Pygmy rabbit</td>
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</tr>
<tr>
<td>36</td>
<td></td>
<td>0.76 ± 0.10*</td>
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<tr>
<td>39</td>
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<td>0.75 ± 0.07*</td>
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<tr>
<td>42</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Mountain cottontail</td>
<td></td>
<td>0.81 ± 0.08*</td>
</tr>
<tr>
<td>36</td>
<td>--</td>
<td>0.77 ± 0.05*</td>
</tr>
<tr>
<td>39</td>
<td>--</td>
<td>0.52 ± 0.05</td>
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<tr>
<td>42</td>
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<td>0.62 ± 0.08</td>
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</table>
Table 1.2. The proportion of the total food eaten that was consumed from the patch containing the lower level of 1,8-cineole concentration (indicated in the row heading) when simultaneously offered a second patch of food containing the higher 1,8-cineole concentration (indicated in the column heading) by pygmy rabbits (*Brachylagus idahoensis*) and mountain cottontail rabbits (*Sylvilagus nuttallii*). Asterisks indicate proportions > 0.5 ($\alpha = 0.05$).

<table>
<thead>
<tr>
<th>Rabbit Species</th>
<th>% Cineole in lower cineole patch</th>
<th>% Cineole in higher cineole patch</th>
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<tr>
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<td></td>
<td>1</td>
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<tr>
<td>Pygmy rabbit</td>
<td>0</td>
<td>0.77 ± 0.06*</td>
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<td></td>
<td>1</td>
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<td></td>
<td>2</td>
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<td>3.5</td>
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<tr>
<td>Mountain cottontail</td>
<td>0</td>
<td>0.90 ± 0.06*</td>
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<tr>
<td></td>
<td>3.5</td>
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</table>
Figure 1.1 The equivalence points (equal dry matter intake) between 1,8-cineole and fiber levels in paired food patches for 9 pygmy rabbits (*Brachylagus idahoensis*, filled circles, solid lines) and 9 mountain cottontail rabbits (*Sylvilagus nuttallii*, open circles, dashed lines) and the associated equivalence curves. Increasing axes from the origin indicate increasing risk, and the slope of the curves indicate the marginal rate of substitution of cineole for fiber.
Figure 1.2 The predicted relative perceived risk values at patches with a range of 1,8-cineole values for pygmy rabbits (*Brachylagus idahoensis*, A) and mountain cottontail rabbits (*Sylvilagus nuttallii*, B), and the associated indifference curves for pygmy rabbits, C) and cottontails, D). The risk values were predicted using the model $E(R) = (0.07F) + (0.10T)$ for pygmy rabbits and $E(R) = (0.05F) + (0.33T)$ for cottontails, where $E(R)$ was the expected relative risk level, $F$ was fiber and $T$ was toxin. The indifference curves were predicted using the equation $T_1 = T_2 - \frac{\beta_1}{\beta_2}(F_1 - F_2)$. Each isoline depicts the combinations of fiber and toxin concentrations in food that the rabbits perceive as equally risky. The predicted relative risk levels
of sagebrush (*Artemisia tridentata wyomingensis*) stems and leaves and mature bluebunch wheatgrass (*Pseudoregneria spicata*) are shown on the indifference curves.
CHAPTER TWO

THE BALANCING ACT OF FORAGING: MAMMALIAN HERBIVORES TRADE-OFF MULTIPLE RISKS WHEN SELECTING FOOD PATCHES

INTRODUCTION

When animals attempt to acquire nutrients from a food patch, they are constrained by properties of the food itself, such as prey size and density or plant fiber and plant secondary metabolites (i.e., toxins), and by the context in which the patch of food occurs, such as exposure to predators, competitors, and environmental conditions (Grubb 1975, Turner 1982, Lucas 1983, Lima 1985, Brown 1988, McArthur et al. 1991, Shipley and Spalinger 1995, Behmer et al. 2002). Each of these properties exacts a penalty or cost to the animal’s potential fitness at a given probability of occurrence (i.e., expected risk), and even small changes in habitat characteristics can result in large changes in the risk perceived by the animal (Heithaus and Dill 2002; Brown and Kotler 2004; Willems and Hill 2009). Therefore, while seeking food in heterogeneous landscapes, animals often make tradeoffs among multiple, simultaneous risks.

The most common examples of tradeoffs focus on how foraging animals weigh the risk of predation against benefits or risks associated with food (Sih 1980; Lima 1985; Houston et al. 1993; Kotler and Blaustein 1995; Schmidt 2000; Grand 2002; Brown and Kotler 2004; McArthur et al. 2012; McArthur et al. 2014). However, tests of herbivore responses to the interplay among risks have often been restricted to qualitative tradeoffs between only two types of risks or benefits, only two levels of each risk type, or a cost-benefit analysis of only one risk (Sih 1980; Lima 1985; Houston et al. 1993; Grand 2002; Brown and Kotler 2004; Nersesian et al. 2011). In contrast, the method of paired comparisons (David 1988), which has been used in social sciences and economics to model preferences and decisions by humans (Fishburn 1968), can be used to
quantify overall “utility” (i.e., level of satisfaction) by animals in habitat patches in relation to any number of multiple variables (i.e., risks) simultaneously. The model can also quantify the marginal rate of substitution (MRS), which is the rate at which the forager will trade off one risk for another while maintaining the same level of utility or expected risk (Camp et al. 2015). To do this, the animal is presented with items (e.g., food patches) in a series of paired choice sets, forcing it to choose between them. The model creates a utility function based on the relative ranking of these items, which reveals the animal’s habitat preferences on a single scale representing an integrated response to a variety of risks and rewards in their environment (Rapport 1971). Because properties of the food patch are integrated by an animal’s choice based on its overall satisfaction, risks can vary not only in cost to fitness, but also the perceived probability that that cost will be incurred while foraging, thus its expected risk. Although the method of paired comparisons has been used to model tradeoffs between two risks, fiber and toxins, in food patches selected by rabbits (Camp et al. 2015), this type of model could be extended to explore the consequences of multiple, diverse types of risks that occur at different intensities and different spatiotemporal scales.

Our objectives were to develop a model that predicts overall perceived risk and quantifies tradeoffs between four competing risks encountered at food patches – two associated with the food patch itself (plant fiber and toxin) and two associated with the context of the patch in terms of perceived predation risk (exposure to predation [inverse of concealment cover] and distance from a burrow refuge). To do this, we conducted a series of paired choice trials with two species of captive rabbits that we expected to differ in their perception of these risks. In our modeling approach, we used the inverse of utility, which we refer to as “risk,” with the expectation that animals should minimize risk.
To predict overall risk at a foraging patch, in the model we included the individual risks of the amount of fiber and toxins in food, exposure to predation, and distance from a refuge. Fiber in the cell wall of plants is risky to foragers because it dilutes nutrients, slows intake, and in some species can slow or impede digestion, thus animals might not assimilate enough nutrients to keep up with metabolic requirements and are therefore at risk of starvation (McArthur et al. 1991; Iason and Van Wieren 1999). Likewise, the risks of ingesting plant secondary metabolites such as terpenes, alkaloids, and phenolics, ranges from small reductions in the nutritional benefits of food (e.g., digestibility; (McArthur et al. 1991; Robbins et al. 1991), to compromised energy budgets (Sorensen et al. 2005) and reproductive success (DeGabriel et al. 2009) to serious physiological costs where the inability to avoid toxin intake result in toxicosis or death (Freeland and Janzen 1974; Fowler 1983; Foley and McArthur 1994).

Habitat features can also influence an animal’s perceived risk of predation. Depending on an animal’s adaptations, actual and perceived predation risk might be greater in more concealed (Götmark et al. 1995, Schooley et al. 1996) or more exposed (Banks et al. 1999, Camp et al. 2012, Nersesian, et al. 2012) patches. For animals that rely on a central place burrow, nest or refuge, the risk of predation might increase with distance from an animal’s refuge (Lima 1985; Bakker et al. 2005), Camp et al. 2012). For both food and predation risks, the potential cost to fitness ranges from very high (death from starvation, toxicosis, or predation) to low (small reduction in energy acquired, missed opportunity costs or increased energy expenditures from modifying other activities to avoid these costs). However, the probability of incurring these costs at a given food patch are likely higher for the food risks than the predation risks.

In this study, we evaluated how pygmy rabbits and mountain cottontail rabbits (hereafter, cottontails), which both inhabit sagebrush (Artemisia spp.) ecosystems in the intermountain
western United States, but differ in size, tolerance for fiber (Shipley et al. 2006) and toxins (Shipley et al. 2012) in food, and use of burrows and concealment cover (Camp et al. 2015, Crowell et al. 2016), weigh these four risks when selecting food patches. Pygmy rabbits are dietary specialists, consuming a diet composed of 50 – 100% sagebrush (Thines et al. 2004), whereas cottontails are dietary and habitat generalists that consume < 4% sagebrush (MacCracken and Hansen 1984). Although relatively high in digestible protein and low in fiber, sagebrush contains high levels of toxins (i.e., monoterpenes, polyphenolics and sesquiterpene lactones; Kelsey et al. 2006) that might interact with digestibility (Kohl et al. 2015). Pygmy rabbits have a greater ability to minimize absorption and maximize detoxification of toxins in sagebrush than do cottontail rabbits (Shipley et al. 2012), but the cottontails have a greater capacity to digest plant fiber, especially in relation to their energy requirements, than pygmy rabbits (Shipley et al. 2006). Pygmy rabbits rely on sagebrush and burrow systems for protection from predators (Camp et al. 2012) and temperatures during the winter (Katzner et al. 1997), whereas cottontail rabbits occupy a wide range of habitats, and use, but do not require, burrows (Orr 1940; Chapman 1975). Both pygmy rabbits and cottontails experience high levels of predation from aerial and terrestrial predators (Bond et al. 2001; Crawford et al. 2010; Price et al. 2010).

Therefore, we predicted that when faced with multiple risks simultaneously, both pygmy rabbits and cottontails would perceive food patches with higher levels of fiber, sagebrush toxins, and exposure to predation as riskier, but only pygmy rabbits would perceive food patches farther from a burrow as riskier. We expected that more toxic food patches would be relatively riskier for cottontails than pygmy rabbits, and food patches farther from a burrow would be relatively riskier for pygmy rabbits than cottontails. We expected that pygmy rabbits would be willing to
consume more toxic food patches to forage closer to their burrows, whereas cottontails would forage further from a burrow to consume food with lower fiber and toxins. In addition, we created a simulated “landscape of risk” to demonstrate a potential future application of our modeling approach in which the model is used with spatially-explicit habitat data to predict use of foraging patches by free-ranging herbivores.

METHODS

Capture and maintenance of study animals

We captured pygmy rabbits in Camas and Lemhi Counties in Idaho, USA, and Beaverhead County in Montana, USA, and mountain cottontail rabbits in Whitman County, Washington, USA (Idaho Wildlife Collection Permits #010813 and 100310, Washington Scientific Collection Permit #13-102, Montana Scientific Collection Permit #2014-062, Washington State University Institutional Animal Care and Use Committee Protocol #4398). While they were not participating in experiments, rabbits were housed individually on pine shavings in 1.2 m × 1.8 m mesh cages located at Washington State University, Pullman, Washington. Rabbits were fed ad libitum a basal diet of water, commercial rabbit pellets (Purina Professional Rabbit Chow, Purina Mills, LLC, St. Louis, MO, 36% NDF, 18% CP), greenhouse-grown or commercially-produced greens, and wild-grown sagebrush. Rabbits were provided with a nest box and a 3-m long, 10-cm diameter tube or a wooden hutch for security cover.

General experimental approach

To evaluate risk levels at foraging patches and tradeoffs that individuals made between risks, we conducted a series of paired choice trials during May 2013 – September 2015 in which we offered rabbits a choice between two food patches that varied in the level of fiber and toxin in food, the level of exposure to predators, and the distance from a burrow. In each set of trials,
rabbits were offered two food patches in outdoor experimental arenas that were sporadically exposed to the sight, smell, and sound of free-ranging predators, including coyotes (*Canis latrans*), badgers (*Taxidea taxus*), great-horned owls (*Bubo virginianus*), and red-tailed hawks (*Buteo jamaicensis*) that inhabit the natural area surrounding the arenas. Although the rabbits within the pens were never at risk of mortality, we controlled for behavioural variation from exposure to predators by randomizing the order in which rabbits received treatments for each feeding trial. Each patch contained a bowl of *ad libitum* of the basal diet of rabbit pellets, or the experimentally-manipulated diet, placed on a rubber mat under a 0.46 × 0.46 × 0.46 m clear acrylic box with a 10-cm diameter opening. We recorded the amount of food offered and remaining after 24 hrs for each patch in each experiment and corrected for dry matter by drying the food remaining and a sample of the food pellets offered at 100° C for ≥ 24 hrs. All of the experiments, except for those in which we manipulated distance from a burrow, were conducted in nine outdoor arenas (≈ 3.8 × 3.6 m) constructed of chain-link fence covered with a 2-cm mesh. Each arena contained two familiar refuges, an insulated nest box placed on one side of the arena and a 120-cm long plastic tube (8-cm diameter) placed on the opposite side of the arena. We conducted trials in which distance of a food patch from a burrow was manipulated in three outdoor arenas (≈ 4 × 12.5 m) constructed of 2-cm mesh, each with the 0.5-m high burrow mound on one end of the arena.

For all trials, we established a baseline level of each variable that was the lowest expected risk level consistent with what the animals might experience in their natural habitat. The lowest fibre level was 36% neutral detergent fiber (NDF), the lowest toxin level was 0% 1,8-cineole (a major monoterpen in sagebrush, hereafter cineole), and the lowest levels of predation risk were 0% exposure to predation (i.e., a transparent box over a food patch) and 1 m from a burrow. In
preliminary experiments, we ensured that rabbits responded to increasing levels of each type of risk when applied separately (i.e., fiber and cineole [Camp et al. 2015], exposure to predation (i.e., increasing opaqueness of the cover box) and distance from a burrow [Crowell et al. 2016]). To create diets that varied in fiber levels from 36% – 50% (dry mass, DM), we added rice hulls (77% NDF) to the basal diet of rabbit pellets diet using a pellet mill (PM605, Buskirk Engineering, Ossian, IN, USA). Grasses, forbs and sagebrush consumed by free-ranging pygmy rabbits and mountain cotontails range from about 30% – 78% NDF (Thines et al. 2004). We measured fiber content of each diet using NDF analysis (Goering and Soest 1970) with filter bags, sodium sulfite, and alpha amylase (Ankom Fiber Analyzer 200/220®, Ankom Technology, Fairport NY). We created toxin diets by adding cineole in levels ranging from 0 – 10% DM to the basal diet. Monoterpenes in sagebrush leaves typically range from 1 – 4% by dry weight (Kelsey et al. 2006). We mixed the pure cineole with the basal diet daily by misting the pellets with a commercial olive oil mister to achieve consistent distribution of the cineole on the pellets.

We varied perceived exposure to predation by attaching a sheet of transparency to each of the five sides of the clear acrylic cover box that was placed over each food patch (Crowell et al. 2016). Each cover transparency was divided into 100 – 0.46 × 0.46-cm squares. To create different levels of perceived exposure to predation, we randomly selected squares that were coloured an opaque black. We varied distance from a burrow refuge by conducting a series of trials with food patches placed at distances from 1 – 8.5 m from artificial burrows located within a soil mound (six entrances, 8 – 10-cm diameters), similar to those used by free-ranging pygmy rabbits and sometimes cotontails, which are typically found on natural soil mounds and have multiple entrances that are 10-12 cm in diameter (Green and Flinders 1980).
For all trials, we used 3 – 9 individual pygmy rabbits (both sexes, $\bar{X} = 412$ g, SD = 67 g) for a total of 913 trials and 3 – 9 individual cottontail rabbits (both sexes, $\bar{X} = 1127$ g, SD = 65 g), for a total of 675 trials. Trials with pygmy rabbits and cottontails were conducted simultaneously. We conducted two types of trials (Table 1). During the first type of trial, only one of the four variables was varied from baseline to the highest risk level, and the other variables remained at baseline. During the second type of trial, we varied two variables and the others remained at baseline levels. For any trial that involved only fibre and distance or only cineole and distance, we conducted trials at the baseline level of 0% exposure to predators (completely covered with opaque black transparency), but also at three addition levels of exposure (50, 75, 100%) because during preliminary trials, exposure and distance interacted such that animals would travel farther to a food patch if they were less exposed (i.e., more concealed resulting from less transparency).

Statistical analysis

Using the method of paired comparisons and data collected during all 1588 trials, we created a model predicting the relative risk of patches containing different levels of fiber, toxin, exposure to predation, and distance from a burrow for each animal. We assumed that

$$E(R_{ijk}) = \beta_0 + \beta_1 F_{ijk} + \beta_2 T_{ijk} + \beta_3 E_{ijk} + \beta_4 D_{ijk} ,$$

(1)

where $E(R_{ijk})$ denoted the expected “risk level” of the $k^{th}$ food patch for the $i^{th}$ animal on the $j^{th}$ trial. $F_{ijk}, T_{ijk}, E_{ijk},$ and $D_{ijk}$ were the levels of fiber (F, %), toxin (T, %), and exposure to predation (E, proportion), and distance to burrow (D, m) in the $k^{th}$ food patch presented to the $i^{th}$ animal in the $j^{th}$ trial. The $\beta_i$ was the rate at which an increase in fiber, toxin, exposure or distance increases relative risk. We assumed a linear relationship between variables and expected risk. Because we offered two food patches ($k = 1, 2$), let $Y_{ij} = R_{ij1} - R_{ij2}$ denote the difference in the risk of each patch, Then, from Equation (1), we have that
\[ E(Y_{ij}) = \beta_{1i}(F_{ij1} - F_{ij2}) + \beta_{2i}(T_{ij1} - T_{ij2}) + \beta_{3i}(E_{ij1} - E_{ij2}) + \beta_{4i}(D_{ij1} - D_{ij2}). \]  

We quantified \( Y_{ij} \) as the difference in intake between the two patches divided by the total intake during each trial.

We used a regression analysis to provide estimates of \( \beta \) for each risk variable for each animal (R Development Core Team 2.15.12). We assumed that any individual risk variable contributed to the overall expected risk in the patch if the 95\% confidence interval of its mean \( \beta \) did not overlap zero. We compared \( \beta \)'s among risks between species by determining if the 95\% confidence intervals of the mean parameter estimates overlapped each other. We computed the MRS for all risk pairs for each animal from the ratio \( \beta_i/\beta_j \) and then averaged the ratios within species. In the MRS, the denominator represents the risk the animal is willing to accept more of to reduce the risk in the numerator. We evaluated whether the MRSs differed between species by calculating 95\% confidence intervals around the mean MRSs for each species.

**Landscape of risk simulation**

Finally, we demonstrated how our model could be used to visualize the risk landscape and predict a priori the selection of foraging patches in a real landscape containing diverse risks. We created a simulated 40 × 40 m “landscape” that reflected the structure of pygmy rabbit burrow systems and plant communities of sagebrush steppe landscapes in which pygmy rabbits and cottontails coexist. Using actual measurements collected during winter and summer from Idaho, Washington, and Montana (Table 2), we assigned average values for plant size (area), fiber and total monoterpenes content (%), and an average of aerial and terrestrial exposure (%) for three dominant plant species (i.e., Wyoming big sagebrush (Artemisia tridentata wyomingensis); black sagebrush (Artemisia nova), and bluebunch wheatgrass (Pseudoroegneria spicata). Because Wyoming big sagebrush growing on burrow systems of pygmy rabbits differs from
sagebrush growing off the burrow system in terms of architecture and nutritional quality (Green and Flinders 1980; Ulappa et al. 2014; Parsons et al. 2016), we used different values for plants on and off burrow systems. We assumed that bluebunch wheatgrass would be covered by snow during winter, hence, unavailable as food or cover. As a measure of risk related to distance from a refuge, we assigned each pixel a value for distance to nearest simulated burrow. We then applied the models for pygmy rabbits and cottontails developed from the paired choice experiments to the simulated landscapes to estimate relative risk at each foraging patch (i.e., plant), and by extension, expected use by pygmy and cottontail rabbits (i.e., higher use at lower risk patches). For each pixel on the landscape consisting of a plant patch, relative risk was calculated using the values for fiber, cineole, exposure to predation, and distance from a burrow.

RESULTS

Risks associated with food and predation explained 45% of the variation in expected relative risk of food patches (i.e., difference in proportion of food consumed between food patches) for both pygmy rabbits (mean $R^2 = 0.45 \pm 0.04$) and cottontails (mean $R^2 = 0.46 \pm 0.04$), but the contribution of each individual risks to expected relative risk at a food patch differed between the rabbit species. As expected, relative risk for both species increased with increased fiber and cineole (Table 3). Relative risk increased with distance from a burrow and exposure for pygmy rabbits, but not for cottontails (Table 3). Regression coefficients ($\beta$) for exposure and distance were greater for pygmy rabbits than cottontails (Table 3), indicating that the variables related to predation risk were riskier for pygmy rabbits than cottontails. The 95% confidence intervals for the fiber $\beta$ for each species barely overlapped, indicating that fiber tended to be riskier for pygmy rabbits than cottontails (Table 3). The $\beta$ for cineole, on the other hand, was greater for cottontails (Table 3), thus cineole was riskier for cottontails than for pygmy rabbits.
Pygmy rabbits and cottontails also had different relative MRS between risk variables, suggesting that they weighed tradeoffs between risks differently. The MRS of fiber for cineole, exposure for cineole, and distance from a burrow for cineole were all greater for pygmy rabbits than for cottontails, indicating that they were willing to accept cineole at a faster rate than cottontails to reduce their intake of fiber or risk of predation (Table 4, Fig. 1). The MRS of distance from a burrow for fiber and for cineole also were relatively greater for pygmy rabbits than cottontails (Table 4), indicating that they traded off fiber or cineole to reduce distance from the burrow at the faster rate than cottontails (Fig. 1). The MRS of fiber for exposure to predation and exposure for distance were relatively similar between pygmy rabbits and cottontails (Table 4, Fig. 1). The large confidence intervals for many of the MRS values are a consequence of the fact that ratios can be imprecise without very large sample sizes, and the estimates for an MRS are less stable than those of the mean coefficients.

Applying the expected risk from our model to a simulated landscape consisting of different sizes and species of sagebrush during the winter, with the addition of grass during the summer, demonstrated how free-ranging pygmy rabbits and cottontails might perceive and trade off food patches differently. Large Wyoming big sagebrush plants were predicted to be the least risky patches for pygmy rabbits and riskiness of all sagebrush patches decreased with patch size and increased with distance from the burrow during both winter and summer (Fig. 2). During the summer, however, small sagebrush plants far from burrows were predicted to be equally risky to pygmy rabbits as patches of bluebunch wheatgrass closer to burrows, despite their higher fiber and exposure to predation (Fig. 2). In contrast, bluebunch wheatgrass patches were less risky for cottontails than any of the more toxic sagebrush patches, regardless of their exposure and distance from burrow systems (Fig. 2).
DISCUSSION

By simultaneously quantifying the responses of rabbits to varying levels of four diverse types of risks associated with food patches, we have demonstrated that foraging decisions within heterogeneous landscapes require nuanced behavioural trade-offs that differ between two sympatric species. We have shown for the first time that the method of paired comparisons can be used to examine how four different risks interact to shape perceptions of risk at foraging patches. We found that pygmy rabbits, which are smaller and specialized for sagebrush habitats with deep soils, consumed lower quality food, containing higher fiber or toxins, to avoid feeding in exposed patches or traveling far from their burrow to forage. In contrast, larger cottontails, which are habitat generalists, fed in exposed patches and traveled farther from the burrow to select food lower in fiber and toxins. Applying our model, which integrates multiple risks at a foraging patch into one relative risk value, to a simulated landscape demonstrated how this modeling framework could be used to predict the spatial distribution of each species. The differences in how the two species perceive and make tradeoffs between risks across a landscape reflects their physiology and evolutionary adaptations to sagebrush habitat.

First, the rabbit species perceived and traded off risks of fiber and toxins in their food differently. Cineole posed a greater relative risk (i.e., higher β) for cottontails than pygmy rabbits. In fact, cottontails were willing to consume nearly five times more fiber in their diets to avoid consuming higher levels of cineole than were pygmy rabbits (i.e., lower MRS). This result was not surprising because pygmy rabbits are less than half the size of cottontails, thus are expected to have a higher mass-specific metabolic rate and a higher energy requirement to gut size ratio than cottontails (Kleiber 1975; Demment and Van Soest 1985). Pygmy rabbits require about 36% more digestible energy per unit body mass than cottontails to maintain body mass and
are less efficient at digesting plant fiber than cottontail rabbits (Shipley et al. 2006). On the other hand, the risk of toxicity was lower for pygmy rabbits than cottontails because pygmy rabbits are twice as tolerant to toxins found in sagebrush (Shipley et al. 2012). Previous studies that have examined tradeoffs among food risks showed that other species also have responded to the interplay between toxin and nutrient concentration. For example, swamp wallabies (Wallabia bicolor) equally valued food with both high fiber and low cineole and food with both low fiber and high cineole (Bedoya-Pérez et al. 2014).

Likewise, pygmy rabbits and cottontails differed in how they perceived two types of predation risks. Exposure to predation (the inverse of concealment cover) increased the relative risk in a patch for pygmy rabbits but not cottontails in our experiments, despite other captive studies that indicated that both species preferred food patches that offered greater artificial concealment provided in a similar manner to our study (Crowell et al. 2016, Utz et al. 2016, Camp et al. 2015), and a field study that found that lower exposure to predation (higher levels of concealment cover) decreased the predation risk perceived by wild pygmy rabbits (Camp et al. 2012). The perceived risk of exposure varied among individual rabbits more than other types of risks we investigated (i.e., had relatively higher confidence intervals), supporting previous studies that implied that individual animals might respond to risks differently depending on the physiological (e.g., detoxification capacity, stress hormones, metabolism), ecological (e.g., competition), or life history strategy of the forager (Stamps 2007; Wolf et al. 2007; McArthur et al. 2014; Mella et al. 2014). In addition, both pygmy rabbits and cottontails in captivity responded more strongly to orientation of exposure to predation (e.g., terrestrial vs. aerial) than the average level of exposure from all sight lines (Crowell et al. 2016), whereas orientation of exposure was not varied in our experiment.
Similar to exposure to predation, foraging further from a burrow was riskier for pygmy rabbits than cottontail rabbits in our experiments. Furthermore, when they were required to make trade-offs between exposure and distance from the burrow, pygmy rabbits relinquished lower levels of exposure (higher concealment) to forage closer to the burrow, indicating that the perceived safety provided by burrow refuges was more important than the perceived safety provided by concealment cover. Our results are not surprising in light of the difference in burrowing behavior between the pygmy rabbits and cottontails. Pygmy rabbits are obligate burrowers and require the deep, sandy or loamy soils in sagebrush landscapes to dig natal and residential burrows, which they rely on for protection from predators and thermal stress (Green and Flinders 1980; Rachlow et al. 2005). In contrast, cottontails will use burrows, but do not typically dig or require them (Orr 1940, Chapman 1975).

Because of their unique adaptations to detoxify sagebrush and construct residential burrows, pygmy rabbits in our study weighed the risk of predation more heavily than food risks than did cottontails. When pygmy rabbits were forced to choose between distance from a burrow and either of the food risks investigated, pygmy rabbits traded off the food risk to forage closer to the burrow at a relatively greater rate than did cottontails. Likewise, when pygmy rabbits were forced to choose between exposure and the toxin risk, the traded off the food risk to forge in the more concealed patches at relatively faster rate than cottontails. In contrast, when cottontails were forced to choose between cineole and any of the other risks investigated, they exposed themselves to the other risk to avoid toxic effects of cineole. Although tradeoffs between fiber and toxins in food were relatively consistent among individuals (i.e., MRS had a narrow confidence interval), tradeoffs between either of the food variables with either of the predation risk variables varied to a greater extent, as was found by Utz et al. (2016). Behaviors associated
with avoiding predation might vary more among individuals than behaviors associated with avoiding fiber and toxins in food because predation risk might vary to a greater degree over time and space and is therefore less predictable than the effects of consuming toxins or fiber.

Like rabbits in our experiments, other species ranging from bottlenose dolphins (Heithaus and Dill 2002), dugongs (*Dugong dugon*; (Wirsing et al. 2007), gerbils (*Gerbillus andersoni allenbyi*; (Kotler et al. 2004), and elk (*Cervus elaphus*; (Hebblewhite and Merrill 2009) trade access to profitable foraging patches for safety. Likewise, other central place foragers trade-off foraging opportunities to remain close to the burrow. For example, European rabbits (*Oryctolagus cuniculus*, Bakker et al. 2005) and collared pikas (*Ochotona collaris*, (Holmes 1991) foraged less with increasing distance from their burrows even when forage biomass increased with increased distance from the burrow. The interactive effects of toxins and predation risk have also been measured in experiments with fox squirrels (*Sciurus niger*; (Schmidt 2000), wood mice (*Apodemus sylvaticus*; (Fedriani and Boulay 2006), brushtail possums (*Trichosurus vulpecula*; (Kirmani et al. 2010; Nersesian et al. 2011), and bushbabies (*Otolemur crassicaudatu*; (McArthur et al. 2012). In each of these studies, foraging decisions depended on food quality (e.g., fiber and toxins). Animals perceived both food quality and security cover (concealment or burrow) as a risk while foraging and were not willing to increase their exposure to predation risk unless high levels of fiber or toxin forced them to do so. Similarly, the common brushtail possum perceived an artificially covered patch containing food with 5% cineole as equal to an exposed cineole-free patch (Nersesian et al. 2011). In fact, the quantitative value of this “equivalence point” is similar to what we documented for pygmy rabbits in our study, which perceived a patch that was about 80% exposed as equally risky to a completely covered patch (0% exposure) with 5% cineole in the food.
Unlike these previous studies, however, our modeling approach allowed us to evaluate the complex ways animal trade-off risks by converting multiple risks that act simultaneously and vary continuously into one relative risk value, and to quantify the MRS between risks. The method of paired comparisons used in this study is rooted in utility theory, which has yet to become commonplace in the wildlife literature. However, Caraco (1980) and Caraco et al. (1980) used utility functions for energetic rewards to evaluate animals’ preferences when food availability was unpredictable (Caraco 1980; Caraco et al. 1980). More recently, we used the method of paired comparisons to examine tradeoffs in dietary risks in pygmy rabbits and cottontails (Camp et al. 2015). Discrete choice models, which are similar to our paired choice models in that they are based on utility theory, have been used to evaluate resource selection by free-ranging animals (Cooper and Millsapauh 1999; McDonald et al. 2006). Discrete choice models, unlike our paired choice models, allow more than two choices. Both paired choice and discrete choice models can be used to rank animals’ preferences over a set of resources, thus can be used to estimate the importance of the attributes of the resource in the selection process or the probability of selecting a given resource. Because a utility function is assumed to be compensatory, researchers using these methods can calculate an MRS between resource attributes to evaluate tradeoffs between resources or evaluate how much one attribute would need to change in response to a change in another attribute for the probability of an individual selecting a resource to remain constant. In our study, we only evaluated the risks at food patches; therefore, our results do not apply to non-food patches such as resting patches or sites use for reproduction (e.g., mating, nesting). However, our method could also be used to evaluate other types of patches by using indices such as fecal counts, bed sites, radio telemetry, or camera traps as a measure of animal choice, rather than food intake. If both resting and foraging locations are
evaluated, researchers could include variables for the rewards of food, thermal properties, and cover and the response variable could be the utility of the patch, rather than the risk level. Finally, for simplification, we assumed a linear relationship between risk variables and expected relative risk as a local approximation of any potential non-linear models. However, the model framework allows logarithmic relationships between expected risk at the patch and individual risk variables or interactions between risks.

Herbivores integrate the influences of environmental cues when selecting foraging locations (Hochman and Kotler 2006; Fedriani and Boulay 2006; Kirmani et al. 2010; Camp et al. 2015). Food quality is associated with cues from the environment such as plant nutrients (e.g., protein) and anti-nutrients (plant fibre and PSMs; (McArthur et al. 1991; Iason and Van Wieren 1999) that can be detected by smell, taste and vision (Provenza et al. 1990; Siitari et al. 2002; Jakubas and Mason). Likewise, predation risk is often associated with indirect cues from the environment, such as low levels of concealment cover (Longland and Price 1991) or moonlight (Kotler et al. 2010), and with direct cues, such as looming objects or the scat and urine of predators (Apfelbach et al. 2005). By demonstrating how our relative risk model can be applied to a landscape, we have shown how these cues can be incorporated into one model that allows animals to demonstrate their perceptions of food and predation risks on a single, continuous scale that could be used to create spatially-explicit topography of the risk landscape. Ecologists have used empirical measures of behavior of free ranging animals, such as food intake and predator avoidance, to create a topography of an animal’s landscape of fear (e.g., (Hernández and Laundré 2005; Searle et al. 2008; Iribarren and Kotler 2012; Coleman and Hill 2014). However, ours is the first study to use behavioural data to create a landscape of multiple risks *a priori* that can be used to predict the perceived value of food and cover resources on the landscape for free-ranging
animals, guide data collection, and provide further insight into the functional relationships between animals and their habitat.

An important next step is to test the predictive ability of our model against animal locations and remotely-sensed and ground measurements of habitat features. Testing the model would require developing landscapes of risks from spatially-explicit maps of fiber and toxins in food patches, exposure to predation (inverse of concealment), and burrow locations paired with animal locations collected using radio or GPS telemetry. Ultimately, this research will advance our ability to assess and predict behavioural responses of animals such as the intensity of use of food patches and other habitats in heterogeneous landscapes, which is increasingly important as habitat is fragmented and altered on a global scale. An understanding of how animals respond to diverse habitat cues provides a basis for predicting consequences of environmental change and for mitigating its effects on species and ecosystems.

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Fedriani JM, Boulay R (2006) Foraging by fearful frugivores: combined effect of fruit ripening


Figure 2.3 The predicted relative perceived risk values at patches with a range of values of 1.8 cineole (%), fiber (% NDF), exposure (% openness) and distance from a burrow (m) for pygmy rabbits (*Brachylagus idahoensis*) and mountain cottontail rabbits (*Sylvilagus nuttallii*). The risk values were predicted using the model $E(R) = 0.064F + 0.076T + 0.509E + 0.098D$ for pygmy rabbits and $E(R) = 0.042F + 0.282T + 0.145E - 0.011D$ for cottontails, where $E(R)$ was the expected relative risk level, $F$ was fiber, $T$ was toxin, $E$ was exposure, and $D$ was distance from the burrow.
Figure 2.4. Relative risks of different types of food patches in a simulated sagebrush landscape in winter and summer for pygmy rabbits (*Brachylagus idahoensis*) and mountain cottontail rabbits (*Sylvilagus nuttallii*). Food patches include Wyoming big sagebrush (*Artemisia tridentata wyomingensis*) on burrow systems (On-WBS) and off burrow systems (off-WBS), black sagebrush (*A.nova*, BLS), and bluebunch wheatgrass (*Pseudoroegneria spicata*, BBWG). Values for plant size, fiber, toxins, and exposure were collected during the summer and winter in
Washington, Idaho, and Montana (Table 2). The risk values were predicted using the model \( E(R) = 0.064F + 0.076T + 0.509E + 0.098D \) for pygmy rabbits and \( E(R) = 0.042F + 0.282T + 0.145E \) + -0.011D for cottontails, where \( E(R) \) was the expected relative risk level, \( F \) was fiber, \( T \) was toxin, \( E \) was exposure, and \( D \) was distance from the burrow. White areas are non-food patches.
Table 2.1. Numbers of animals and trials used in paired preference trials (Patch 1 and Patch 2) with different levels of two types of dietary risks (fiber and toxin) and two types of predation risks (exposure to predation and distance from burrow) conducted with pygmy rabbits (*Brachylagus idahoensis*) and mountain cottontail rabbits (*Sylvilagus nuttallii*) between August 2013 and 2015 at the Small Mammal Research Facility at Washington State University, Pullman, WA. Fiber is % neutral detergent fiber, Toxin is % 1,8 cineole, Exposure is % openness and Distance is distance (m) from the burrow entrance.

<table>
<thead>
<tr>
<th>Trial type</th>
<th>No. animals/no. trials</th>
<th>Patch 1</th>
<th>Patch 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Food risk</td>
<td>Predation risk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fiber</td>
<td>Toxin</td>
</tr>
<tr>
<td>Fiber</td>
<td></td>
<td>36 - 50</td>
<td>0</td>
</tr>
<tr>
<td>Toxin</td>
<td></td>
<td>36</td>
<td>0 - 5</td>
</tr>
<tr>
<td>Exposure</td>
<td></td>
<td>36</td>
<td>0</td>
</tr>
<tr>
<td>Distance</td>
<td></td>
<td>36</td>
<td>0</td>
</tr>
<tr>
<td>Fiber-toxin</td>
<td></td>
<td>36 - 50</td>
<td>0</td>
</tr>
<tr>
<td>Fiber-exposure</td>
<td></td>
<td>36 - 50</td>
<td>0</td>
</tr>
<tr>
<td>Toxin-exposure</td>
<td></td>
<td>36</td>
<td>0 - 9</td>
</tr>
<tr>
<td>Fiber-distance</td>
<td></td>
<td>36 - 50</td>
<td>0</td>
</tr>
<tr>
<td>Toxin-distance</td>
<td></td>
<td>36</td>
<td>0 - 10</td>
</tr>
<tr>
<td>Exposure-distance</td>
<td></td>
<td>36</td>
<td>0</td>
</tr>
</tbody>
</table>

a A subset of these data were used in Crowell *et al.* 2015.

b These data were used in Camp *et al.* 2015.

<table>
<thead>
<tr>
<th>Plant type</th>
<th>Winter</th>
<th>Summer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area (cm²)</td>
<td>Fiber (%)</td>
</tr>
<tr>
<td>Wyoming big sagebrush on burrow system</td>
<td>3432</td>
<td>40</td>
</tr>
<tr>
<td>Wyoming big sagebrush off burrow system</td>
<td>2368</td>
<td>41</td>
</tr>
<tr>
<td>Black sagebrush</td>
<td>1528</td>
<td>44</td>
</tr>
<tr>
<td>Bluebunch wheatgrass</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
Table 2.3. Mean estimated $\beta$s from the relative risk model (equation 2) and 95% confidence intervals (CI) for pygmy rabbits (*Brachylagus idahoensis*) and mountain cottontail rabbits (*Sylvilagus nuttallii*). Fiber is % neutral detergent fiber, Toxin is % 1, 8 cineole, Exposure to predation is % openness, and Distance is distance (m) from the burrow entrance. An asterisk next to a $\beta$ indicates that the 95% confidence interval of the mean does not overlap 0, and differences between $\beta$s between species were determined from non-overlapping confidence intervals.

<table>
<thead>
<tr>
<th>Risk variables</th>
<th>Pygmy rabbits</th>
<th></th>
<th>Cottontail rabbits</th>
<th></th>
<th>Difference between species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>n</td>
<td>95% CI</td>
<td>$\beta$</td>
<td>n</td>
</tr>
<tr>
<td>Fiber</td>
<td>0.064*</td>
<td>13</td>
<td>0.050 – 0.077</td>
<td>0.042*</td>
<td>9</td>
</tr>
<tr>
<td>Toxin</td>
<td>0.076*</td>
<td>13</td>
<td>0.046 – 0.107</td>
<td>0.282*</td>
<td>9</td>
</tr>
<tr>
<td>Exposure</td>
<td>0.509*</td>
<td>8</td>
<td>0.304 – 0.714</td>
<td>0.145</td>
<td>9</td>
</tr>
<tr>
<td>Distance</td>
<td>0.098*</td>
<td>11</td>
<td>0.071 – 0.126</td>
<td>-0.011</td>
<td>8</td>
</tr>
</tbody>
</table>
Table 2.4. Mean estimated marginal rates of substitution (MRS) and 95% confidence intervals (CI) between pairs of risk variables for pygmy rabbits (*Brachylagus idahoensis*) and mountain cottontail rabbits (*Sylvilagus nuttallii*). Fiber is % neutral detergent fiber, Toxin is % 1, 8 cinoele, Exposure is % openness, and Distance is distance (m) from the burrow entrance. MRS is the ratio of a pair of $\beta$s from the relative risk model (equation 2) developed for each animal. In the MRS, the denominator represents the risk the animal is willing to accept more of to reduce the risk in the numerator. Differences between risk coefficients between species were determined from non-overlapping confidence intervals.

<table>
<thead>
<tr>
<th>Risk variables</th>
<th>Pygmy rabbits</th>
<th>Cottontail rabbits</th>
<th>Difference between species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRS</td>
<td>n</td>
<td>95% CI</td>
</tr>
<tr>
<td>Fiber vs. toxin</td>
<td>1.301</td>
<td>12</td>
<td>0.814 – 1.788</td>
</tr>
<tr>
<td>Fiber vs. exposure</td>
<td>0.310</td>
<td>8</td>
<td>-0.020 – 0.641</td>
</tr>
<tr>
<td>Exposure vs. toxin</td>
<td>9.669</td>
<td>8</td>
<td>2.209 – 17.128</td>
</tr>
<tr>
<td>Distance vs. fiber</td>
<td>2.075</td>
<td>10</td>
<td>1.137 – 3.013</td>
</tr>
<tr>
<td>Distance vs. toxin</td>
<td>2.120</td>
<td>9</td>
<td>1.148 – 3.092</td>
</tr>
<tr>
<td>Distance vs. exposure</td>
<td>0.173</td>
<td>6</td>
<td>0.035 – 0.312</td>
</tr>
</tbody>
</table>
CHAPTER THREE

BOTH TEMPERATURE AND FOOD QUALITY INFLUENCE FORAGING AND DIETS OF SMALL MAMMALIAN HERBIVORES

INTRODUCTION

Rising global temperatures are projected to influence the distribution of small mammalian herbivores (Parmesan and Yohe, 2003) because environmental temperature influences their energy and time budgets (Chappell, 1980; Porter and Gates, 1969). Elevated environmental temperature increases energy expended to maintain homeothermy in endotherms (Porter et al., 1994) and time spent using behavioral methods to buffer against these effects (Caraco et al., 1990). Warming temperatures, coupled with decreased precipitation also can change the composition and nutritional quality of forage plants available to herbivores, thus decreasing their energy acquisition and increasing the time needed to find nutritious food (Gershenzon, 1984; Jensen et al., 2016). Therefore, information about how interactions between environmental temperature and food quality affect selection of food patches and diets by small mammalian herbivores, and the physiological mechanisms governing these choices, is required to predict future distributions and inform conservation and restoration strategies.

Mammalian herbivores can reduce energy costs while foraging by selecting food patches with thermally suitable environments, the range of ambient temperatures without regulatory changes in metabolic heat production or evaporative heat loss (Huey, 1991). For example, alpine ibex (*Capra ibex*) in Italy, which have a low tolerance for heat, fed most actively during the coolest time of day and moved to higher elevations and spent less time feeding as the temperature and solar radiation increased (Aublet et al. 2009). Similarly, white-footed mice (*Peromyscus leucopus*) adjusted their foraging behavior according to temperature. They spent
more time foraging on nights when the soil temperature was higher, presumably reducing their thermoregulatory costs (Orrock and Danielson, 2009).

When ambient temperature or habitat conditions forces herbivores to feed in patches above their TNZ; however, they might attempt to consume foods that generate heat during digestion or detoxification or affect heat dissipation through vasoconstriction or dilatation. Consuming higher fiber diets at temperatures in colder temperatures might allow animals to offset heat loss with endogenous heat generated through microbial fermentation (Noblet et al., 1985; Young, 1981, NRC, 1981, West, 1997), but the role of dietary fiber and heat of fermentation in thermoregulation, especially in small mammals is not clear (Mader et al., 2001). Likewise, consuming food with high monoterpenes, a PSM, seemed to benefit white-throated woodrats (Neotoma albigula) at low temperatures by providing endogenous heat production, and harm them at warmer temperatures because the increase in metabolic rate resulted in an increase in body temperature (Dearing et al. 2008). The ingestion of some types of PSMs can help endotherms maintain body temperatures within their TNZs. This might be achieved through a reduction in heat dissipation through vasoconstriction or through heat production through detoxification (Forbey et al. 2009). However, animals might not be able to consume higher levels of PSMs in cold environments because they are limited by the rate of their detoxification system. For example, the intake of juniper by woodrats (Dearing et al., 2000; Sorensen et al., 2005a) and food containing the monoprene 1,8 cineole by pygmy rabbits (Brachylagus idahoensis) and mountain cottontail rabbits (Sylviagus nuttallii) was limited by PSM concentrations (Shipley et al., 2012).

Herbivores forced to feed in patches below their TNZ might attempt to increase intake of digestible energy to meet the energetic demands of thermoregulation. Animals can do this by
eating more total food, choosing higher quality foods (i.e., forage higher digestible energy), or digesting food more thoroughly. However, cold temperatures often occur in winter when forage plants senesce and become more fibrous, thus less digestible. Although herbivores can obtain energy from plant fiber through microbial fermentation, this process is relatively slow and inefficient, especially in small hindgut-fermenting herbivores (Demment and Van Soest, 1985). When consuming high fiber diets over a period of days, small mammals can adjust to elevated energy demands in cold temperatures by increasing retention time in the digestive tract, thereby increasing microbial fermentation and the digestion and absorption of nutrients (Felicetti et al., 2000; García et al., 1999; Gross et al., 1985; Hammond and Wunder, 1991; Jørgensen et al., 1996). Retention time can be increased by slowing the rate of passage through the digestive tract or by expanding the size of the tract (Gross et al., 1985; Hammond and Wunder, 1995; Jørgensen et al., 1996; Nagy and Negus, 1993; Zhao et al., 1996). As herbivores increase their intake during exposure to cold temperatures, the rate of passage through the digestive tract also increases, thereby decreasing nutrient digestibility (Christopherson and Kennedy, 1983). Therefore, retention time must be further increased to maintain digestibility (Hammond and Wunder, 1991; Veloso and Bozinovic, 1993).

Because the functional relationships between ambient temperature and selection of diets and food patches are likely complex and have yet to be fully understood, we examined preferences for temperature during foraging, and the effect of temperature on diet selection, intake, digestion, passage rate, and metabolism in two species of small, hindgut herbivores, pygmy rabbits and mountain cottontail rabbits. These species co-occur in the semi-arid sagebrush (Artemisia spp.) steppe in the intermountain west of the United States. Animals inhabiting these landscapes are exposed to extreme seasonal temperature variation from as low
as -40 °C during winter and to as high as 45 °C during summer (Knapp, 1997). These rabbits differ in body size and tolerance for PSMs and plant fiber, thus would be expected to respond differently to food quality in different ambient temperatures. Pygmy rabbits are nearly half the size of cottontails, with higher mass-specific energy requirements, lower ability to digest fiber (Shipley et al., 2006), and potentially, a narrower TNZ. The lower critical temperature for winter acclimatized pygmy rabbits is between 15 °C – 18 °C and the upper critical temperature is predicted to be between 23 °C – 28 °C (Katzner et al. 1997). The TNZ for mountain cottontails has not been documented, but the TNZ for the desert cottontail (Sylvilagus audubonii) is 28 °C – 40 °C during summer and 25 °C – 36 °C during winter (Hinds, 1973). In addition, pygmy rabbits are a dietary specialist on sagebrush (Thines et al 2004), which is high in monoterpenes and other PSMs (Kelsey et al., 2006), and they can detoxify and eliminate these PSMs to a greater extent than cottontails (Shipley et al. 2012). Based on these known physiological differences, we hypothesized that warm temperatures would be perceived as riskier than cooler patches for cottontail rabbits, pygmy rabbits would consume a greater proportion of PSMs in cold temperatures and both species would consume a greater proportion of the high fiber diet at colder temperatures.

To test this hypothesis, we used a series of feeding experiments with captive pygmy rabbits and cottontails, we first investigated perceived riskiness of food patches that varied in temperature. We predicted that rabbits would perceive patches within their TNZ as least risky, and that the larger cottontails would be more sensitive to warmer patches and pygmy rabbits more sensitive to cold patches. Second, we determined the effects of temperature on selection of PSMs and plant fiber in their diet. Because terpenes have been shown to increase RMR (Dearing et al., 2008), we predicted that rabbits would consume a diet relatively lower in PSMs in
temperatures above their TNZ and relatively higher in temperatures below their TNZ. We expected the effect of PSMs would be more pronounced in the larger cottontails at warm temperatures and in the smaller, PSM-tolerant pygmy rabbits at lower temperatures. We also predicted that both species would consume a greater proportion of the high fiber diet at temperatures below their TNZ because of the potential for microbial fermentation to increase heat production, but the effect would be more pronounced in the smaller pygmy rabbit. Third, we measured the effects of temperature on daily intake, digestion and passage of food. We predicted that rabbits would either consume more food or digest it more thoroughly in cold temperatures relative to warm temperatures in response to the increase in thermoregulatory requirements. Because forage digestibility in herbivores depends on time exposed to microbial fermentation, we expected the passage rate of food would increase with an increase in intake and a decrease in temperature. Lastly, we examined the effects of PSMs and plant fiber on RMR. We predicted that when both species of rabbits consumed PSMs they would have higher RMR because of the energy cost of detoxification (Sorensen et al., 2005b), but that the effect would be more pronounced in mountain cottontails because they are less resistant to PSMs in sagebrush and use more energetically expensive pathways of detoxification (Shipley et al., 2012). We also expected that consuming higher amounts of fiber would increase RMR because of the increased costs of mechanical and chemical processing of food. Understanding these basic relationships will increase our understanding of the relative value, and interactions between, thermal refugia and food quality.

METHODS

Capture and maintenance of study animals
We captured pygmy rabbits for these experiments in Camas and Lemhi Counties in Idaho and Beaverhead County in Montana, USA (Idaho Wildlife Collection Permits #010813 and 100310 and Montana Scientific Collection Permit #2014-062) and mountain cottontail rabbits in Whitman County, Washington (Washington Scientific Collection Permit #13-102, Washington State University Institutional Animal Care and Use Committee Protocol #4398). While they were not participating in experiments, rabbits were housed individually on pine shavings in 1.2 m × 1.8 m enclosures located at the Small Mammal Research Facility at Washington State University, Pullman, Washington, USA. Rabbits were fed ad libitum a basal diet of water, completely-balanced commercial rabbit pellets (Purina Professional Rabbit Chow, Purina Mills, LLC, St. Louis, MO, 36% Neutral Detergent Fiber, NDF), and greenhouse-grown forbs. Rabbits were provided with a nest box and a 3-m long, 4-cm diameter tube or a wooden hutch for security cover.

Assessing perceived risk of temperature at food patches

To determine whether the temperature of foraging patches influenced perception of riskiness of patches, we conducted a series of double choice preference trials with 16 pygmy rabbits (457 g, SD = 40.19 g) and 10 cottontails (1099 g, SD = 60.43). The trials were conducted in 3.8 × 3.6 m covered, outdoor pens constructed of chain-link fence with a 2-cm mesh. During each 24-h trial, each animal was offered a choice between two food patches that each contained bowls of their basal diet placed inside temperature-controlled 0.46-m³ dark acrylic boxes, which were only capable of heating, or 56 × 73 × 84 cm compact freezers modified to heat or cool with 10-cm diameter holes as entryways. We conducted these trials across a range of ambient and experimental temperatures during two winters (January 2015 – March 2015, December 2015 – February 2016) and one summer/autumn (August 2016 – October 2016) to provide the greatest
temperature range above and below their estimated TNZ as possible. During the first winter, rabbits were given a choice between two boxes that were set for 4.4 °C and 18.3 °C, 18.3 °C and 29.4 °C, and 4.4 °C and 29.4 °C in a series of three trials. During the second winter, rabbits were offered a choice between boxes that were set for 1.7 °C and 15.6 °C, 15.6 °C and 26.7 °C, and 1.7 °C and 26.7 °C. During the summer/autumn rabbits were offered a choice between boxes that were set for -1.7 °C and 15.6 °C, 15.6 °C and 43.3 °C, and -1.1°C and 43.3 °C. The actual temperatures in the boxes were recorded using HOBO Temperature Data Loggers (Model UX100-001, Onset Computer Corporation, Bourne, MA) every 15 min. The temperatures in the boxes were averaged over each 24 h trial period for analyses. We allowed the rabbits three days of acclimation in the trial pens before beginning each series of trials. We corrected fresh mass of food pellets offered from the dry matter content by drying a 10-g sample at 100 °C for ≥ 24 h. We weighed the food remaining in each food patch each morning, dried the refusals at 100 °C ≥ 24 h, and calculated intake as the difference between DM offered and refused.

Using the method of paired comparisons, we modeled the expected relative risk of food patches at different temperatures. We assumed that

\[ E(R_{ijk}) = \beta_{0i} + \beta_{1i}T_{ijk} + \beta_{2i}T_{ijk}^2, \]

where \( E(R_{ijk}) \) denoted the expected “risk level” of the \( k \)th food patch for the \( i \)th animal on the \( j \)th trial and \( T_{ijk} \) was the temperature of the box in the \( k \)th food patch presented to the \( i \)th animal in the \( j \)th trial. \( \beta_{1i} \) was the rate at which a change in temperature changes relative risk. Because we expected some minima in the relationship between temperature and “riskiness” with in the TNZ of the rabbits over the range of temperatures that we offered, we included a quadratic function in the model. Because we offered two food patches (\( k = 1,2 \)), let \( Y_{ij} = R_{ij1} - R_{ij2} \) denote the difference in the risk of each patch, thus
\[ E(Y_{ij}) = \beta_{1i}(T_{ij1} - T_{ij2}) + \beta_{2i}(T_{ij1}^2 - T_{ij2}^2). \]

We quantified \( Y_{ij} \) as the difference in intake between the two bowls divided by the total intake during each trial. We used a regression analysis to provide estimates of \( \beta_{1i} \) and \( \beta_{2i} \) for each animal (R Development Core Team 2008). To evaluate if temperature influenced perceived risk, we tested if \( \bar{\beta}_{1i} \) and \( \bar{\beta}_{2i} \) for each species were significantly different than zero using a one sample t-test. To compare \( \bar{\beta}_{1i} \) and \( \bar{\beta}_{2i} \) between species, we used a Mann-Whitney Rank Sum Test.

**Assessing the effects of temperature on voluntary intake of PSMs and fiber**

To determine if rabbits would voluntarily consume a diet lower in PSMs or fiber with increased ambient temperature, between 05 December 2015 and 22 January 2015, we conducted a series of feeding trials with 6 pygmy rabbits (453 g, SD = 42.95 g) and 6 cottontails (1137 g, SD = 73 g) housed at 3 temperatures. Rabbits were housed in 50 × 50 × 40 cm stainless steel crates in temperature controlled greenhouses at either 10.0 °C (below lower critical temperature for pygmy rabbits), 18.3 °C (within TNZ), or 26.7 °C (above TNZ; Katzner et al., 1997), which reflect temperatures in the animals’ natural habitat. For the temperature-PSM trials, we created diets with high PSMs by mixing liquid 1,8-cineole (a major monoterpene in sagebrush, hereafter, cineole) with the basal diet of rabbit pellets. Because of their higher tolerance for cineole (Shipley et al., 2012), pygmy rabbits were offered a choice between the basal diet and the basal diet with 5% cineole, whereas cottontail rabbits were offered a choice between the basal diet and the basal diet with 3% cineole. For the temperature-fiber trials, we created diets of high fiber (50% NDF) by adding rice hulls (77% NDF) to the basal diet using a pellet mill (PM605, Buskirk Engineering, Ossian, IN, USA). Both rabbit species were offered a choice between the basal diet and the high fiber diet. The levels of cineole were within the range of the total PSMs consumed by pygmy rabbits and fiber levels were within the range consumed by pygmy rabbits.
and cottontails (Kelsey et al., 2006; MacCracken and Hansen, 1984; Thines et al., 2004; Ulappa et al., 2014). For both sets of trials, we used a crossover experimental design, so each animal was subjected to all temperature-diet combinations in a different randomly-assigned order. We corrected fresh mass of food pellets as described above. Each trial lasted 5 days and we calculated the average daily intake for each animal during the captive trial.

We used mixed effects linear models to separately evaluate the influence of temperature on the average daily proportion of the high cineole or fiber diets consumed by the rabbits (SAS Institute Inc. 2015). The fixed effects were temperature and species and their interaction and individual animal was included as a random effect. Significant results were followed by pairwise comparisons among temperature and diet treatments using a Tukey-Kramer adjusted α.

Assessing the effects of temperature on food digestion

To determine if ambient temperature influenced dry matter intake (g/g body mass/day), dry matter digestibility (%), digestible dry matter intake (g/g body mass/day), and mean retention time (h) of foods consumed by rabbits, we conducted single choice digestion trials with 4 pygmy rabbits (482 g, SD = 35.49 g) and 4 cottontail rabbits (1099 g, SD = 48.26 g) between 02 and 28 June, 2015. Because of the potential interaction between temperature and fiber on intake, digestion, and mean retention time, we used two temperatures (10.0 °C and 26.7 °C) and two diets (36% NDF basal diet and 50% NDF high fiber diet), resulting in four temperature-diet combinations in this experiment. We used a crossover experimental design so that each individual received each of the temperature-diet combinations in a different randomly assigned order. During these trials, the rabbits were housed in 50 x 50 x 40 cm stainless steel crates in a standard animal room with a modified air conditioner unit for heating and cooling. The rabbits were acclimated to their assigned diet-temperature treatment for 3 days before data collection.
To determine mass adjusted daily dry matter intake (g/g body mass/d), dry matter digestibility (%), and mass adjusted daily digestible dry matter intake (g/g body mass/d) of the diets for both pygmy rabbits and cottontails, we conducted total collection digestion trials over a 4-d period. During the trial period, we weighed a ration of pelleted diet and fed it \textit{ad libitum}, along with water \textit{ad libitum} to each rabbit each morning. We weighed a fresh sample of food and dried it at 100 °C to correct for dry matter composition. Residual food and feces were collected after each 24 h during a four-day period, dried at 100 °C and weighed. We calculated dry matter intake as the difference between the dry mass of food offered and refused, dry matter digestibility as the difference between dry matter intake and the dry mass of feces produced divided by dry matter intake. Digestible dry matter intake was the product of dry matter intake and dry matter digestibility. Dry matter intake and digestible dry matter intake were adjusted for body mass by dividing by body mass before analysis.

To determine the mean retention time of solid digesta within the rabbits at each combination of temperature and fiber content, we marked a sample of each pelleted diet with YbNO$_3$. On the first day of the digestion trial, we gave each animal a 0.5-g oral pulse-dose of the labeled sample from their assigned diet. For the following 72 h, the rabbits were fed their assigned diet and water \textit{ad libitum}. After dosing, feces were collected at 2-h intervals for 40 h, after which feces were collected every 4 h through the end of the trial at hour 72. Because the frequent fecal sampling associated with the passage rate trials stressed the cottontail rabbits and decreased their food intake, they were removed from those trials. In addition, one pygmy rabbit was removed from the fiber 10.0 °C trial because of a decrease in intake and abnormally soft feces.
To determine the Yb concentration of fecal samples, we digested the samples using HCL and HNO₃. The concentration of Yb (ppm) in the fecal samples was determined with inductively coupled plasma mass spectrometry at WSU’s GeoAnalytical Laboratory. We estimated mean retention time for each animal by fitting the following equation to the downward portion of the marker concentration curve:

\[ Y = Y_0 e^{-kt}, \]

where \( Y \) is the marker concentration in feces at time \( t \), \( Y_0 \) is constant, \( k \) is the rate-constant, and \( t \) the time interval after feeding the marker. Mean retention time was calculated as the sum of the reciprocal and \( k \) and the transit time, which is the initial appearance of the marker after dosing (Skaguchi et al., 1987).

We used mixed effects models to separately evaluate the response variables of dry matter intake, dry matter digestibility, digestible dry matter intake, and mean retention time (SAS Institute Inc. 2015). The fixed effects were temperature, diet, species, and all possible interactions, and the random effect was individual animal. Significant results were followed by pairwise comparisons among diet and temperature treatments using a Tukey-Kramer adjusted \( \alpha \).

**Assessing the effects of PSMs and fiber on resting metabolic rate**

To determine whether fiber and cineole increases the RMR of rabbits, we measured rates of oxygen consumption (\( \dot{V}O_2 \), mL O₂ hr⁻¹) of animals exposed to different diet treatments using flow-through respirometry. We conducted trials with 4 pygmy rabbits (483 g, SD = 49.87 g) and 6 cottontail rabbits (1077 g, SD = 58.73 g) between 29 March 2016 and 21 April 2016. During the feeding stage of the trials, the rabbits were housed in their normal enclosures in the barn where the ambient temperature ranged from -1.6 °C to 27.7 °C. Three diets were used during the experiment. One diet was the control diet, which was their basal diet (36% NDF). The first
treatment diet was the basal diet mixed with 3% cineole and the second treatment diet was the high fiber diet (50% NDF). We used a crossover experimental design in which each individual received each diet in a different and random order. The rabbits were fed their assigned diet for 3 days before we measured their RMR. We recorded the rabbits’ daily food intake on each day and calculated the average daily mass adjusted intake on each diet. On the fourth day of each trial, each animal was placed inside an airtight, plexiglass metabolic chamber sized for each species (pygmy rabbit = 4500 cm$^3$, cottontail = 8505 cm$^3$). The chamber was placed inside a darkened environmental cabinet to control the ambient temperature at 18 °C. The RMR of rabbits was collected between 0800 and 1700 hours to avoid potentially confounding circadian rhythms in metabolic rate. Trials lasted 1 h, with the first 30 min used for acclimation and the second 30 min for data collection. Room air was dried of water vapor using a Drierite (W.A. Hammond Drierite Co. LTD., Xenia, OH) column and forced into the chamber at a constant flow rate of 4000 ml/min (Lighton 2014) using a mass flow controller (model number 32907-71, Cole Parmer, Vernon Hills, IL). Excurrent air was scrubbed of water vapor using Drierite and carbon dioxide using indicating soda-lime, and a subsample was pushed to a fuel cell oxygen analyzer (model FC-10, Sable Systems, North Las Vegas, NV). Flow rate into the chamber and oxygen concentrations were averaged over 5 s intervals, converted to digital signal by an A-D converter (UI-2, Sable Systems), and recorded using Warthog LabHelper Software (Warthog Systems, Riverside, California). We collected baseline concentrations of oxygen in room air for approximately 3 min at the start of every trial and immediately before beginning the sampling interval to correct for drift in the oxygen analyzer. The $\dot{V}O_2$ was calculated by Warthog Lab Analyst Software using the equation:

$$\dot{V}O_2 = \frac{(FiO2 - FeO2) \times FR}{(1 - FeO2)}$$
where FR is the incurrent mass flow rate of dry air, FiO2 is the fractional oxygen concentration in dry, CO2-free air (0.2095), and FeO2 is the fractional oxygen concentration of excurrent air scrubbed of water vapor and CO2. Precision of the instrumentation was verified via ethanol combustion (Lighton 2008).

We divided $\dot{V}O_2$ for each animal by its body mass to adjust for mass. We used a mixed effects linear model to separately evaluate the response variables of mass-adjusted RMR and mass-adjusted daily intake, with the fixed effects of dietary treatment, rabbit species and their interaction, and the random effect of individual animal (SAS Institute Inc. 2015). Significant results were followed by pairwise comparisons using a Tukey-Kramer adjusted $\alpha$.

RESULTS

Perceived risk of temperature at food patches

Contrary to our prediction that perceived risk would be lowest at patches within their TNZs, risk tended to increase with increasing temperature for both species (Fig. 1). Risk associated with the temperature of a food patch explained 74% of the variation in expected relative risk of food patches (i.e., difference in proportion of food consumed between food patches of differing temperatures) for pygmy rabbits (mean $R^2 = 0.735 \pm 0.066$) and 63% of the variation for cottontails (mean $R^2 = 0.627 \pm 0.099$). We originally included ambient temperature outside the boxes covering food patches in the model, but it did not improve the fit of the model nor were the coefficients for ambient temperature significant. The regression coefficients for $T$ and $T^2$ were significantly different than zero for cottontails ($T$: $t = 2.905$, DF = 9, $P = 0.016$, $T^2$: $t = 2.40$, DF = 9, $P = 0.039$), but not pygmy rabbits ($T$: $t = 0.558$, DF = 14, $P = 0.586$, $T^2$: $t = 0.91$, DF = 15, $P = 0.379$). Regression coefficients for $T$ and $T^2$ were lower for cottontails than pygmy
rabbits (T: U = 36 P = 0.03, T²: U = 34, P = 0.016), indicating that increasing temperature was riskier for cottontails than pygmy rabbits.

Effects of temperature on voluntary intake of PSMs and fiber

Contrary to our predictions, when rabbits were offered a choice between the cineole diet and the control diet, neither temperature (F_{2,18} = 2.05, P = 0.158) nor the temperature × species interaction (F_{2,18} = 0.23, P = 0.794) influenced the proportion of the cineole diet consumed by either pygmy rabbits or cottontails, but pygmy rabbits consumed twice the proportion of the cineole diet across temperatures as did cottontails (F_{1,18} = 4.61, P = 0.046; Fig. 2). Because the pygmy rabbit experimental diet was 5% cineole and the cottontail diet was 3% cineole, pygmy rabbits actually ate about 4 time more cineole than cottontails. However, as predicted, when rabbits were given a choice between the high fiber diet and the control diet, temperature influenced the proportion of fiber diet consumed by both pygmy rabbits and cottontails similarly (F_{2,18} = 13.98, P < 0.001), but proportion of fiber consumed did not differ between the species (F_{1,18} = 2.11, P = 0.164) nor with the temperature × species interaction (F_{2,18} = 2.70, P = 0.094). Across species, the proportion of high fiber diet consumed was greater when housed in 10 °C compared to 18.3 °C (t = 2.63, DF = 18, P = 0.043) and when housed in 18.3 °C compared to 26.7 °C (t = 2.62, DF = 18, P = 0.043; Fig. 3).

Effects of temperature on intake, digestion, and passage rate of food

Temperature and diet influenced the amount of food consumed by both pygmy rabbits and cottontails, but not how well it was digested. Dry matter intake was significantly influenced by the temperature × diet (F_{1,18} = 18.80, P = 0.0004) and diet × species (F_{1,18} = 12.69, P = 0.002) interactions. Both species had a higher dry matter intake of both diets when in 10 °C than in 26.7 °C (control: t = 3.04, DF = 18, P = 0.0322, fiber: t = 9.18, DF = 18, P < 0.0001), and a higher dry
matter intake on the high fiber than the control diets in 10 °C (t = 9.27, DF = 18, P < 0.0001) and 26.7 °C (t = 3.14, DF = 18, P = 0.0266; Fig. 4). Pygmy rabbits consumed about twice as much mass adjusted dry matter per day on the control and fiber diet within both temperature than the cottontails (control: t = -5.14, DF = 18, P 0.0004, fiber: t = -7.59, DF = 18, P < 0.0001; Fig. 4). Dry matter digestibility of diets differed between diet treatments for both rabbit species (F_{1,18} = 76.90, P < 0.0001), but not between temperatures (F_{1,18} = 1.03, P = 0.325), species (F_{1,18} = 0.04, P = 0.843), nor any interaction (all P-values ≥ 0.28). When animals were housed at both temperatures, the control diet was about 25% more digestible than the high fiber diet (t = 8.77, DF = 18, P < 0.0001; Fig. 5). Digestible dry matter intake was influenced by the temperature × diet interaction (F_{1,18} = 11.36, P = 0.0034) and the temperature × diet × species interaction (F_{1,18} = 4.11, P = 0.057). In 10.0 °C, pygmy rabbits and cottontails had a higher digestible dry matter intake on the high fiber diet compared to the control (pygmy rabbit: t = 4.23, DF = 18, P = 0.009, cottontail: t = 3.52, DF = 18, P = 0.04, Fig. 6). In 10.0 °C, pygmy rabbits had almost double the digestible dry matter intake of the high fiber diet than that of the cottontails (t = -7.99, DF = 18, P <0.0001, Fig. 6).

Mean retention time of particles within the digestive tract of pygmy rabbits was significantly influenced by temperature (F_{1,8} = 7.88, P = 0.023), but not fiber content of diets (F_{1,8} = 0.32, P = 0.586), nor was the interaction between temperature and fiber content significant (F_{1,8} = 1.42, P = 0.158). Mean retention time of solid digesta within the digestive tract was significantly shorter when pygmy rabbits were housed at 10 °C than 26.7 °C (t = -2.81, DF = 8, P = 0.023; Fig. 7).

Effects of PSMs and fiber on resting metabolic rate
Contrary to our expectations, RMR of pygmy rabbits and cottontail rabbits, as measured by oxygen consumption, did not significantly differ among control, high fiber, or cineole diets within species, but there was a diet × species interaction ($F_{2,18} = 3.83$, $P = 0.041$). Pygmy rabbits and cottontails had a similar mass-adjusted RMR on the control ($t = 0.88$, $DF = 18$, $P = 0.947$) and high fiber diets ($t = 1.00$, $DF = 18$, $P = 0.911$), but pygmy rabbits had a higher mass-adjusted RMR on the cineole diet than did the cottontails ($t = 3.80$, $DF = 18$, $P = 0.014$; Fig. 8).

Mass-adjusted daily intake during the metabolic trials was significantly influenced by diet ($F_{2,18} = 5.15$, $P = 0.017$), species ($F_{1,18} = 33.84$, $P < 0.001$), and diet × species ($F_{2,18} = 4.80$, $P = 0.021$). Pygmy rabbits had a higher mass-adjusted intake across all diets than cottontails (control: $t = -3.31$, $DF = 18$, $P = 0.039$, cineole: $t = -4.84$, $DF = 18$, $P = 0.002$, fiber: $t = -6.31$, $DF = 18$, $P < 0.001$). Pygmy rabbits had a higher mass-adjusted intake on the fiber diet compared to the control diet ($t = -3.74$, $DF = 18$, $P = 0.002$).

**DISCUSSION**

Pygmy rabbits and mountain cottontail rabbits in our study demonstrated both behavioral and physiological responses to differences in temperature and diet quality. Our results suggest that warmer temperatures might be risker for pygmy rabbits and cottontails and they have mechanisms to cope with colder temperatures by altering diet composition and intake. Both species generally chose to feed in patches that were relatively colder. In cold environments rabbits ate more total food, a greater proportion of high fiber food, and passed food more quickly through the digestive system compared to warm environments. Temperature did not affect how much PSMs rabbits consumed nor how thoroughly they digested food. Fiber reduced dry matter digestibility, but neither fiber or PSMs influenced RMR of the rabbits.
Regardless of ambient temperature, the perceived relative risk was lowest in food patches held at -5 °C for pygmy rabbits and -9 °C for cottontails, temperatures which are below the lower critical temperature for pygmy rabbits in winter (15 °C – 20 °C; Katzner et al., 1997), and presumably below the summer and winter lower critical temperature for cottontails (Hinds, 1973). Despite fitting a quadratic model, risk tended to increase for both species as temperature increased. This finding was contrary to our expectation that risk would be lowest in patches within their TNZ, increasing as temperatures increased and decreased, especially for the smaller pygmy rabbit. However, as expected, warmer temperatures contributed more strongly to risk for cottontails than pygmy rabbits. Because cottontails are about 45% larger than pygmy rabbits, they have a smaller surface area to volume ratio, thus they retain heat better.

Neither species responded as strongly to the risk of temperature as they did to the risks associated with diets and predation in previous studies (i.e., coefficients were not significantly different than zero, Camp et al., 2015, Camp et al., in review). Several possibilities might account for the weak preference for colder food patches by rabbits.

First, both rabbit species might be more sensitive to warm temperatures than cold temperatures. Domestic rabbits (*Oryctolagus cuniculus*) are extremely sensitive to heat stress, but less so to cold stress (De la Fuente et al., 2007; Marai et al., 2002) because they have few functional sweat glands and have difficulty eliminating excess body heat when environmental temperature is high (Marai et al., 2002). Domestic rabbits that were exposed to heat stress for 4.5 h had higher plasma concentrations of cortisol than those that were exposed to cold stress for the same amount of time (De la Fuente et al., 2007). Similarly, exposure of domestic rabbits to high temperature-humidity indices reduced their growth and reproduction (e.g., conception rate, litter size, milk production, sperm motility) and resistance to disease (Marai et al., 2002).
Similar to other lagomorphs, pygmy rabbits and cottontails might be more resistant to cold than hot temperatures. Pygmy rabbits and mountain cottontails are both endemic to the intermountain Western United States, which is characterized by extreme diurnal and seasonal temperature fluctuations (Knapp, 1997). Unlike many small mammals, they do not hibernate during the winter and are active at all times of the day over the year (Larrucea and Brussard, 2009). Despite their small body size, both species can survive extremely cold winters. Pygmy rabbits, unlike other lagomorphs in North America, dig their own burrows, which provide refugia from temperature extremes across seasons (Milling 2017). Similar to our findings with captive rabbits, free-ranging pygmy rabbits did not select resting sites based on temperature during the winter, but selected cooler sites relative to availability during the summer (Milling et al., in review), demonstrating that they might be more sensitive to hot temperatures than cold. Furthermore, a pygmy rabbit acclimatized to winter temperatures became heat stressed at 25 °C (Katzner et al., 1997). In our study, both rabbit species almost completely avoided temperatures above 35 °C.

In addition, temperatures at the artificial food patches we provided might be relatively unimportant because the rabbits chose to feed in the temperature-controlled food patches frequently throughout the day and night, but each feeding bout was short (about 1-2 min, M. Camp, unpublished data). These foraging bouts might not have been long enough to affect energy or heat balance. Domestic rabbits that have shown responses to heat stress or cold stress are often exposed to the temperatures for several hours or days at a time (De la Fuente et al., 2007). Furthermore, we only varied ambient temperature within the boxes covering the food patches. While they were feeding, these boxes shielded the rabbits from wind, solar radiation, and precipitation, which can greatly influence the operative temperature experienced by an
animal (Bakken, 1980). Although the lowest risk for both species appeared to be below their lower critical temperatures, the microclimate provided by the boxes might have imposed lower thermoregulatory costs than the unmeasured operative temperature outside of the boxes.

Contrary to our prediction, although both rabbit species generally chose to feed in colder food patches, they did not voluntarily consume more of the cineole diet when housed temperatures below their TNZ. Therefore, our findings contrast with studies showing white-throated woodrats consumed more PSMs when housed in colder temperatures compared to warm temperatures (Dearing et al., 2008; McLister et al., 2004). White-throated woodrats ate a greater proportion of juniper relative to a PSM-free control diet when housed at 20 °C than at 28 °C (Dearing et al., 2008). Moreover, RMR was 1.3 times higher in 20 °C and 1.4 times higher in the 28 °C when consuming a diet exclusively of juniper compared to a control diet with no PSMs. These studies suggested that mammalian herbivores avoid PSMs in warmer temperatures to reduce the additional heat created from heat generated during detoxification. In another study, white-throated woodrats that were acclimated to 25 °C and fed a juniper diet had relatively higher costs of thermoregulation (50%, as measured by BMR) than woodrats housed at the same temperature on a control diet (McLister et al., 2004). However, woodrats consuming a juniper diet acclimated to 18 °C had lower thermoregulatory costs (24%) than woodrats acclimated to the same temperature consuming a control diet (McLister et al., 2004).

Rabbits in our study might not have responded to the interaction between temperature and PSMs the same way as woodrats for several reasons. Although our temperature range was wider and included lower temperatures (10 °C – 27 °C) than those used in the woodrat studies (18 °C – 28 °C), our acclimation periods might not have been long enough to elicit physiological responses by the animals. Furthermore, the woodrats were offered juniper as the experimental
diet, which contains a suite of monoterpenes and phenolics, whereas we only offered the rabbits one monoterpene (cineole) and at a low enough concentration that allowed animals to maintain their body mass. The increase in RMR in the woodrats might have been caused by a different PSM. In addition, in our study, diets with 3% cineole did not increase RMR in either rabbit species, so likely did not elicit sufficient thermogenesis to influence diet choice.

Surprisingly, both pygmy rabbits and cottontails voluntarily chose to eat a portion of the cineole diet when housed at all three temperatures (up to an average of about 23% for pygmy rabbits and 12% for cottontails), despite previous observations that both species select patches that have relatively lower levels of cineole (Camp et al., 2015) and eat very low proportions of fresh sagebrush (8% for pygmy rabbits and 0.7% for cottontails) when high quality, terpene-free diets were available (Shipley et al. 2006). Pygmy rabbits have a greater ability to regulate absorption and detoxify cineole than cottontails (Shipley et al., 2012), which explains the two-fold higher consumption of the cineole diet, and four-fold consumption of cineole by pygmy rabbits, but consuming cineole might have some health benefits at low concentrations that outweigh potential thermoregulatory costs. For example, clinical evidence suggests that cineole has anti-inflammatory properties, and it has been shown to be useful for the treatment of inflammatory airway diseases in humans, such as asthma and chronic obstructive pulmonary disease (Juergens, 2014). Cineole also possesses gastro-protective activity and reduces blood pressure through vascular relaxation in rats (Lahlou et al., 2002; Santos and Rao, 2001), and protects against liver failure of mice with endotoxemic shock (Santos et al., 2001). A variety of medicinal benefits have been reported for plants of the *Artemisia* genus because of the anti-parasitic effects of the monoterpenes (Ferreira, 2009). For example, extracts from *Artemisia absinthium* L. reduced the severity of coccidial infection (*Eimeria tenella*) in chickens (*Arbor*
The idea of animals using plants to self-medicate is not completely novel (Huffman, 2003) and the possible therapeutic properties of cineole for wild herbivores requires further investigation.

As we predicted, temperature did influence voluntary consumption of plant fiber in both rabbit species. When housed in temperatures below their TNZ (10.0 °C), pygmy rabbits and cottontails consumed a greater proportion of the high fiber diet than when they were housed at warmer temperatures within and above their TNZ. Furthermore, pygmy rabbits and cottontails ate similar proportions of the high fiber diet regardless of temperature, despite previous studies that showed cottontails digest fiber more efficiently (Shipley et al., 2006) and perceive fiber as less risky than do pygmy rabbits (Camp et al., 2015). One possible explanation for this finding is that higher fiber diets have a higher heat increment associated with digestion than lower fiber diets. The heat increment of feeding is the increase in metabolic rate associated with digestion following ingestion of a meal. Several studies have suggested that endotherms might use the heat increment of feeding to substitute for thermostatic heat production in cold environments and can thus reduce the energetic cost of thermoregulation (Chappell et al., 1997; Hawkins et al., 1997; MacArthur and Campbell, 1994). For ruminants, the heat increment of feeding includes the heat of fermentation. Because increasing fiber in the diets of ruminants increases the degree of microbial fermentation in the rumen, high fiber diets can increase the heat increment of feeding (NRC, 1981). Rabbits are hindgut fermenters, and though they rely on microbial fermentation to digest high fiber diets, they are less efficient at digesting fiber than ruminants. However, they do rely on microbial fermentation, so we hypothesize that this could be a substantial source of heat for a small bodied mammal. The role of the heat of fermentation in the heat increment of feeding for small hindgut fermenters is currently unclear, but our results indicate that consuming a
relatively greater proportion of dietary fiber in cold temperatures might provide thermoregulatory benefits for a small hindgut fermenter.

If higher levels of dietary fiber did increase the heat increment of feeding for rabbits in our study, we would expect an increase in RMR when rabbits were on a higher fiber diet. This was not the case in our study. One reason could be that it was impossible to control the exact timing of food consumption by the rabbits. To accurately measure the effects of the control and experimental diets on RMR, the rabbits should be in a fed state (postingestive) rather than a postabsorptive state. Even though the rabbits were provided the experimental and control diets ad libitum right up to the time when they were placed in the metabolic chamber, we could not guarantee that animals were in a postingestive state during the metabolic trials. Previous studies on the metabolism of lagomorphs have assumed that relatively short periods of fasting (< 3 h) are needed to reach a postabsorptive state (Hinds, 1973). However, pygmy rabbits that were fasted for 5 h did not have significantly different metabolic rates than animals with free access to food (Katzner et al., 1997). Therefore, the heat of fermentation might play a beneficial role by reducing acute thermoregulatory costs, but further experiments measuring the heat of fermentation relative to fiber levels and how it might be used to reduce these costs in small hindgut fermenters are required to fully understand these relationships.

Temperature influenced not only where rabbits chose to feed and what they chose to eat, but also how much they ate. During the digestion trials, both pygmy rabbits and cottontails consumed about 50% more total food when they were housed in 10 °C compared to 26.7 °C, regardless of the fiber content of the diet. Because dry matter digestibility did not differ with temperatures, rabbits also had a greater digestible dry matter intake in the colder temperature. This result is similar to a study that showed a 42 – 73% increase in the amount of food eaten by
prairie voles (*Microtus ochrogaster*) and Northern collared lemmings (*Dicrostonyx groenlandicus*) when they were housed in 5 °C compared to 23 °C, but both rodent species maintained between 57% and 62% dry matter digestibility (Hammond and Wunder, 1995). The greater digestible dry matter intake by rabbits in the colder temperature in our study presumably provided additional energy to offset the energy lost from the increased thermoregulatory costs. Both rabbit species also consumed more total food when they were offered the high fiber diet with a 20% lower dry matter digestibility than the control diet, thus maintained the same digestible dry matter intake across diets.

Despite their 45% smaller body mass, pygmy rabbits had a higher mass-adjusted dry matter intake, during the metabolic and digestion trials, and higher digestible dry matter intake than cottontails but similar mass-adjusted RMRs. The results of the current study corroborate a previous study on pygmy rabbits and Eastern cottontails (*Sylvilagus floridanus*). Shipley et al. (2006) found the daily energy requirement of cottontails (549.2 kJ/kg\(^{0.75}\)/d) was consistent with the scaling of daily energy expenditure (basal metabolic rate + activity + thermoregulation + heat increment, kJ/d) for eutherian mammals (591.6 kJ/kg\(^{0.75}\), Robbins 1993). However, pygmy rabbits required digestible energy of 750.8 kJ/kg\(^{0.75}\)/d to maintain their body mass, which is 27% more than predicted from the scaling relationship for eutherian mammals and 37% higher per unit metabolic mass than cottontails (Shipley et al., 2006). In our study, both pygmy rabbits and cottontails increased dry matter intake in the colder temperature (10.0 °C) as the fiber level in the pellets increased, but pygmy rabbits consumed more dry matter and digestible dry matter relative to their body mass than did cottontails. Shipley et al. (2006) also found that pygmy rabbits had a higher dry matter intake relative to their body mass than cottontails. Consequently, pygmy
rabbits might need to either eat higher quality food to spend more time searching for and harvesting food than cottontails.

To consume more food when confronted with either colder temperatures or higher plant fiber, rabbits should either pass food more quickly through the digestive tract and presumably digest food to a lesser extent, or increase the size of the gut. Our results indicated that pygmy rabbits passed food more quickly through the digestive tract when housed in the colder temperature compared to the warmer temperature. Although the faster passage was likely caused by the higher intake in the colder temperature, we did not observe a lower dry matter digestibility in the colder temperature for either diet. Other studies have shown mammalian herbivores increase food intake and rate of passage of digesta, at the cost of decreased digestibility, as energy demands increase or diet quality decreases (Loeb et al., 1991; Nagy and Negus, 1993; Woodall, 1989). This strategy is problematic for small herbivores, however, because they have greater energy needs per unit body mass than larger herbivores, and are limited by the size of their digestive tract and rate of fermentation. Some small mammalian herbivores, including rabbits, overcome this problem by separating large, indigestible particles from solutes and small particles in the proximal colon, and retaining the latter in their cecum (Davies and Davies, 2003; Hume, 1989). This allows animals to concentrate digestive effort on the more digestible fractions of the digesta, whereas the increased rate of passage of indigestible large particles from the colon allows animals to maintain higher rates of food intake. This differential digestion partially compensates for the increase in energy demands or the lower nutrient density of high fiber forage (Hume, 1989). In some cecal fermenters, such as rabbits, selective retention of digesta components is combined with cecotrophy (ingestion of nutrient dense feces derived from the
cecum), which allows them to obtain more nutrients from high fiber diets (Davies and Davies, 2003).

The ability of rabbits in our study to increase intake and passage rate in the cold temperatures, without a decrease in digestibility of diets, is likely a result of selective digesta retention in the cecum and cecotrophy. Likewise, rabbits could be maintaining intake of digestible dry matter on the high fiber diet through selective retention in the cecum, such as was documented in Brandt’s voles (*Microtus brandti*), which are also small cecal fermenters (Hume et al., 1993; Pei et al., 2001). Fiber content of the diet did not influence mean retention time of solid digesta in our rabbits. Similarly, a study on Brandt’s voles did not detect a significant difference in mean retention times of liquid or solid digesta of a high fiber (37.6% NDF) and low fiber (13.6% NDF) diets (Pei et al., 2001). However, they noted the liquid digesta marker, Co-EDTA, was recycled to the stomach by cecotrophy and voles showed a significant increase in gut capacity on the high fiber diet (Pei et al., 2001).

An increase in gut capacity in response to high fiber diets is often observed in small herbivores (Gross et al., 1985; Hammond and Wunder, 1995), but this is often coupled with an increase in retention time of digesta. We observed an increase in intake in response to cold temperatures and high fiber diets by both species, yet we observed a decrease in retention time with colder temperatures by pygmy rabbits. This suggests the rabbits did not increase their gut capacity over the course of our study. However, the lack of increase in gut capacity could be a result of the relatively short period of time the rabbits were on each treatment diet (7 d). In the aforementioned study on Brandt’s voles, gut capacity increased on the high fiber diet after 14 d, but it was not sufficient to increase retention time, and the increase in gut size was small relative to similar studies on voles (Pei et al., 2001). In other studies that documented notable increases
in gut capacity in response to high fiber diets, animals were acclimated to the diets for >18 d (Gross et al., 1985; Hammond and Wunder, 1995). Further exploration into how temperature and diet quality influence passage rate and gut size in pygmy rabbits and cottontails is needed to fully understand these relationships.

In conclusion, our study establishes the importance of considering the interactions between temperature and food quality when investigating the behavior, physiology, and ecology of small mammalian herbivores. Such species usually demonstrate seasonal shifts in diet and space use, and although forage availability can be the leading cause of such shifts, temperature differences across the day or seasons also might influence diet or patch selection. Our results suggest that warmer temperatures might be riskier for pygmy rabbits and cottontails than colder temperatures. Moreover, results suggest that these rabbits have mechanisms to cope with colder temperatures and low quality foods (PSMs and fiber) by altering diet composition and intake.

Temperature increases engendered by climate change are expected to reduce the amount of suitable habitat for up to 87% of mammalian species (Schloss et al., 2012). The pygmy rabbits’ bioclimatic envelope is predicted to decline by 87% by 2080 under the current trend in observed climate (Leach et al., 2015). Projected warming of the climate could tip the balance of energy costs and acquisition, which could exacerbate effects of habitat loss on small mammal populations. Therefore, understanding the interactions between temperature, diet, and patch selection will be necessary to predict the response of mammalian herbivores to climate change.

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Figure 3.1. The predicted relative risk values at food patches with a range of temperatures for pygmy rabbits (*Brachylagus idahoensis*) and mountain cottontail rabbits (*Sylvilagus nuttallii*). The risk values were predicted using the model $E(R) = -0.19T + 0.00024T^2$ for pygmy rabbits and $E(R) = 0.054T - 0.00033T^2$ for cottontails, where $E(R)$ was the expected relative risk level and $T$ was the temperature at a foraging patch. Risk coefficients for $T$ and $T^2$ were lower for pygmy rabbits than cottontails.
Figure 3.2. Proportion ± SE consumed of the diet containing cineole (5% for pygmy rabbits and 3% for cottontails) relative to the control diet (0% cineole) by pygmy rabbits (*Brachylagus idahoensis*) and mountain cottontail rabbits (*Sylvilagus nuttallii*) housed at three different temperatures (10.0 °C, 18.3 °C, and 26.7 °C). Pygmy rabbits ate a greater proportion of the cineole diet than did cottontails, but temperature did not influence diet composition. Significant differences in the proportion of the cineole diet consumed between species and temperatures are indicated by different lowercase letters above the bars.
Figure 3.3. Proportion consumed of the high fiber diet (50% neutral detergent fiber) relative to the control diet (36% neutral detergent fiber) ± SE by pygmy rabbits (*Brachylagus idahoensis*) and mountain cottontail rabbits (*Sylvilagus nuttallii*) housed at three different temperatures (10.0 °C, 18.3 °C, and 26.7 °C). Both species consumed a greater proportion of the high fiber diet with decreasing temperature. Significant differences in the proportion of the high fiber diet consumed among temperatures are indicated by different letters above the bars.
Figure 3.4. Mass-adjusted daily dry matter intake ± SE for control (36% neutral detergent fiber) and high fiber (50% neutral detergent fiber) diets for pygmy rabbits (*Brachylagus idahoensis*) and mountain cottontail rabbits (*Sylvilagus nuttallii*) housed at two different temperatures (10.0 °C and 26.7 °C). Both pygmy rabbits and cottontails ate more dry mass of food at 10.0 °C than 26.7 °C, and ate more when consuming high fiber food than low fiber control diet. Pygmy rabbits had a higher mass-adjusted dry matter intake than cottontails. Significant differences in dry matter intake between temperatures and diets are indicated by lowercase letters.
Figure 3.5. Dry matter digestibility ± SE for control (36% neutral detergent fiber) and high fiber (50% neutral detergent fiber) diets for pygmy rabbits (*Brachylagus idahoensis*) and mountain cottontail rabbits (*Sylvilagus nuttallii*) housed at two different temperatures (10.0 °C and 26.7 °C). When consuming the high fiber diet, both pygmy rabbits and cottontails had a lower dry matter digestibility than when consuming the control diet, but dry matter digestibility did not vary with temperature or species. Significant differences in dry matter digestibility between diets are indicated by different lowercase letters above the bars.
Figure 3.6. Mass-adjusted daily digestible dry matter intake ± SE for control (36% neutral detergent fiber) and high fiber (50% neutral detergent fiber) diets for pygmy rabbits (*Brachylagus idahoensis*) and mountain cottontail rabbits (*Sylvilagus nuttallii*) housed at two different temperatures (10.0 °C and 26.7 °C). Both pygmy rabbits and cottontails had higher digestible dry matter intakes when housed in 10.0 °C than in 26.7 °C, and digestible dry matter intake differed between species but not between diets. Significant differences in digestible dry matter intake between temperatures and species are indicated by different lowercase letters above the bars.
Figure 3.7. Mean retention times (h) ± SE of food particles in the digestive system for control (36% neutral detergent fiber) and high fiber (50% neutral detergent fiber) diets for four pygmy rabbits (*Brachylagus idahoensis*) housed at two different temperatures (10.0 °C and 26.7 °C). Mean retention times differed by temperature, but not diet. Significant differences in mean retention time between temperatures are indicated by different lowercase letters above the bars.
Figure 3.8. Mass-adjusted resting metabolic rate as measured by oxygen consumption ± SE for control (36% neutral detergent fiber), high fiber (50% neutral detergent fiber), and cineole diets (3% 1,8 cineole) consumed by pygmy rabbits (*Brachylagus idahoensis*) and mountain cottontail rabbits (*Sylvilagus nuttallii*). Oxygen consumption did not differ among diets for either species, but oxygen consumption by pygmy rabbits when fed the cineole diet was higher than oxygen consumption of cottontails fed the cineole and high fiber diets. Significant differences in oxygen consumption between species and diets are indicated by different uppercase letters above the bars.