

Using the Specialization Framework to Determine Degree of Dietary Specialization in a Herbivorous Woodrat

Michele M. Skopec¹ · Kevin D. Kohl² · Katharina Schramm^{1,2} · James R. Halpert³ · M. Denise Dearing²

Received: 19 August 2015 / Revised: 9 November 2015 / Accepted: 19 November 2015 / Published online: 2 December 2015
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Abstract To be considered a dietary specialist, mammalian herbivores must consume large quantities of a plant species considered “difficult” with respect to nutrient or toxin content, and possess specialized adaptations to deal with plant defensive compounds or low nutritional content. Populations of *Neotoma lepida* in the Great Basin consume *Juniperus osteosperma*, a plant heavily defended by terpenes, but a detailed dietary analysis of this population is lacking. Therefore, we investigated the extent of dietary specialization in this species in comparison with the better-studied specialist species, *N. stephensi*. Microhistological analysis of feces from *N. lepida* revealed that greater than 90 % of their diet in nature was comprised of juniper. In laboratory tolerance trials, *N. lepida* tolerated a diet of 80 % *J. osteosperma*, similar to that observed for *N. stephensi*. There was no difference in the abilities of *N. lepida* and *N. stephensi* to metabolize hexobarbital, a proxy compound for terpene metabolism. In preference tests of native and non-native juniper species, *N. lepida* did not exhibit a preference for its native or co-occurring juniper, *J. osteosperma*, over the non-native species, *J. monosperma*, whereas *N. stephensi* preferred its native or co-occurring juniper *J. monosperma* over non-native *J. osteosperma*. Behavioral and habitat differences between these woodrat species lead to the categorization of *N. stephensi* as an obligate juniper specialist with a small range that overlaps that of its

preferred food, *J. monosperma*, and *N. lepida* as a facultative juniper specialist with a large range, and only a portion of its distribution containing populations that feed extensively on *J. osteosperma*.

Keywords Dietary specialization · *Neotoma* · Juniper · Herbivory

Introduction

While herbivory is a common feeding strategy for mammals, dietary specialization, i.e., feeding nearly exclusively on an individual species of plant, is rare. For decades, the dominant theory for the preponderance of generalist over specialist mammalian herbivores has been the detoxification-limitation theory (Dearing et al. 2000; Freeland and Janzen 1974; Marsh et al. 2006), which posits that most mammalian herbivores tend to be generalists that avoid overwhelming any particular biotransformation pathway with a high dose of plant secondary compounds (PSC). However, dietary specialists do occur. Shipley et al. (2009) developed a framework for placing mammalian herbivores along the specialist-generalist continuum and separated specialization into two types, obligate and facultative.

In this framework, mammalian herbivores are considered specialists when one plant species comprises >60 % of the diet, the herbivore possesses specialized anatomical, physiological, or behavioral mechanisms to deal with the plant defenses, and the herbivore experiences reduced competition due to specialization (Shipley et al. 2009). Obligate specialists have narrow fundamental and realized niches, limited available diets, and difficult and restricted realized diets. Facultative specialists have broader fundamental and realized niches than obligate specialists, though they may encounter

✉ Michele M. Skopec
micheleskopec@weber.edu

¹ Department of Zoology, Weber State University, 1415 Edvalson Dr., Ogden, UT 84408, USA

² Department of Biology, University of Utah, Salt Lake City 84112, UT, USA

³ School of Pharmacy, University of Connecticut, Storrs, CT, USA

limited available diets in parts of their range leading to restricted realized diets (ShIPLEY et al. 2009).

Woodrats (genus *Neotoma*) are a group of small herbivorous rodents ideal for testing the specialization paradigm developed by ShIPLEY et al. (2009). since different species within the genus have dietary habits that span the generalist – specialist continuum, from the obligate generalist *N. cinerea* to the obligate juniper specialist *N. stephensi* (Bentacourt et al. 1990). Most woodrat species that are dietary specialists feed primarily on plants that are heavily defended either chemically, such as *N. stephensi* consuming one-seed juniper (*J. monosperma*), or structurally defended, such as *N. lepida* consuming cactus in inland California (Brown et al. 1972; MacMillen 1964; Vaughan and Czaplewski 1985). Numerous studies have investigated the behavioral, physiological, and microbial mechanisms that different species and populations in the *Neotoma* genus use to deal with their specialized diets (Kohl et al. 2014b; Malenke et al. 2014; Magnanou et al. 2009; Skopec et al. 2007; Sorensen and Dearing 2003; Torregrossa and Dearing 2009; Torregrossa et al. 2011). However, we still lack an understanding of what factors constrain some species such as *N. stephensi* to narrow specialization on a single species, whereas other species like *N. lepida* display a more flexible or facultative specialization throughout their range.

While a range of dietary specialization occurs within the genus *Neotoma*, one species, the desert woodrat, stands out in its ability to not only specialize locally on difficult plants, but also its ability to specialize on a wide variety of difficult plants across habitats. *Neotoma lepida* has one of the largest ranges in the genus, encompassing parts of the Great Basin, Mojave, and Sonoran deserts (Patton et al. 2014; Verts and Carraway 2002) and specializes on different plant species across its range. For example, *N. lepida* populations in the Mojave Desert specialize either on creosote bush (*Larrea tridentata*, Cameron and Rainey 1972). or mesquite (Smith et al. 2014) depending on plant availability. In contrast, populations from inland California specialize on Cholla cactus (*Opuntia bigelovii*, Brown et al. 1972) or Prickly pear cactus (*Opuntia occidentalis*, MacMillen 1964). Conversely, *N. stephensi* feeds nearly exclusively on one-seed juniper (*Juniperus monosperma*) and has a much smaller range than *N. lepida*, and the distribution of *N. stephensi* overlaps the range of its preferred food, *J. monosperma* (Dial 1988; Vaughan and Czaplewski 1985).

Dietary specialists experience tradeoffs. Specialization is beneficial to woodrats because their preferred species of plant tends to be in high abundance in the environment, is present year round, and acts as water source during dry months (Smith et al. 2014; Stones and Hayward 1968; Vaughan and Czaplewski 1985). Adaptions to plant defenses allow woodrats to experience less competition from other herbivores in their

environment (Smith et al. 2014; Stones and Hayward 1968; Vaughan and Czaplewski 1985). In addition, many of the *Neotoma* dietary specialists build middens, or stick homes, in their preferred plant species, leading to lower foraging costs and predation risk (Smith et al. 2014; Stones and Hayward 1968; Vaughan and Czaplewski 1985). However, woodrat specialists also experience significant costs. The energetic cost of biotransformation and excretion of PSCs has been estimated to be as high as 35 % of maintenance expenditure in *N. stephensi*, a juniper specialist (Sorensen et al. 2004). Many of the PSCs consumed by woodrats, such as terpenes and phenolics, are diuretics, which can be problematic for desert dwelling woodrats (Dearing et al. 2001, 2002). Dietary specialists may be more prone to temperature dependent toxicity of PSCs, a phenomenon where warmer ambient temperatures slow the metabolism of toxins (Dearing 2013; Kurnath and Dearing 2013). Additionally, specialists may be less able to deal with novel PSCs (Sorensen et al. 2005).

Here, we explored dietary specialization in a population of *N. lepida* in the Great Basin desert. In this location, *N. lepida* is known to feed on Utah juniper (*Juniperus osteosperma*). We compared the degree of dietary specialization to that of the well described juniper specialist, *N. stephensi* (Dial 1988; Vaughan and Czaplewski 1985). Previous work by Stones and Hayward (1968) suggests that *N. lepida* in the Great Basin may be a juniper specialist based on the contents of their food caches, but a quantitative analysis of *N. lepida*'s diet and ability to consume high levels of *J. osteosperma* has not been undertaken. We used microhistological analysis of fecal pellets from wild-caught *N. lepida* and laboratory tolerance trials to determine how much juniper *N. lepida* does and can consume in comparison to that of the known specialist *N. stephensi*. We also compared *N. lepida*'s biotransformation capabilities to *N. stephensi*, by measuring *in vivo* hexobarbital clearance time (Skopec et al. 2013). Preference trials with *J. osteosperma* and *J. monosperma* were performed to determine the flexibility in the feeding behaviors of both woodrat species. Woodrats were collected from populations where only one species of juniper is present, *J. osteosperma* for *N. lepida*, and *J. monosperma* for *N. stephensi*. If *N. lepida* from the Great Basin is a dietary specialist, we expected that it would consume >60 % juniper diet in nature, it could tolerate a similar level of its native juniper compared to *N. stephensi* in the laboratory, and it would have similar biotransformation capabilities for the terpenes present in juniper compared to *N. stephensi*. Finally, we predicted that the obligate specialist, *N. stephensi*, would prefer its native juniper, *J. monosperma*, while the putative facultative specialist, *N. lepida*, would display more behavioral flexibility with less of a preference for its native juniper, *J. osteosperma*.

Methods and Materials

Animals Great Basin *N. lepida* were trapped near White Rocks in Tooele County, Utah, USA (40°19'N, 112°54'W). *Neotoma stephensi* were trapped on Woodhouse Mesa, near Wupatki National Park, 45 km northeast of Flagstaff, Arizona, USA (35°30' N, 111°27' W). Woodrats were housed in individual shoebox cages (48 × 27 × 20 cm) with pine shavings on a 12L: 12D cycle at 28 °C and with a relative humidity of 15 %. The woodrats were fed high-fiber rabbit chow (Harlan Teklad formula 2031) and water ad libitum. Average body mass (± SD) did not differ between the two species and was 149.1 ± 30.6 for *N. lepida* and 175.4 ± 29.7 for *N. stephensi* ($T = 1.11$ $P = 0.28$). All experimental procedures involving woodrats were approved by the University of Utah's Institutional Animal Care and Use Committee protocol number 10-01013.

Microhistological Analysis of Feces Fresh *N. lepida* fecal pellets were collected from the traps of 22 individuals that were live-trapped November 2013. Pellets were dried in a 50 °C oven and then ground to pass through a 1 mm screen. Ground samples were affixed to microscope slides using Permount Mounting Medium, and 20 fields per slide (50× magnification) were examined for plant fragments. Plant fragments in feces were identified as juniper or non-juniper by comparison to reference slides of juniper samples collected from the area that had been processed by the same protocol as the pellets. Relative percentages were calculated for each individual as in Flinders and Hansen (1972).

Diets The junipers used in the dietary treatments were collected from trees at woodrat trapping sites (*J. monosperma* from *N. stephensi* trapping site and *J. osteosperma* from *N. lepida* trapping site) and frozen at -20 °C in sealed plastic bags until use. The juniper foliage was ground in a Waring blender (model CB-5) with dry ice until it passed through a 1.0 mm screen. Diets were ground to prevent sorting by the woodrats. Samples of the ground juniper were sent to Dairy Forage One (Ithaca NY, USA) for nutrient analysis. Terpene composition and content of the ground juniper was analyzed, and compounds were identified against authentic standards and by database searches as outlined in Adams et al. 2014. The diet treatments contained increasing percentages of ground juniper homogenized with ground high-fiber rabbit chow. The diets were comprised of 0, 20, 40, 60, 80, and 100 % juniper on a dry matter basis. The dry matter content of each diet fed was determined daily by placing 5 g samples of each diet in a drying oven at 50 °C. The dry matter (DM) content of the juniper was 55 % for *J. monosperma* and 54 % for *J. osterosperma*.

Tolerance Trials To compare the two woodrat species' tolerances for their native vs. non-native juniper, animals were fed

diets with increasing concentrations of juniper (0–100 % in 20 % increments) for 3 d each. Diets were fed ad libitum. Dry matter intake and body mass were measured daily. Any animal that lost more than 10 % of its initial body mass was removed from the trial. A total of 16 animals were used in the tolerance trials ($N = 8$ *N. lepida* and $N = 8$ *N. stephensi*) in a repeated measures design. Each trial was preceded by a 21 d wash-out period during which animals were fed only their maintenance diet of high fiber rabbit chow. The amount of time that the two species of woodrats remained in the trial while consuming diets of each of the two different juniper species was compared using Kaplan-Meier survival analysis (JMP 10). The mean persistence time for each woodrat species consuming each juniper was compared using a log rank test (JMP 10). Survival analysis methods are used to compare the time to any discrete event, while death is a common end point, other end points such as body mass loss are often used, and a 10 % loss of initial body mass was used as the end point in this study (Lee and Wang 2003). The amount of juniper consumed per gram body mass when feeding on the 20 %–100 % juniper diets was calculated as a three day average and compared between the two woodrats on the two juniper species using a repeated measures MANOVA. Percent juniper was the repeated measure, and woodrat and juniper species were the independent variables.

Hexobarbital Clearance Assays Hexobarbital is a hypnotic agent that is metabolized by the cytochromes P450, a super-family of biotransformation enzymes that are used by woodrats to metabolize terpenes present in juniper (Skopec et al. 2013). Hexobarbital clearance assays measure the length of time a rodent remains unconscious after injection with hexobarbital, where the length of the hypnotic state is inversely proportional to the activity of the relevant cytochromes P450 activity in an animal. These assays have been used in multiple studies to gauge *in vivo* biotransformation capabilities of woodrats (Dearing et al. 2006; Kurnath and Dearing 2013; Skopec et al. 2013). Therefore, we compared hexobarbital clearance times in *N. lepida* and *N. stephensi* to two species of woodrats, *N. floridana* and *N. cinerea*, that have generalist feeding strategies (Bentacourt et al. 1990; Post et al. 1993). For the assays, hexobarbital (100 mg/kg) was injected into the intraperitoneal (IP) cavity of *N. lepida* ($N = 10$, average body mass 128 ± 31.28), *N. stephensi* ($N = 8$, average body mass 168 ± 34.69), *N. cinerea* ($N = 5$, average body mass 272.8 ± 49.45), and *N. floridana* ($N = 5$, average body mass 359.2 ± 51.82), and the length of the resulting hypnotic state was measured as the duration of time after injection during which an animal is unable to right itself twice within 30 s of being placed on its back (Dearing et al. 2006). Hexobarbital clearance times were compared between the species using an ANOVA, and *post-hoc* Tukey's HSD were used to determine significant differences between each species.

Preference Trials To compare the flexibility of the two woodrats species with respect to feeding behavior, a series of three preference trials was performed where animals were given a choice between 60 % *J. monosperma* and 60 % *J. osteosperma* diets. A total of 16 animals were used in the preference trials ($N = 8$ *N. lepida* and $N = 8$ *N. stephensi*). Each trial was preceded by a 21 d wash-out period during which animals were fed only their maintenance diet of high fiber rabbit chow.

In the first preference trial, hereon referred to as “non-induced”, animals were not exposed to juniper prior to the trial to determine if there was innate preference for native juniper. Animals were placed in shoebox cages that contained two external feeders. Each feeder contained ground rabbit chow for a three day pre-treatment to acclimate animals to the feeding set-up. For the next three days, one feeder contained a 60 % *J. monosperma* diet and the other contained 60 % *J. osteosperma* diet. The location of the juniper diets was switched each night to prevent the animals from displaying a preference for a specific feeder. The 60 % juniper concentration was selected because both woodrat species tolerated both juniper species at this level in the tolerance trials (Figs. 1 and 2).

The second preference trial involved feeding the woodrats with their native juniper for the three day pretreatment period to determine if pre-exposure to the native juniper increased preference for and consumption of the native juniper. Animals were fed 20, 40, and 60 % juniper diets for 1 d each, prior to being given a choice of 60 % *J. monosperma* diet and 60 % *J. osteosperma* diet for 3 d. Induction periods are commonly used in feeding trials with woodrats to induce biotransformation enzymes needed to metabolize PSCs in foliage (Skopec et al. 2013).

The third preference trial involved feeding woodrats with the non-native juniper for the 3 d pretreatment period to

determine whether pre-exposure to the non-native juniper increased preference for and consumption of the non-native juniper. A change in preference due to pre-exposure could mean that the animals were using different biotransformation pathways to deal with the native versus non-native juniper. Animals were fed 20, 40, and 60 % juniper diets for one day each prior to being given a choice of 60 % *J. monosperma* diet and 60 % *J. osteosperma* diet for 3 d.

Dry matter intake of each diet and body mass was measured daily for all trials. A two-way repeated measures ANOVA was used to compare dry matter intake per gram body mass (three-day average) between woodrat species and juniper diets. Induction status (non-induced, induced with *J. monosperma*, or induced with *J. osteosperma*) was the repeated variable, and woodrat and juniper species were the independent variables. Paired *T*-tests were used to determine whether the woodrats showed a significant preference for one juniper species over the other in each woodrat.

Results

Juniper Consumption by *Neotoma lepida* in Nature A total of 93.8 ± 4.6 % of the plant fragments in the fecal pellets from 22 animals trapped during November 2013 were identified as juniper. On average, 231 ± 25 plant fragments were analyzed from each set of pellets.

Nutritional Content of Juniper The nutrient content of the two species of juniper was similar with both containing ~6 % protein, ~27 % acid detergent fiber, ~36 % neutral detergent fiber as well as similar contents of trace minerals (Table 1). However, terpene concentrations and profiles differed between the two junipers, with *J. osteosperma* containing twice the level of terpenes as *J. monosperma* (Table 2). The

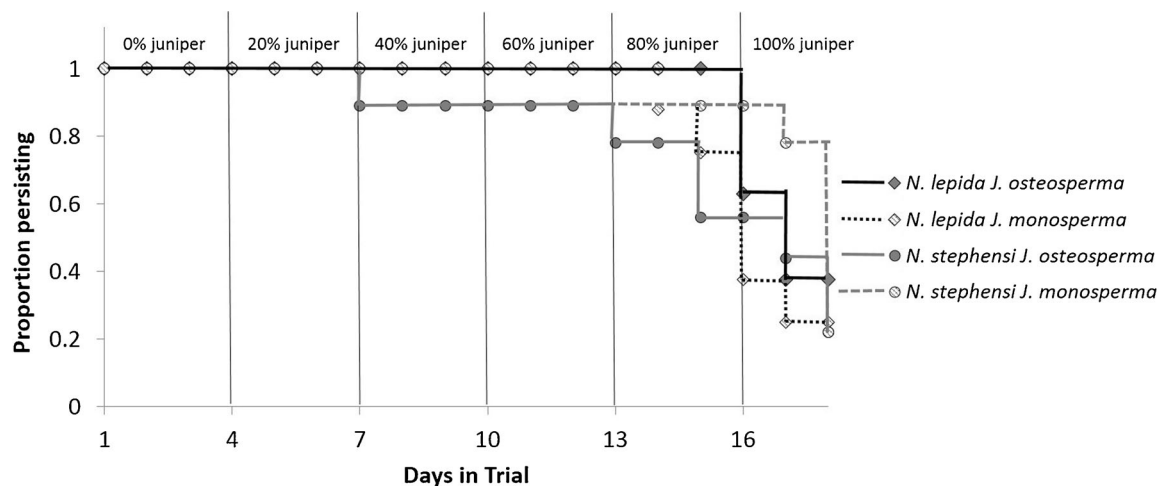
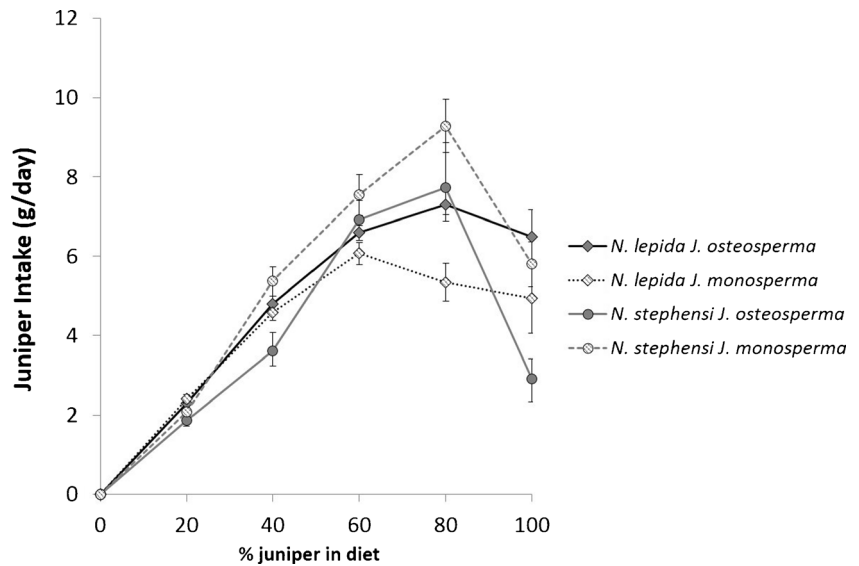


Fig. 1 Proportion of *Neotoma lepida* and *N. stephensi* persisting in the juniper tolerance trials. Woodrats were removed from the experiment when they lost more than 10 % of their initial body mass. There was no

difference in persistence between woodrat species or by juniper diet Wilcoxon $X^2 = 2.35$ $P = 0.5$

Fig. 2 Juniper intake of *Neotoma lepida* and *N. stephensi* in the juniper tolerance trials. Juniper intakes are 3 d averages (mean ± SE) for each diet consumed



dominant terpene in *J. monosperma* was α -pinene, while *J. osteosperma* had a lower concentration of four different terpenes, α -pinene, sabinene, camphor, and bornyl acetate.

Tolerance Trials Persistence, as measured by ability to maintain body mass during the trial, did not differ between the two species of woodrat on either juniper diet (Fig. 1, Wilcoxon $\chi^2 = 2.35 P = 0.5$). Animals persisted in the 18 day trials an average of 16.6 ± 2.2 days, which is when the 100 % juniper diets were offered.

Juniper intake per gram body mass differed between woodrat species but not by juniper species (Table 3, Fig. 2). There was a significant difference in juniper intake (per gram body mass) as a function of juniper in the diet (Table 3, Fig. 2), and peak juniper consumption occurred when the juniper concentration reached 80 % for both juniper diets. There were

significant woodrat by juniper species, % juniper by woodrat species and % juniper by juniper species by woodrat species interactions (Table 3, Fig. 2), because at the higher concentrations of juniper (>80 %), *N. stephensi* consumed more of its native juniper, *J. monosperma*, and *N. lepida* consumed more of its native juniper, *J. osteosperma*.

Hexobarbital Clearance Assays There was a significant difference in hexobarbital clearance times among the four species of woodrats ($F_{3,24} = 28.24, P < 0.001$, Fig. 3). The hexobarbital clearance times of *N. lepida* and *N. stephensi* did not differ from each other (Tukey’s HSD $P > 0.05$). Moreover, the hexobarbital clearance times of *N. cinerea* and *N. floridana* were significantly longer than both *N. lepida* and *N. stephensi* (Tukey’s HSD $P < 0.05$).

Table 1 Nutrient content of juniper fed to woodrats

	<i>J. monosperma</i>	<i>J. osteosperma</i>
Crude protein (% DM)	6.3	6.1
Acid detergent fiber (% DM)	26.2	27.4
Neutral detergent fiber (% DM)	36.6	36.3
Calcium (% DM)	1.08	1.44
Phosphorus (% DM)	0.18	0.10
Magnesium (% DM)	0.14	0.14
Potassium (% DM)	0.51	0.53
Sodium (% DM)	0.019	0.014
Iron (ppm)	161	92
Zinc (ppm)	13	10
Copper (ppm)	3	3
Manganese (ppm)	15	17
Molybdenum (ppm)	0.8	0.5

Preference Trials During the preference trials, there was a significant difference in dry matter intake per gram body mass between the woodrat species ($F_{1,24} = 8.42 P = 0.008$) but not juniper species ($F_{1,24} = 0.057 P = 0.81$, Fig. 4). Prior to induction, *N. stephensi* had juniper intakes that were significantly higher than *N. lepida*. This result was due to *N. stephensi*’s ability to ingest 3-fold more of its native juniper than *N. lepida*, which ingested less and roughly equal amounts of both species of juniper. Exposure to juniper for three days prior to the preference trials increased intake in both woodrat species, demonstrating a significant effect of induction ($F_{2,23} = 35.41 P < 0.001$). In *N. stephensi*, induction with *J. monosperma* increased consumption of the *J. monosperma* diet during the preference trial, and induction with *J. osteosperma* increased consumption of the *J. osteosperma* diet in the preference trial. Thus, there was a significant juniper X induction effect ($F_{2,23} = 8.60 P = 0.002$) in *N. stephensi* but not in *N. lepida*, leading to a

Table 2 Relative terpene content of juniper fed to woodrats (Adams et al. 2014)

	<i>J. monosperma</i>	<i>J. osteosperma</i>
Oil yields, 24 h dist. - % DW	4.48 %	8.9 %
α -pinene ^a - % oil	61.8	10.5
Sabinene - % oil	0.1	12.7
Camphor - % oil	0.3	8.5
Bornyl acetate - % oil	0.5	16.1
Elemol - % oil	2.4	5.2
β -eudesmol - % oil	4.3	0.7

^a All terpenes were identified based on authentic reference compounds and database searches

significant woodrat species X induction X juniper effect ($F_{2,23} = 12.54$ $P < 0.001$).

In both the non-induced and induced trials with *J. monosperma*, *N. stephensi* preferred *J. monosperma*, the native juniper ($P = 0.02$, Fig. 5). When induced with *J. osteosperma*, *N. stephensi* switched its preference to *J. osteosperma* ($P = 0.01$). *Neotoma lepida* never exhibited a preference for either juniper in the tolerance trials ($P = 0.8$ non-induced and *J. osteosperma* induced, $P = 0.1$ *J. monosperma* induced).

Discussion

Here, we used the framework of Shipley et al. (2009) to determine if *N. lepida* is a dietary specialist in the Great Basin Desert. According to this framework, a specialist mammalian herbivore is defined to be one that predominately consumes a single “difficult” plant species, and has mechanisms to deal with the challenges of this food source. Shipley et al. (2009) also differentiated between “obligate” and “facultative” specialists based on niche size and dietary flexibility, with obligate specialists having narrower niches and more restricted diets than facultative specialists. Based on these criteria and

the results herein, we propose that the feeding strategy of *N. lepida* fits the description of a facultative specialist.

The first in depth dietary analysis of a population of *N. lepida* from the Great Basin, described herein, revealed that not only do these woodrats consume upwards of 90 % *J. osteosperma* in nature, but they can also tolerate 80 % *J. osteosperma* diets in a laboratory setting. These results are similar to those for the well described *J. monosperma* specialist, *N. stephensi*. Additionally, *N. lepida*'s biotransformation abilities did not differ from *N. stephensi* as measured by hexobarbital clearance times. Unlike *N. stephensi*, which is an obligate specialist, *N. lepida* did not show a preference for its native juniper, and therefore, according to Shipley et al. (2009), should be categorized as a facultative juniper specialist. As a facultative specialist, *N. lepida* is able to consume large quantities of *J. osteosperma* when it co-occurs with the plant, but is not dependent on juniper and therefore occurs in habitats that lack this plant species.

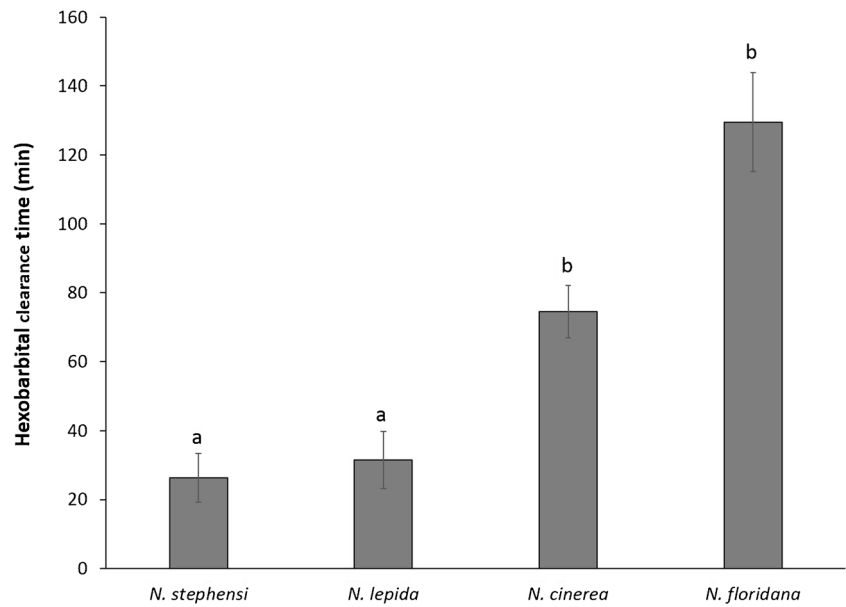
Neotoma lepida's facultative specialization on juniper in the Great Basin Desert is similar to the population level specialization that occurs in insects (Fox and Morrow 1981). Mammalian dietary specialists are considered to be rare, while insect dietary specialists are abundant (Forister et al. 2015; Freeland and Janzen 1974), and it has been proposed that selection favors specialization in insects and generalization in mammals (Freeland and Janzen 1974; Rausher 1992). However, localized specialization has been documented for other woodrats species considered generalists at the species level (Karasov 1989; McEachern et al. 2006). Thus, local specialization may be the norm and not the exception among woodrat species.

Juniper is a difficult food. The two species of juniper used in the study are low in protein (~6 % by dry weight) and high in fiber (~36 % neutral detergent fiber by dry weight). Furthermore, they are heavily defended with PSCs, particularly monoterpenes (4.5 % terpenes for *J. monosperma* and 8.9 % terpenes for *J. osteosperma* by dry weight). Terpenes are used as defensive compounds by plants (Gershenson and Dudareva 2007) and are known to have neuro-, hepato-, and

Table 3 Summary of repeated measures MANOVA for juniper intake during juniper tolerance trials

Source of variation	<i>F</i>	<i>df</i>	<i>P</i> -value
Between subjects			
Woodrat species	16.44	1, 15	0.001
Juniper species	1.32	1, 15	0.27
Woodrat × juniper species	11.75	1, 15	0.004
Within subjects			
% juniper	144.17	4, 12	<0.001
% juniper × woodrat species	3.31	4, 12	0.048
% juniper × juniper species	4.75	4, 12	0.16
% juniper × juniper species × woodrat species	4.52	4, 12	0.02

Fig. 3 Hexobarbital clearance times in woodrats. Means \pm SE of *Neotoma stephensi* ($N = 9$), *N. lepida* ($N = 9$), *N. cinerea* ($N = 5$) and *N. floridana* ($N = 5$) are shown. Letter *a*, *b* and *c* denote means that are significantly different ($P < 0.05$) as determined by Tukey's HSD



nephrotoxic effects in vertebrates (Falk et al. 1990; Savolainen and Pfäffli 1978; Sperling et al. 1967). However, as an evergreen, juniper is a year round food and water source for woodrats. Very few vertebrates are known to specialize on conifers due to their low nutritional value and abundant defenses such as PSCs. Woodrats (*N. stephensi* and *N. lepida*), red tree voles (*Arborimus longicaudus*), Aberts' squirrels (*Sclerurus aberti*), and wooly flying squirrels (*Eupetaurus cinereus*) are the only described examples of mammals specializing on conifers (Dial 1988; Hayes 1996; Murphy and Linhart 1999; Vaughan and Czaplewski 1985; Zahler and

Khan 2003). Other mammalian herbivores consume juniper but in much smaller quantities, and often with major digestive and/or toxic effects (Anderson et al. 2013; Estell et al. 2014; Schwartz et al. 1980; Zlatnik 1999). Therefore, *N. lepida*'s ability to consume large quantities of juniper likely decreases the amount of competition it experiences from other mammalian herbivores present in the Great Basin.

Neotoma lepida can efficiently utilize juniper as a major dietary constituent both in nature and in the laboratory. More than 90 % of the plant fragments in the feces of wild-caught *N. lepida* were juniper. *Neotoma lepida* also performed

Fig. 4 Dry matter intake of 60 % juniper diets by *Neotoma stephensi* and *N. lepida* in the juniper preference trials. Mean \pm SE of 3 d dry matter intakes are shown. * denotes within woodrat species differences in dry matter intake of two juniper diets offered ($P < 0.05$). + denotes between woodrat species differences in dry matter intake of juniper diets ($P < 0.05$)

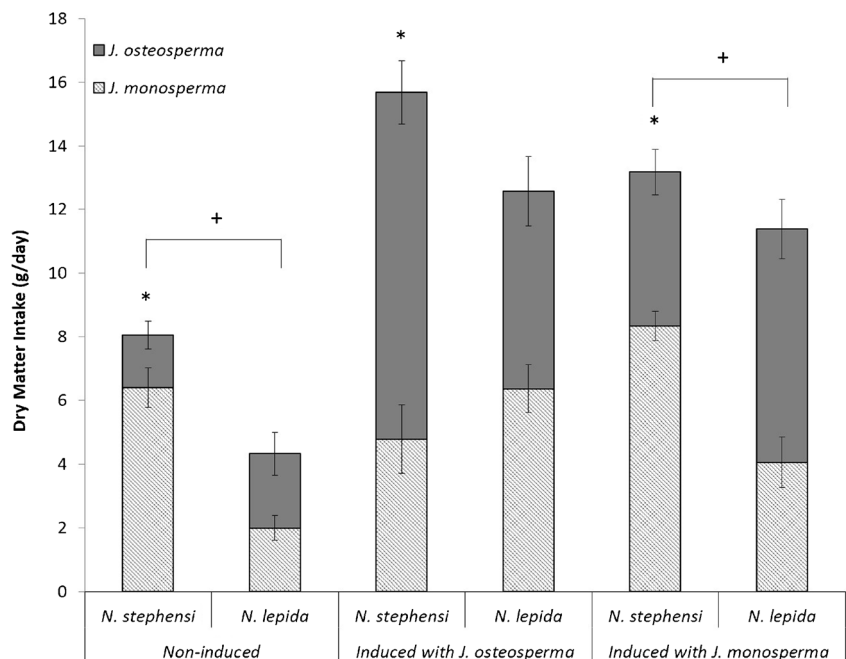
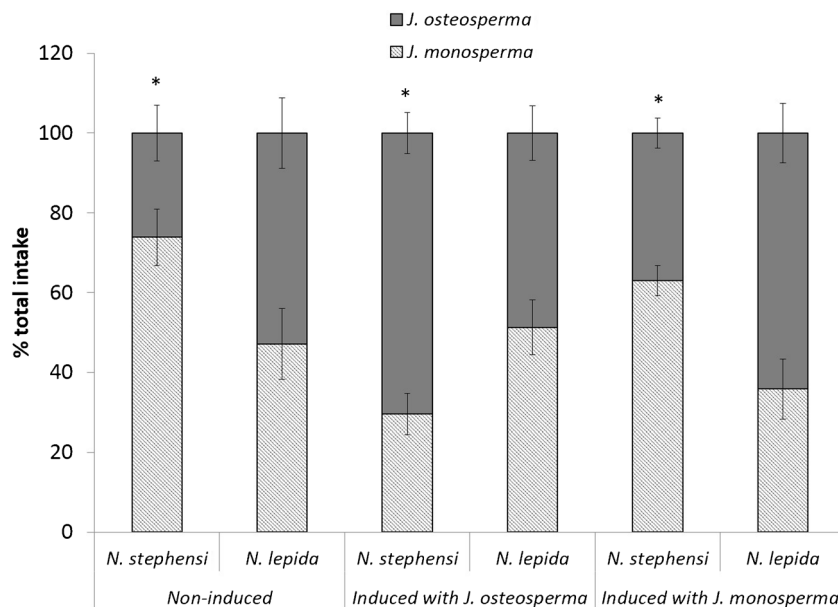


Fig. 5 Relative percent of total intake of the 60 % juniper diets by *Neotoma stephensi* and *N. lepida* in the juniper preference trials. Mean \pm SE of 3 d percent of total intakes are shown. * denotes differences in percent intake of two juniper diets offered ($P < 0.05$)



similarly to *N. stephensi* in the tolerance trials, with the exception of *N. stephensi* consuming more of the 80 % *J. monosperma* diet than *N. lepida*, and *N. lepida* consuming more of the 100 % *J. osteosperma* diet than *N. stephensi* (Fig. 2). Both woodrat species tolerated diets containing up to 60 % of both juniper species and diets containing 80 % of the native juniper species. While a high tolerance for the respective native juniper was expected, a high tolerance for the non-native juniper was not expected, especially given the large difference in the terpene profiles of the two junipers. This result may be due to common biotransformation enzymes for dealing with terpenes shared between the two woodrat species and is the subject of a current investigation.

Like *N. stephensi*, *N. lepida* possesses enhanced biotransformation abilities to deal with the PSCs present in juniper. In this study, we found that *N. lepida* had similar hexobarbital clearance times to *N. stephensi*, and both species had clearance times that were 2–3 times faster than the generalist, *N. cinerea*, and 4–5 times faster than another generalist, *N. floridana*. In previous work, *N. lepida* exhibited longer hexobarbital clearance times and decreased consumption of juniper when given a P450 suicide inhibitor (Skopec et al. 2013), demonstrating that P450s play an integral role in biotransforming the PSCs present in juniper. Furthermore, juniper feeding *N. lepida* have differential expression of detoxification genes compared to populations that do not have access to juniper (Magnanou et al. 2009). *Neotoma lepida* also have pregastric fermentation chambers that may allow for better digestion of fibrous juniper or harbor bacteria that aid in the biotransformation of PSCs (Kohl et al. 2014a). Lastly, the cytochrome P450 2B enzymes of *N. lepida* differ with population and diet composition (Malenke et al. 2012). These CYP2B variants have different biochemical structures and

functional properties that could play a key role in the metabolism of juniper terpenes by *N. lepida* (Wilderman et al. 2014).

While both woodrat species performed similarly in the tolerance trials, their responses differed in the preference trials. In the preference trial where the woodrats were not exposed to juniper prior to the trial (“non-induced” trials), *N. stephensi* consumed more *J. monosperma*, the native juniper, than *J. osteosperma*, while *N. lepida* did not show a preference for its native juniper, *J. osteosperma*. The preference for native juniper, as well as higher juniper consumption overall, suggests that *N. stephensi* constitutively maintains higher levels of biotransformation enzymes when not on a toxic diet compared to *N. lepida*. Both species increased their overall food and juniper consumption when fed an increasing amount of juniper over three days before the trials (induced trials), indicating that both woodrats have inducible mechanisms to deal with the juniper consumption. Interestingly, *N. lepida* never demonstrated a preference for its native juniper, *J. osteosperma*, while *N. stephensi* exhibited a preference for the juniper species with which it was induced. We have taken this lack of preference as evidence that, unlike the obligate specialist *N. stephensi*, *N. lepida* is a facultative *J. osteosperma* specialist and as such acted like a generalist during the preference trials by trying to mix diets. Diet mixing is an important behavior to avoid overwhelming any particular biotransformation pathway with a high dose of a single PSC (Dearing and Cork 1999; Freeland and Janzen 1974). This lack of preference and inherent desire to diet mix may be why *N. lepida* is more flexible in terms of diet and habitat than the obligate specialist *N. stephensi*. *Neotoma stephensi*’s preference switching, i.e., preferred *J. monosperma* when induced with *J. monosperma* and preferred *J. osteosperma*

when induced with *J. osteosperma*, suggests that *N. stephensi* may utilize different inducible biotransformation enzymes for the terpenes in *J. monosperma* vs. *J. osteosperma*.

We speculate that the difference in the terpene profiles between *J. monosperma* and *J. osteosperma* may have led to a higher degree of specialization in *N. stephensi*. *Juniperus monosperma*'s terpene profile is dominated by a single terpene, α -pinene (60 % of total oil yield), while *J. osteosperma*'s has four different terpenes making up 50 % of the oil yield, α -pinene (10.5 %), sabinene (12.7 %), camphor (8.5 %), and bornyl acetate (16.1 %) (Adams et al. 2014). Additionally, *J. monosperma* exhibits extremely low variability in its terpene profile across its range (Adams 1994). Specializing on *J. monosperma* may have resulted in the evolution of unique enzymes in *N. stephensi* to be especially efficient at biotransforming a single secondary compound, α -pinene, whereas *N. lepida*'s specialization on *J. osteosperma* with its cocktail of terpenes may have led to *N. lepida* retaining more varied pathways for biotransforming terpenes as well as other types of plant secondary compounds (Wilderman et al. 2014).

Neotoma stephensi has one of the smallest ranges of any woodrat species and its range overlays that of its preferred plant species, *J. monosperma*, (Dial 1988; Vaughan and Czaplewski 1985). In contrast, *N. lepida* has one of the broadest ranges of any woodrat species and, as stated previously, has developed different dietary specializations throughout its range. As a facultative specialist, *N. lepida* may have broken the old adage “jack of all trades, master of none” and become a “jack of all trades, master of many”. A better understanding of the behavioral, physiological and microbial mechanisms it uses to master multiple dietary specializations will allow for better understanding of how a species can adapt to new and potentially novel environments.

Acknowledgments We thank Andrew Corbin for his adept technical assistance. Support for this research came from NSF (IOS 1256383 and IOS 1461359).

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