



# Interacting effects of ambient temperature and food quality on the foraging ecology of small mammalian herbivores

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## ABSTRACT

Both temperature and diet quality play an important role in the time and energy budgets of small mammalian herbivores. However, little is known about how temperature and diet quality interact to influence diet selection, nutrient extraction, and energetics, and how these effects might differ among species. Therefore, we examined the effects of diet quality and temperature on aspects of the foraging ecology of two species of lagomorphs, pygmy rabbits (*Brachylagus idahoensis*), which are small dietary specialists, and mountain cottontail rabbits (*Sylvilagus nuttallii*), which are larger dietary and habitat generalists. In a series of feeding experiments, we investigated 1) the effects of temperature on selection of plant fiber and the plant secondary metabolite 1,8 cineole in their diets, 2) effects of temperature and plant fiber on daily intake, digestion, and passage of food, 3) effects of plant fiber and 1,8 cineole on resting metabolic rate, and 4) how these interactions differ between the rabbit species. Both species chose to eat more total food and a greater proportion of high fiber food that passed more quickly through the digestive system in colder temperatures. However, temperature did not affect how much 1,8 cineole the rabbits consumed nor how thoroughly they digested food. Food quality affected how well they digested the dry matter in the food, but not their resting metabolic rate. Understanding how the interactions between ambient temperature and food quality affect selection of diets and intake by small mammalian herbivores, and the physiological mechanisms governing these choices, is useful for predicting how these species might respond to changes in both temperature and food quality and inform conservation and restoration strategies.

## 1. Introduction

To survive and reproduce in variable environments, small mammalian herbivores must adapt strategies to efficiently ingest and allocate energy. Their success depends on both the intrinsic properties of their food, such as concentrations of plant fiber and plant secondary metabolites, and on properties of the environment, such as food availability, the risk of predation, and thermal conditions. Consuming fibrous and chemically-defended food can reduce the rate and extent of digesting and metabolizing energy (Demment and Van Soest, 1985a, 1985b; Sorensen et al., 2005b). Likewise, foraging in temperatures outside of its thermal neutral zone (TNZ) increases a homeotherm's energy expenditure (Porter et al., 1994) or requires the animal to spend additional time using behavioral methods to buffer against these effects (Caraco et al., 1990). Furthermore, changing temperatures, both seasonal and long-term climatic trends, can influence the nutritional quality of forage plants available to herbivores, such as concentrations

of nitrogen, fiber and chemical defenses (i.e., plant secondary metabolites, Gershenson, 1984; Chapin et al., 1995; Jensen et al., 2016). Although the influence of dietary quality on foraging by herbivores has been well-studied, few studies have directly explored how interactions between environmental temperature and food quality affect the foraging ecology of small mammalian herbivores.

When possible, herbivores can increase their net energy gain by selecting food that is low in fiber and plant secondary metabolites and by foraging at sites or times when the temperature they experience is within their TNZ (Huey, 1991; Aublet et al., 2009; Orrock and Danielson, 2009). 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*) spent more time foraging on nights when the soil temperature was higher, presumably reducing their thermoregulatory costs (Orrock and Danielson, 2009).

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However, when ambient temperature or habitat conditions force herbivores to feed in patches below or above their TNZ, they might attempt to consume foods that generate or dissipate heat. Consuming higher fiber diets when temperatures are colder might allow animals to offset heat loss with endogenous heat generated through microbial fermentation of plant fiber (Noblet et al., 1985; Young, 1981; NRC, 1981; West, 1997). That said, the role of dietary fiber in thermoregulation, especially in small mammals, is unclear (Mader et al., 2001). In addition, ingesting some types of plant secondary metabolites might help endotherms maintain body temperatures within their TNZs by reducing heat dissipation through vasoconstriction or by producing heat during detoxification (Forbey et al., 2009). However, because ingesting fiber and plant secondary metabolites can cause animals to reduce their food intake, they might not be able to consume high enough levels of either to reduce thermoregulatory costs in cold temperatures (Dearing et al., 2000; Demment and Van Soest, 1985a, 1985b; Shipley et al., 2012; Sorensen et al., 2005a).

Another strategy herbivores might use when forced to feed in patches below their TNZ is to increase their intake of digestible energy to meet the increase in energetic demands of thermoregulation. Animals can do this by eating more total food, choosing higher quality foods (i.e., forage with higher digestible energy), or digesting food more thoroughly. However, cold temperatures often occur during winter when forage plants senesce and become more fibrous, thus less digestible. Herbivores can obtain energy from plant fiber through microbial fermentation, but this process is relatively slow and inefficient, especially in small, hindgut-fermenting herbivores (Demment and Van Soest, 1985a, 1985b). However, small herbivores have mechanisms that help them cope with lower quality diets. For example, small mammals can adjust to elevated energy demands in cold temperatures by increasing retention time of fibrous food in the digestive tract over a period of days, 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).

Because these complex functional relationships are not fully understood, we studied how temperature and food quality interact to shape diet selection, food intake and digestion, and energetics. We compared responses to diet quality and temperature of two species of lagomorphs, pygmy rabbits (*Brachylagus idahoensis*) and mountain cottontails (*Sylvilagus nuttallii*), that differ in life history characteristics, but 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  $-40^{\circ}\text{C}$  during winter and as high as  $45^{\circ}\text{C}$  during summer (Knapp, 1997). Pygmy rabbits are habitat and dietary obligates (Green and Flinders, 1980), thus are restricted to sagebrush steppe, whereas generalist mountain cottontails use a wide variety of foods and habitats, and neither rabbit species has adapted hibernation to cope with temperature extremes and seasonal changes in food quality.

Because they differ in body size and tolerance for plant secondary metabolites, pygmy rabbits and cottontails would be expected to respond differently to food quality in relation to ambient temperature. Pygmy rabbits ( $\sim 450$  g) are less than half the size of cottontails ( $\sim 1100$  g), with higher mass-specific energy requirements, lower ability to digest fiber (Shipley et al., 2006), and potentially a narrower TNZ and greater tolerance to heat (McNab, 2002). Although the TNZ for mountain cottontails has not been documented, the lower critical temperature for winter-acclimatized pygmy rabbits is between  $15^{\circ}\text{C}$  and  $18^{\circ}\text{C}$  and the upper critical temperature is predicted to be between  $23^{\circ}\text{C}$  and  $28^{\circ}\text{C}$  (Katzner et al., 1997). In addition, pygmy rabbits, unlike cottontails, have special adaptations for efficiently detoxifying and eliminating the high levels of monoterpenes in sagebrush, their primary food source (Shipley et al., 2012).

To examine the interactions between temperature and food quality, we conducted a series of feeding experiments with captive pygmy

rabbits and cottontails. We first investigated the effects of temperature on selection of plant fiber and plant secondary metabolites (i.e., monoterpenes) in their diets. Next, we measured the effects of temperature on daily intake, digestion, and passage of food in relation to fiber content. Finally, we examined the effects of plant fiber and plant secondary metabolites on resting metabolic rates. We predicted that both rabbit species would choose to consume diets higher in fiber and monoterpene concentration in relatively colder temperatures to help generate heat for thermoregulation, but that the pygmy rabbits would increase their consumption of fiber to a lesser degree, and their consumption of monoterpenes to a greater degree, than would cottontails. To compensate for the increased energy cost of thermoregulation, we predicted that both species of rabbits would either consume more food or digest it more thoroughly in cold temperatures relative to warm temperatures. Forage digestibility in herbivores depends on time exposed to microbial fermentation (Cork, 1994); therefore, we expected the passage rate of food to increase with an increase in intake and a decrease in temperature. Because consuming higher fiber requires additional mechanical and chemical processing of food (Cork, 1994) and consuming monoterpenes requires using energetically expensive detoxification pathways (Sorensen et al., 2005b), we predicted that by consuming lower quality diets, both rabbit species would respond with an increase in resting metabolic rate.

## 2. Methods

### 2.1. 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), at elevations ranging from 1463m to 1819m. We captured mountain cottontail rabbits in Whitman County, Washington (Washington Scientific Collection Permit #13-102, Washington State University Institutional Animal Care and Use Committee Protocol #4398), at an elevation of 717 m. Capture weights for pygmy rabbits ranged from 350g to 500 g and 1080–1120 g for cottontails. Animals had lived in captivity at the Small Mammal Research Facility at Washington State University, Pullman, Washington, USA, for at least a year before experiments commenced. While they were not participating in experiments, rabbits were housed individually on pine shavings in  $1.2\text{ m} \times 1.8\text{ m}$  enclosures. We fed the rabbits 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), greenhouse-grown forbs, 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.

### 2.2. Assessing the effects of temperature on selection of food with plant fiber and plant secondary metabolites

To determine if rabbits would respond to lower temperatures by voluntarily consuming diets higher in plant fiber or plant secondary metabolites, we conducted a series of feeding trials with 6 pygmy rabbits (453 g, SD = 43 g) and 6 cottontails (1137 g, SD = 73 g) housed at 3 temperatures. These experiments took place during the winter, between 05 December 2015 and 22 January 2016. Rabbits were housed in  $50\text{ cm} \times 50\text{ cm} \times 40\text{ cm}$  stainless steel crates in temperature controlled greenhouses at either  $10.0^{\circ}\text{C}$  (below lower critical temperature for winter acclimatized pygmy rabbits),  $18.3^{\circ}\text{C}$  (within TNZ), or  $26.7^{\circ}\text{C}$  (above TNZ; Katzner et al., 1997), which reflect temperatures in the animals' natural habitat. For the trials with plant fiber, 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. For the trials with plant secondary metabolites, we created diets with a high monoterpene concentration 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. Both the levels of plant fiber and cineole used in these experiments 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 that allowed each animal to experience all temperature-diet combinations in a different, randomly-assigned order. We weighed a fresh sample of food and dried it at 100 °C to correct for dry matter composition. The animals were acclimated to each combination of temperature and diet for 5 days, which immediately preceded the trial period. Each trial lasted 5 days and we calculated the average daily intake for each animal during the trial.

### 2.3. Assessing the effects of temperature and plant fiber on digestion

To determine if ambient temperature influenced daily dry matter intake (g intake/g body mass/day), dry matter digestibility (%), daily digestible dry matter intake (g intake/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 g) and 4 cottontail rabbits (1099 g, SD = 48 g) during the summer between 02 and 28 June 2015. Because of the potential interaction between temperature and fiber on intake, digestion, and mean retention time, we used 2 temperatures (10.0 °C and 26.7 °C) and 2 diets (36% NDF and 50% NDF, high fiber diet), resulting in 4 temperature-diet combinations in this experiment. We used a crossover experimental design so that each rabbit received each of the temperature-diet combinations in a different randomly-assigned order. During these trials, the rabbits were housed in 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 directly before data collection.

To examine daily dry matter intake, dry matter digestibility, and daily digestible dry matter intake 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 ad libitum, along with water ad libitum to each rabbit every morning. We corrected fresh mass of food pellets as described above. Residual food and waste feces were collected after each 24 h during a 4-d period, dried at 100 °C and weighed. We calculated dry matter intake as the difference between the dry mass of food offered and refused, and 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 measure the mean retention time of particulate digesta within the rabbits at each combination of temperature and fiber content, we marked a sample of each pelleted diet by soaking it in a solution with Ytterbium Mononitrate ( $\text{YbNO}_3$ ). Ytterbium Mononitrate binds to the solid portion of the food sample (Skaguchi et al., 1987). 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 ad libitum. After dosing, waste 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 caused the cottontails to decrease their food intake beyond pre-determined safe levels, 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 Ytterbium concentration of fecal samples, we digested the samples using HCL and  $\text{HNO}_3$ . The concentration of Ytterbium (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 Ytterbium concentration curve:

$$Y = Y_0 e^{-kt},$$

where Y is the Ytterbium 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 of k and the transit time, which is the initial appearance of the marker after dosing (Skaguchi et al., 1987).

### 2.4. Assessing the effects of plant fiber and plant secondary metabolites on resting metabolic rate

To investigate whether fiber and cineole increased the resting metabolic rates of rabbits, we measured rates of oxygen consumption ( $\dot{V}O_2$ , mL  $\text{O}_2$   $\text{h}^{-1}$ ) of animals exposed to different diet treatments using flow-through respirometry. We conducted trials with 4 pygmy rabbits (483 g, SD = 50 g) and 6 cottontail rabbits (1077 g, SD = 59 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 semi-enclosed barn where the ambient temperature ranged from -1.6 °C to 27.7 °C. Three diets were used during the experiment, a control diet and 2 treatment diets. The control diet was the rabbits' basal diet that contained 36% fiber. The first treatment diet was the high fiber diet (50% NDF) and the second treatment diet was the basal diet mixed with 3% cineole. We used a crossover experimental design in which each rabbit received each diet in a random order. The rabbits were fed their assigned diet for 3 days before we measured their resting metabolic rates. We recorded the rabbits' daily food intake on each day and calculated the average daily intake, adjusted for body mass, on each diet.

On the 4th day of each trial, each animal was placed inside an airtight, plexiglass metabolic chamber sized for each species (pygmy rabbit = 4500  $\text{cm}^3$ , cottontail = 8505  $\text{cm}^3$ ). The chamber was placed inside a darkened environmental cabinet to control the ambient temperature at 18 °C. The resting metabolic rates of rabbits were measured between 0800 and 1700 h 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 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 LabAnalyst Software using the equation:

$$\dot{V}O_2 = \frac{(\text{FiO}_2 - \text{FeO}_2) \times \text{FR}}{(1 - \text{FeO}_2)},$$

where FR is the incurrent mass flow rate of dry air,  $\text{FiO}_2$  is the fractional oxygen concentration in dry,  $\text{CO}_2$ -free air (0.2095), and  $\text{FeO}_2$  is the

fractional oxygen concentration of excurrent air scrubbed of water vapor and CO<sub>2</sub>. Precision of the instrumentation was verified via ethanol combustion. We divided  $\dot{V}O_2$  for each animal by its body mass to adjust for mass.

### 2.5. Statistical analyses

We used a series of mixed effects linear models to examine the influence of diet quality and temperature on the rabbit's foraging ecology (SAS Institute Inc, 2015). To determine the effect of temperature on the proportion rabbits consumed of the high fiber diet and the cineole diet, we modeled those response variables as a function of fixed effects for temperature, rabbit species, and their interaction, and individual animal was included as a random effect. The models evaluating the influence of temperature and dietary fiber on daily dry matter intake, dry matter digestibility, and digestible dry matter intake included temperature, fiber level, species, and all possible interactions as fixed effects, and the individual animal as a random effect. Because cottontails ate too little food to allow us to evaluate mean retention time, we did not model this response for this species. For pygmy rabbits, we modeled the response of mean retention time with fixed effects for temperature, fiber, and their interaction, and the random effect was individual animal. To evaluate the effects of dietary fiber and cineole on the response variables of mass-adjusted resting metabolic rates and mass-adjusted daily intake, the fixed effects were dietary treatment (control, fiber, or cineole), rabbit species, and their interaction, and the random effect was individual animal. All significant results were followed by pairwise comparisons among diet or temperature treatments using a Tukey-Kramer adjusted  $\alpha$ . For all linear models, we used the identity link function and residual maximum likelihood estimation methods. We examined residual plots to assess for normality and equal variance.

## 3. Results

### 3.1. Effects of temperature on selection of food with plant fiber and plant secondary metabolites

Temperature influenced the proportion of plant fiber, but not plant secondary metabolites, consumed by rabbits. As expected, when rabbits were given a choice of how much of the control diet and the high fiber diet to consume at 3 different temperatures, they chose a greater proportion of fiber in colder temperatures ( $F_{2,18} = 13.98$ ,  $P < 0.001$ ). When housed in 10 °C, both rabbit species consumed a greater proportion of the high fiber diet than when housed at 18.3 °C ( $t = 2.63$ ,  $DF = 18$ ,  $P = 0.043$ ; Fig. 1). Contrary to our predictions, however, when rabbits were offered a choice of how much control diet and cineole diet to consume, neither temperature ( $F_{2,18} = 2.05$ ,  $P = 0.158$ ) nor the temperature  $\times$  species interaction ( $F_{2,18} = 0.23$ ,  $P = 0.794$ ) influenced the proportion of the cineole diet consumed by either pygmy rabbits or cottontails. Regardless of temperature, the specialist pygmy rabbits consumed twice the proportion of the cineole diet than did cottontails ( $F_{1,18} = 4.61$ ,  $P = 0.046$ ; Fig. 1). Because the experimental diet offered to pygmy rabbits was 5% cineole and the cottontail diet was 3% cineole, this meant that pygmy rabbits ate about 4 times more total cineole than did cottontails.

### 3.2. Effects of temperature and plant fiber 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 food was digested. Dry matter intake was influenced by the temperature  $\times$  diet ( $F_{1,18} = 18.80$ ,  $P = 0.0004$ ) and diet  $\times$  species ( $F_{1,18} = 12.69$ ,  $P = 0.002$ ) interactions. When housed at 10 °C, and regardless of diet, both species had a higher dry matter intake than they did when in 26.7 °C (control:  $t = 3.04$ ,  $DF = 18$ ,  $P = 0.0322$ , fiber:  $t = 9.18$ ,  $DF = 18$ ,  $P < 0.0001$ ).

Both species also had a higher dry matter intake on the high fiber than the control diet in 10 °C ( $t = 9.27$ ,  $DF = 18$ ,  $P < 0.0001$ ) and 26.7 °C ( $t = 3.14$ ,  $DF = 18$ ,  $P = 0.0266$ ; Fig. 2). At all temperatures and on both diets, the smaller pygmy rabbits consumed about twice as much dry matter per day, when adjusted for their body weight, as did the larger cottontails (control:  $t = -5.14$ ,  $DF = 18$ ,  $P = 0.0004$ , fiber:  $t = -7.59$ ,  $DF = 18$ ,  $P < 0.0001$ ; Fig. 2). 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 ( $F_{1,18} = 1.03$ ,  $P = 0.325$ ), or species ( $F_{1,18} = 0.04$ ,  $P = 0.843$ ). The control diet was about 25% more digestible than the high fiber diet, regardless of temperature ( $t = 8.77$ ,  $DF = 18$ ,  $P < 0.0001$ ; Fig. 3). Digestible dry matter intake was influenced by the temperature  $\times$  diet interaction ( $F_{1,18} = 11.36$ ,  $P = 0.0034$ ) and the temperature  $\times$  diet  $\times$  species interaction ( $F_{1,18} = 4.11$ ,  $P = 0.057$ ). In 10.0 °C, pygmy rabbits had a higher digestible dry matter intake on the high fiber diet compared to the control (pygmy rabbit:  $t = 4.23$ ,  $DF = 18$ ,  $P = 0.009$ , Fig. 4). In 10.0 °C, pygmy rabbits had almost twice the digestible dry matter intake of the high fiber diet as that of the cottontails, when adjusted for body mass ( $t = -7.99$ ,  $DF = 18$ ,  $P < 0.0001$ , Fig. 4).

Mean retention time of particles within the digestive tract of pygmy rabbits was influenced by temperature ( $F_{1,8} = 7.88$ ,  $P = 0.023$ ), but not dietary fiber ( $F_{1,8} = 0.32$ ,  $P = 0.586$ ), nor was there an interaction between temperature and fiber content ( $F_{1,8} = 1.42$ ,  $P = 0.158$ ). Mean retention time of particulate digesta within the digestive tract was significantly shorter when pygmy rabbits were housed at 10 °C than at 26.7 °C ( $t = -2.81$ ,  $DF = 8$ ,  $P = 0.023$ ; Fig. 5).

### 3.3. Effects of plant fiber and plant secondary metabolites on resting metabolic rate

Contrary to our expectations, resting metabolic rate of both species, as measured by oxygen consumption, did not significantly differ among control, high fiber, or cineole diets. However, there was a diet  $\times$  species interaction ( $F_{2,18} = 3.83$ ,  $P = 0.041$ ). Pygmy rabbits and cottontails had a similar mass-adjusted resting metabolic rate 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 resting metabolic rate on the cineole diet than did the cottontails ( $t = 3.80$ ,  $DF = 18$ ,  $P = 0.014$ ; Fig. 6).

Daily dry matter 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  $\times$  species ( $F_{2,18} = 4.80$ ,  $P = 0.021$ ). Pygmy rabbits had a higher intake, when adjusted for body mass, for all three diets than did cottontails (control:  $t = -3.31$ ,  $DF = 18$ ,  $P = 0.039$ , cineole:  $t = -4.84$ ,  $DF = 18$ ,  $P = 0.002$ , fiber:  $t = -6.31$ ,  $DF = 18$ ,  $P < 0.001$ ). On the high fiber diet, pygmy rabbits also had a higher intake compared to when they were on the control diet ( $t = -3.74$ ,  $DF = 18$ ,  $P = 0.002$ ).

## 4. Discussion

Pygmy rabbits and mountain cottontail rabbits in our study demonstrated both behavioral and physiological responses to changes in ambient temperature and diet quality. Although we expected the smaller dietary and habitat specialist to respond more intensely to colder temperatures and lower quality food than the larger generalist, their responses were similar in magnitude. In colder environments, rabbits of both species ate more food, ate more total food, ate a greater proportion of high fiber food, and passed food more quickly through the digestive system. When food was higher in fiber, the rabbits digested less of the food they consumed, but they maintained their intake of digestible dry matter across diets and temperatures by adjusting their total dry matter intake accordingly. Contrary to our expectations, however, temperature did not affect how much of the plant secondary metabolite, cineole, rabbits consumed.

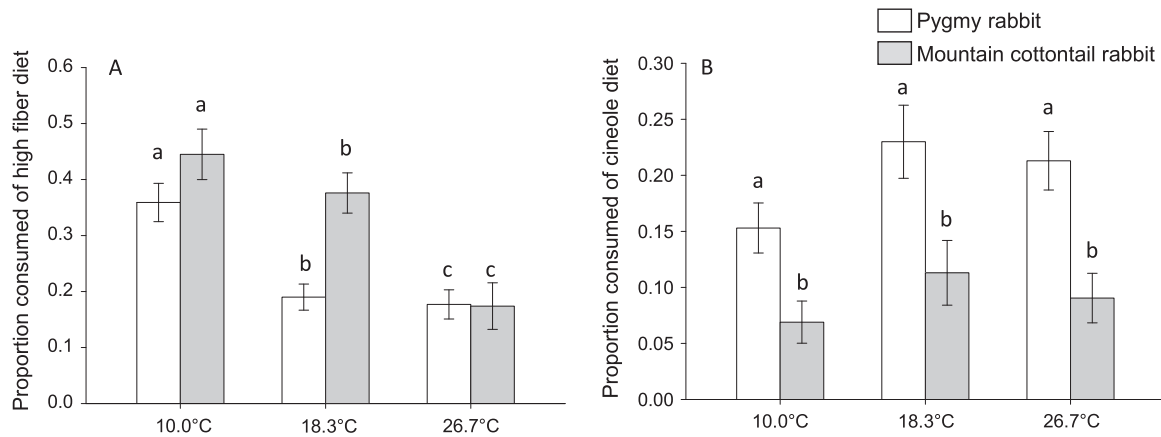


Fig. 1. Proportion  $\pm$  SE consumed of the high fiber diet (50% neutral detergent fiber) relative to the control diet (36% neutral detergent fiber) by pygmy rabbits (*Brachylagus idahoensis*, n = 6) and mountain cottontail rabbits (*Sylvilagus nuttallii*, n = 6) housed at three different temperatures (10.0 °C, 18.3 °C, and 26.7 °C, A). 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. Proportion  $\pm$  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*, n = 6) and mountain cottontail rabbits (*Sylvilagus nuttallii*, n = 6) housed at three different temperatures (10.0 °C, 18.3 °C, and 26.7 °C, B). 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.

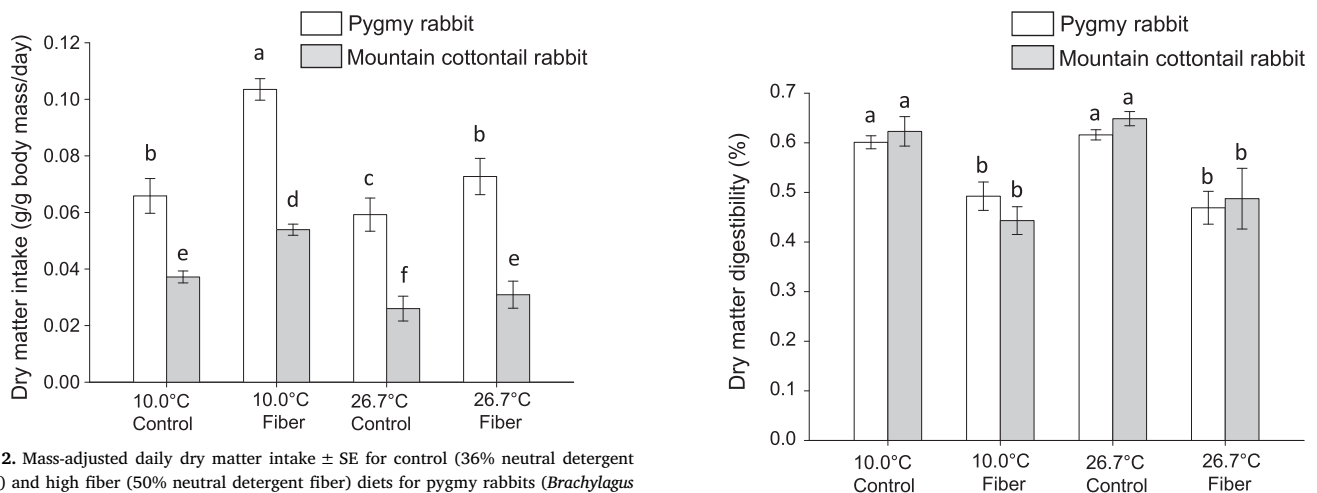
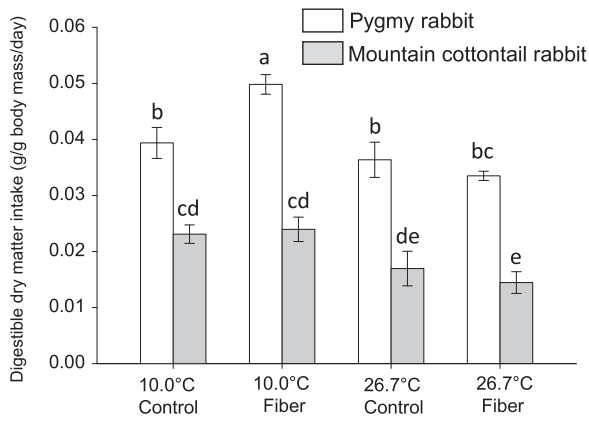


Fig. 2. Mass-adjusted daily dry matter intake  $\pm$  SE for control (36% neutral detergent fiber) and high fiber (50% neutral detergent fiber) diets for pygmy rabbits (*Brachylagus idahoensis*, n = 4) and mountain cottontail rabbits (*Sylvilagus nuttallii*, n = 4) housed at two different temperatures (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.

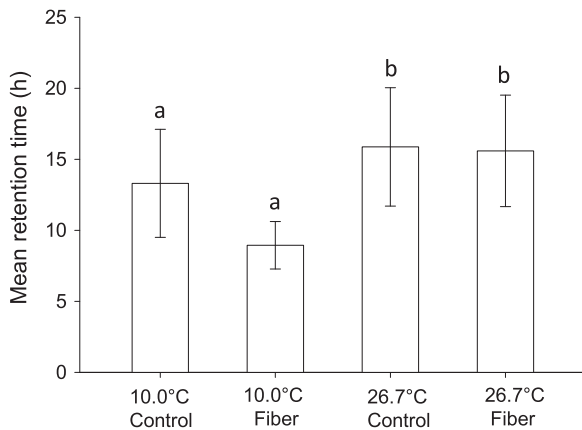
As we predicted, temperature influenced voluntary consumption of plant fiber by both rabbit species. When housed in a relatively colder temperature (10.0 °C), pygmy rabbits and cottontails consumed a greater proportion of the high fiber diet than when they were housed at warmer temperatures. Furthermore, regardless of the temperature, pygmy rabbits and cottontails ate similar proportions of the high fiber diet. This runs contrary to findings in previous studies that showed cottontails digest fiber more efficiently (Shipley et al., 2006) and perceive fiber as less risky (Camp et al., 2015) than do pygmy rabbits. One possible explanation our finding is that higher fiber diets have a higher heat increment associated with digestion than do lower fiber diets. The heat increment of feeding is the increase in metabolic rate associated with digestion following ingestion of a meal (Chappell et al., 1997). Several studies have suggested that endotherms in cold environments can reduce the energetic cost of thermoregulation by using the heat increment of feeding to substitute for thermostatic heat production (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

Fig. 3. Dry matter digestibility  $\pm$  SE for control (36% neutral detergent fiber) and high fiber (50% neutral detergent fiber) diets for pygmy rabbits (*Brachylagus idahoensis*, n = 4) and mountain cottontail rabbits (*Sylvilagus nuttallii*, n = 4) 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.

increases the degree of microbial fermentation in the rumen, high fiber diets can increase the heat increment of feeding (NRC, 1981). As hindgut-fermenters, rabbits are less efficient at digesting fiber than ruminants (Demment and Van Soest, 1985a, 1985b). However, we suspect that microbial fermentation in the cecum may also provide a substantial source of heat for a small-bodied herbivore. The role of fermentation in the heat increment of feeding for small hindgut fermenters is currently unclear, but ours is the first study to indicate that consuming a relatively greater proportion of dietary fiber in cold temperatures might provide some thermoregulatory benefits. Because the fiber content of forage available to herbivores in temperate climates is often the lowest in winter when temperatures are the coldest, herbivores might be able to compensate for reduced energy extracted from the food by reducing energy lost through thermoregulatory costs. For small herbivores that do not hibernate, such as rabbits, this compensation might be particularly valuable.

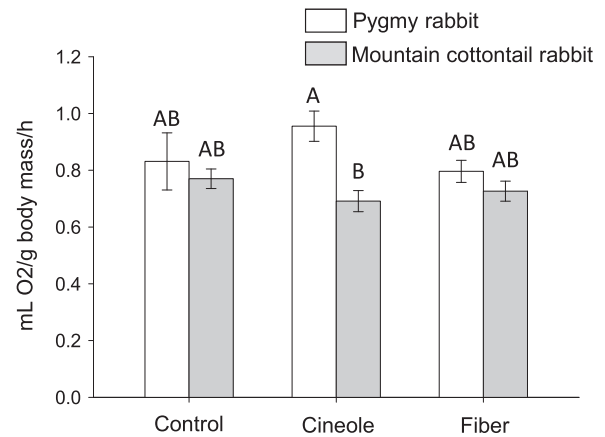


**Fig. 4.** Mass-adjusted daily digestible dry matter intake  $\pm$  SE for control (36% neutral detergent fiber) and high fiber (50% neutral detergent fiber) diets for pygmy rabbits (*Brachylagus idahoensis*,  $n = 4$ ) and mountain cottontail rabbits (*Sylvilagus nuttallii*,  $n = 4$ ) housed at two 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.



**Fig. 5.** Mean retention times (h)  $\pm$  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*,  $n = 4$ ) 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.

If higher levels of dietary fiber do increase the heat increment of feeding, we might expect that consuming a higher fiber diet would result in an increase in resting metabolic rate. However, we did not detect an effect of fiber on resting metabolic rates of rabbits in our study. One reason for our finding 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 resting metabolic rate, the rabbits should be in a fed state (post-ingestive) rather than a post-absorptive 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 post-ingestive state during the metabolic trials. However, only a short period of fasting (< 3 h) might be required for lagomorphs to reach a post-absorptive state (Hinds, 1973) and, in a previous study, 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, our results suggest that the heat of fermentation might play a beneficial role in small herbivores by reducing acute thermoregulatory costs without increasing metabolic costs. Further experiments measuring the effect of fiber consumption on



**Fig. 6.** Mass-adjusted resting metabolic rate as measured by oxygen consumption  $\pm$  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.

the heat of fermentation, and the heat of fermentation on thermoregulatory costs, at a greater range of environmental temperatures are required to fully understand these relationships.

In contrast to their selection for higher fiber diets, rabbits of neither species voluntarily consumed more of the cineole diet when housed at a relatively colder temperature. Our finding contrasts with studies that suggest other species of small mammalian herbivores avoid plant secondary metabolites in warmer temperatures and consume more plant secondary metabolites in colder temperatures. For example, white-throated woodrats consumed more juniper, which contains high levels of plant secondary metabolites, when housed in colder temperatures (20 °C) compared to warm temperatures (28 °C, Dearing et al., 2008; McLister et al., 2004). Moreover, resting metabolic rates of woodrats were 1.3 times higher in 20 °C and 1.4 times higher in 28 °C when consuming a diet exclusively of juniper compared to a diet free of plant secondary metabolites.

We suggest several reasons that rabbits in our study might have had a less pronounced response to the interaction of plant secondary metabolites and temperature than did woodrats. Although our temperature range was wider and included lower temperatures (10–27 °C) than those used in the woodrat studies (18–28 °C), our acclimation periods (5 days for the voluntary intake trials and 3 days for the metabolic rate trials) were shorter than those of the woodrats (14 days) and might not have been long enough to elicit strong 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) at a concentration equal to all the individual monoterpenes combined in sagebrush. The increase in resting metabolic rate of the woodrats might have been caused by a different plant secondary metabolite, or by a suite of plant secondary metabolites acting in concert. Because the diet containing 3% cineole did not increase resting metabolic rate in either rabbit species, it likely did not elicit sufficient thermogenesis to influence diet choice. Although higher levels of cineole may have increased resting metabolic rate, these levels would generally exceed those found in natural forage encountered by these rabbits.

Surprisingly, regardless of temperature, both pygmy rabbits and cottontails voluntarily chose to eat a portion of the cineole diet, even when a terpene-free food was available (i.e., pygmy rabbits averaged 23% and cottontails averaged 12% of their diet). This finding contrasts with 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, monoterpene-free diets were available (Shipley et al., 2006). Pygmy rabbits have a greater ability to regulate absorption and detoxify cineole than do cottontails (Shipley et al., 2012), which explains their two-fold higher consumption of the cineole diet, and four-fold consumption of cineole. Both rabbit species, however, might choose to consume a minimum level of monoterpenes when available to provide potential health benefits (e.g., anti-parasitic, anti-inflammatory, and gastro-protective benefits; Jurgens, 2014; Kostadinovic et al., 2012; Lahlou et al., 2002; Santos and Rao, 2001) that outweigh potential thermoregulatory and detoxification costs.

Temperature influenced not only what rabbits 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 temperature, 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. Like our rabbits, however, both rodent species maintained between 57% and 62% dry matter digestibility across temperatures (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 they 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 and digestible dry matter intake than cottontails, but similar mass-adjusted resting metabolic rates. Both pygmy rabbits and cottontails increased their intake of dry matter in the colder temperature (10.0 °C) and when fed a higher fiber diet, 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, compared to eastern cottontails (*Sylvilagus floridanus*), pygmy rabbits had a higher dry matter intake and daily digestible energy requirement relative to their body mass. Consequently, pygmy rabbits might need either to eat higher quality food or to spend more time searching for and harvesting food than cottontails. This requirement might partly explain why pygmy rabbits are restricted to sagebrush steppe landscapes that provide abundant evergreen sagebrush that is high in digestible energy throughout the year (Green and Flinders, 1980; Welch and Pederson, 1981).

Our results indicated that pygmy rabbits passed food more quickly through the digestive tract when housed in the colder temperatures. Although the faster passage of food through the digestive tract was likely caused by the higher intake in the colder temperature, we did not observe a concurrent reduction in dry matter digestibility of either diet in the colder temperature. Other studies have shown mammalian herbivores increase food intake and rate of passage of digesta, at the expense of decreased digestibility, as energy demands increase or diet quality decreases (Loeb et al., 1991; Nagy and Negus, 1993; Woodall, 1989). However, 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 retention of digesta components in the cecum and cecotrophy (i.e., the ingestion of nutrient dense feces derived from the cecum that allows small herbivores to more efficiently extract nutrients from high fiber diets; Davies and Davies, 2003). Consequently, these digestive processes might also allow the rabbits to maintain their intake of digestible dry matter when consuming high fiber food in cold temperatures, as has been documented for another small cecal fermenter, the Brandt's vole (*Microtus brandti*; Hume et al., 1993; Pei et al., 2001). Fiber content of the diet did

not influence mean retention time of particulate digesta in our rabbits. Similarly, Brandt's voles (*Microtus brandti*) did not vary in their mean retention times of liquid or solid digesta of high fiber (37.6% NDF) and low fiber (13.6% NDF) diets (Pei et al., 2001). However, 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).

We observed an increase in intake in response to cold temperatures and high fiber diets by both species of rabbits, yet we observed that pygmy rabbits decreased their retention time in colder temperatures. 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. (Pei et al., 2001). Further exploration into how temperature and diet quality influence passage rate and gut size in gut size is needed to fully understand how small herbivores increase intake while maintaining gut size.

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. Although forage availability and quality can be the leading cause of such shifts, daily or seasonal temperature differences also might influence diet or patch selection. Our results suggest that these rabbits have mechanisms to cope with colder temperatures and foods containing plant fiber and plant secondary metabolites by altering diet composition and intake.

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