Small Mammal Response to Weed Treatment with Bayer Rejuvra™ Herbicide

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1.0 ABSTRACT

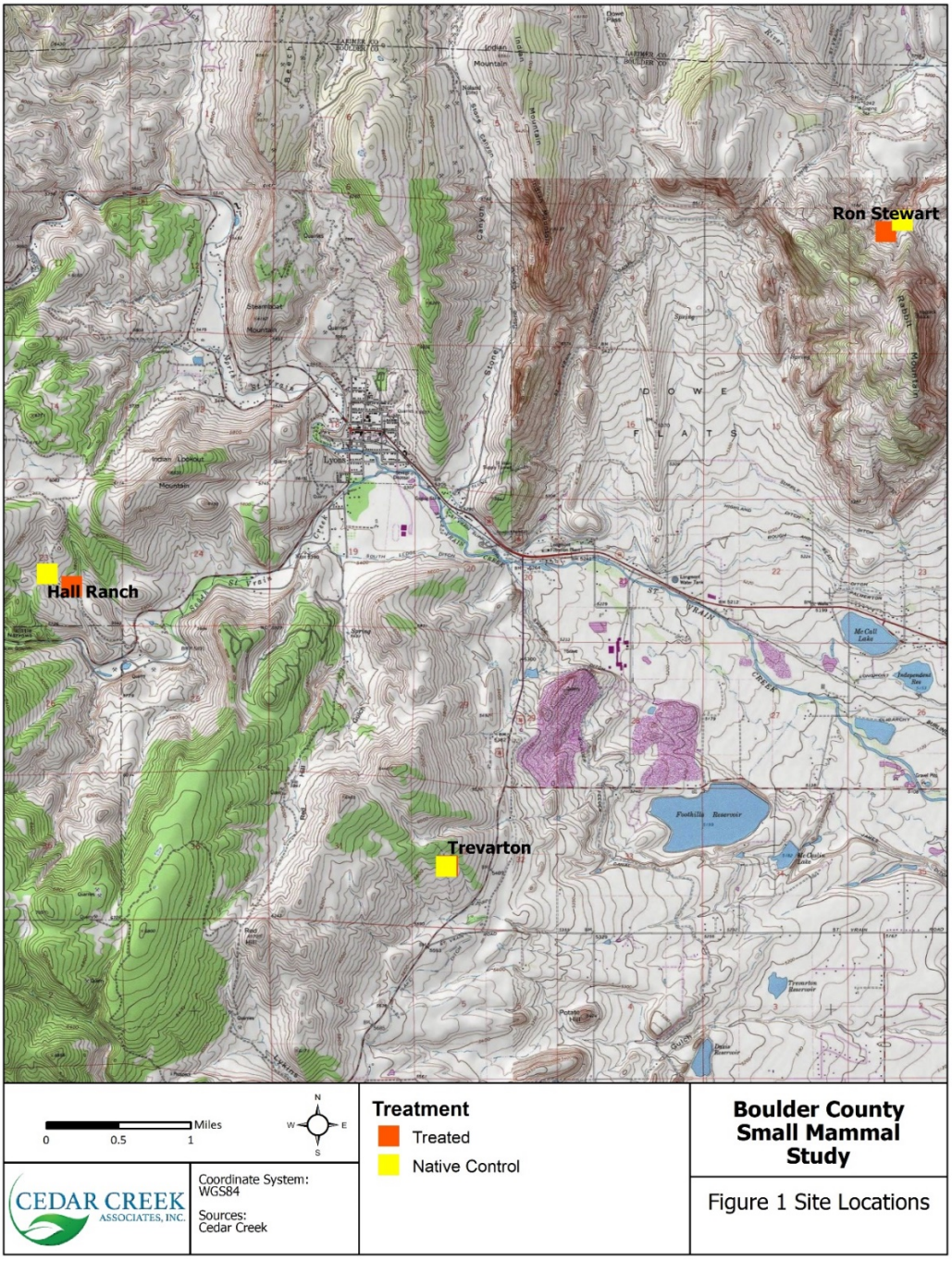
Bayer Rejuvra™ (indaziflam), a pre-emergent herbicide, has shown promising results in its ability to control cheatgrass (*Bromus tectorum*), an invasive annual grass that has altered landscapes and increased fire danger throughout the western United States. Previous studies have shown the efficacy of Bayer Rejuvra™(Rejuvra™) on vegetation, but non-target wildlife species impacts have not been thoroughly examined. This study aims to evaluate the effects of Rejuvra™ at a higher trophic level by studying the effects on small mammals. Live-trapping of small mammals was used to compare species richness and species diversity between native habitats and habitats restored with Rejuvra™. Trapping grids comprised of Sherman traps and Tomahawk traps were established at three sampling locations, with a paired native site and restored sites at each sampling location. Sites that were treated with Rejuvra™ displayed no significant impacts on small mammal diversity. This study's findings can be used to strengthen the understanding of Rejuvra's™ impacts on non-target small mammal species.

2.0 INTRODUCTION

Habitats dominated by cheatgrass (*Bromus tectorum*) decrease native species richness and diversity (Hall 2012, Freeman et al. 2014, Ceradini and Chalfoun 2017). A relatively new herbicide, Rejuvra™, is effective at controlling cheatgrass, thereby restoring native vegetation communities (Brabham et al. 2014, Sebastian et al. 2017, Clark et al. 2019). In restored habitats, when cheatgrass is removed, vegetation communities are returned to a more native state (Sullivan et al. 1998, Raybuck et al. 2012). Further study is needed into Rejuvra's™ effects on non-target species including other short-lived vegetation and wildlife. This study compared small mammal diversity and richness in native habitats unaffected by cheatgrass with habitats restored with Rejuvra™. The null hypothesis states that plots treated with Rejuvra™ have the same species diversity and/or species richness as native plots. The alternate hypothesis states that the presence of Rejuvra™ in

the environment impacts species diversity and/or species richness. If Rejuvra™ is shown to have no impact, it would further demonstrate the restoration value of this management tool on Boulder County Parks and Open Space (BCPOS) properties. Effectively restored lands exhibit greater ecological goods and services and habitat function to wildlife and visitors of BCPOS lands.

Map 1. Site Locations



3.0 MATERIALS AND METHODS

3.1 Study Sites

Study sites were selected in collaboration with BCPOS biologists to identify treatment and control locations. Treatment sites consisted of habitat where Rejuvra™ herbicide had been applied. Each treatment site was provided equal time for Rejuvra™ to control cheatgrass and for the native vegetation community to return (Freeman et al. 2014). Control sites were within habitat that have not been treated with Rejuvra™, or any other known herbicide. These sites were selected based on their close resemblance to a native, unaltered vegetation community. Treatment and the paired native control site for each sampling location had similar elevations, aspects, slopes, and habitat types to control for environmental variables. Each study site that was selected was located within a BCPOS property (Map 1) and was within an upland grassland/shrubland vegetation community. These areas within BCPOS properties are most heavily affected by cheatgrass inundation and were areas that could be accessed by a tractor with a spray attachment. Maps and descriptions of each study site's vegetation were recorded and are presented below.

3.1.1 Ron Stewart Preserve at Rabbit Mountain (Map 2):

Dominant vegetative cover at the treated site consisted of perennial grasses and native shrubs. The dominant grasses observed were blue grama (*Bouteloua gracilis*), western wheatgrass (*Pascopyrum smithii*) and needle and thread grass (*Hesperostipa comata*). Dominant shrubs included native species such as skunkbush (*Rhus trilobata*), yucca (*yucca glauca*), fringed sage (*Artemisia frigida*), and broome snakeweed (*gutierrezia sarothrae*).

Dominant vegetative cover at the native control site consisted of perennial grasses and native shrubs. Similarly, the dominant grasses were blue grama and western wheatgrass. The dominant forb was the annual species, *Alyssum sp.,* which was not observed in the treated site. Dominant shrubs included skunkush, yucca and fringed sage. Isolated patches of the noxious weed mullein (*Verbascum thapsus*) were also present.

3.1.2 Trevarton (Map 3):

Dominant vegetative cover at the treated site consisted of perennial grasses and native shrubs. The dominant grasses included western wheatgrass, needle and thread grass and smooth brome (*Bromus inermis*). Dominant shrubs were skunkbush, yucca, winterfat (*Krascheninnikovia lanata*) and prickly pear cactus (O*puntia polyacantha*). Native forbs such as prairie sage (*Artemisia ludoviciana*) and goldenaster (*Heterotheca villosa*) were also present. Portions of the native control site had isolated stands of ponderosa pine (*Pinus ponderosa*) trees.

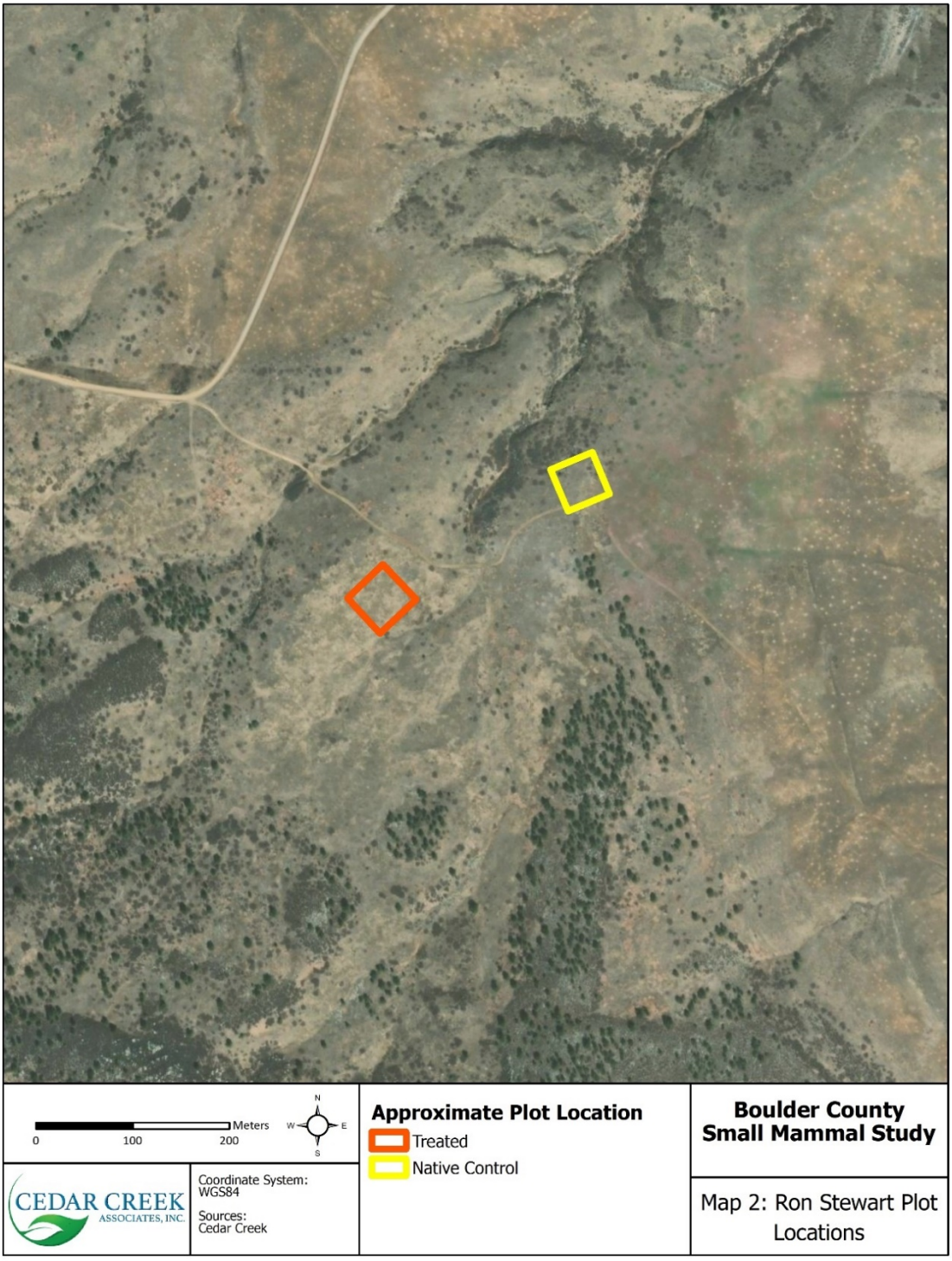
Dominant vegetative cover at the native control site consisted of perennial/annual grass, annual forbs and native shrubs. Dominant grasses included blue grama, western wheatgrass and cheatgrass with the annual forb *Alyssum sp.* contributing to overall cover. The dominant shrubs included native species such as skunkush, broome snakeweed, yucca and prickly pear cactus. Portions of the native control site had isolated stands of ponderosa pine trees.

3.1.3 Hall Ranch (Map 4):

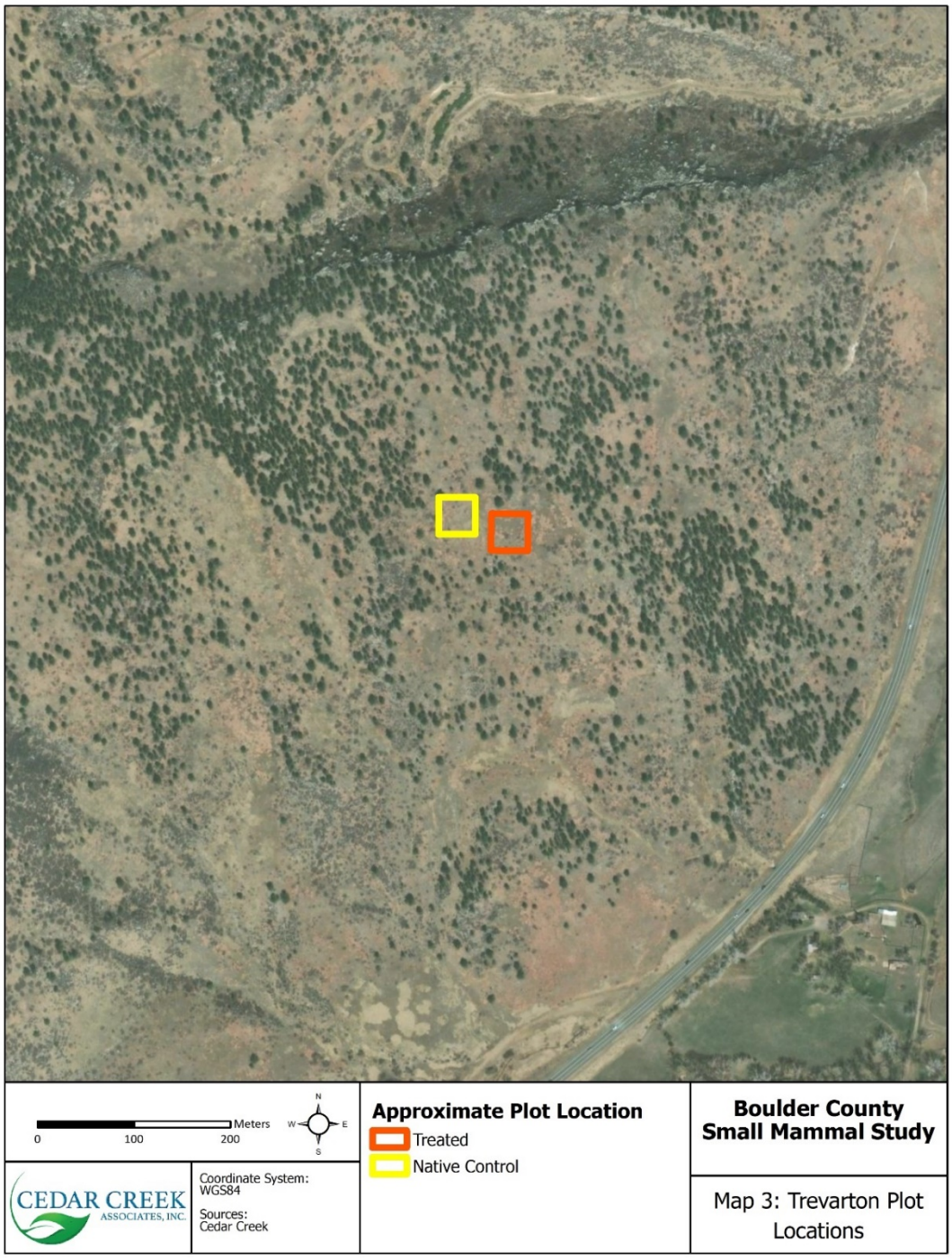
Dominant vegetative cover at the treated site consisted of perennial grasses and native shrubs. Dominant grasses were *Poa sp*., western wheatgrass, and Canada bluegrass (*Poa compressa*). Dominant shrubs were skunkbush, yucca, winterfat (*Krascheninnikovia lanata*) and prickly pear cactus (O*puntia polyacantha*). Native forbs such as prairie sage (*Artemisia ludoviciana*) and goldenaster (*Heterotheca villosa*) also contributed to overall cover. Dominant shrubs included native species such as fringed sage, fragrant sumac, and prickly pear cactus.

Dominant vegetative cover at the native control site consisted of perennial grasses, annual grasses/forbs, and native shrubs. The dominant grasses were cheatgrass, blue grama and *Poa sp*. The dominant forb was the annual species *Alyssum sp*., at a much higher percent cover than the treated stie. Dominant shrubs included native species including rabbitbrush (C*hrysothamnus viscidiflorus*), fringed sage and prickly pear cactus. Bordering the native control site were stands of ponderosa pine.

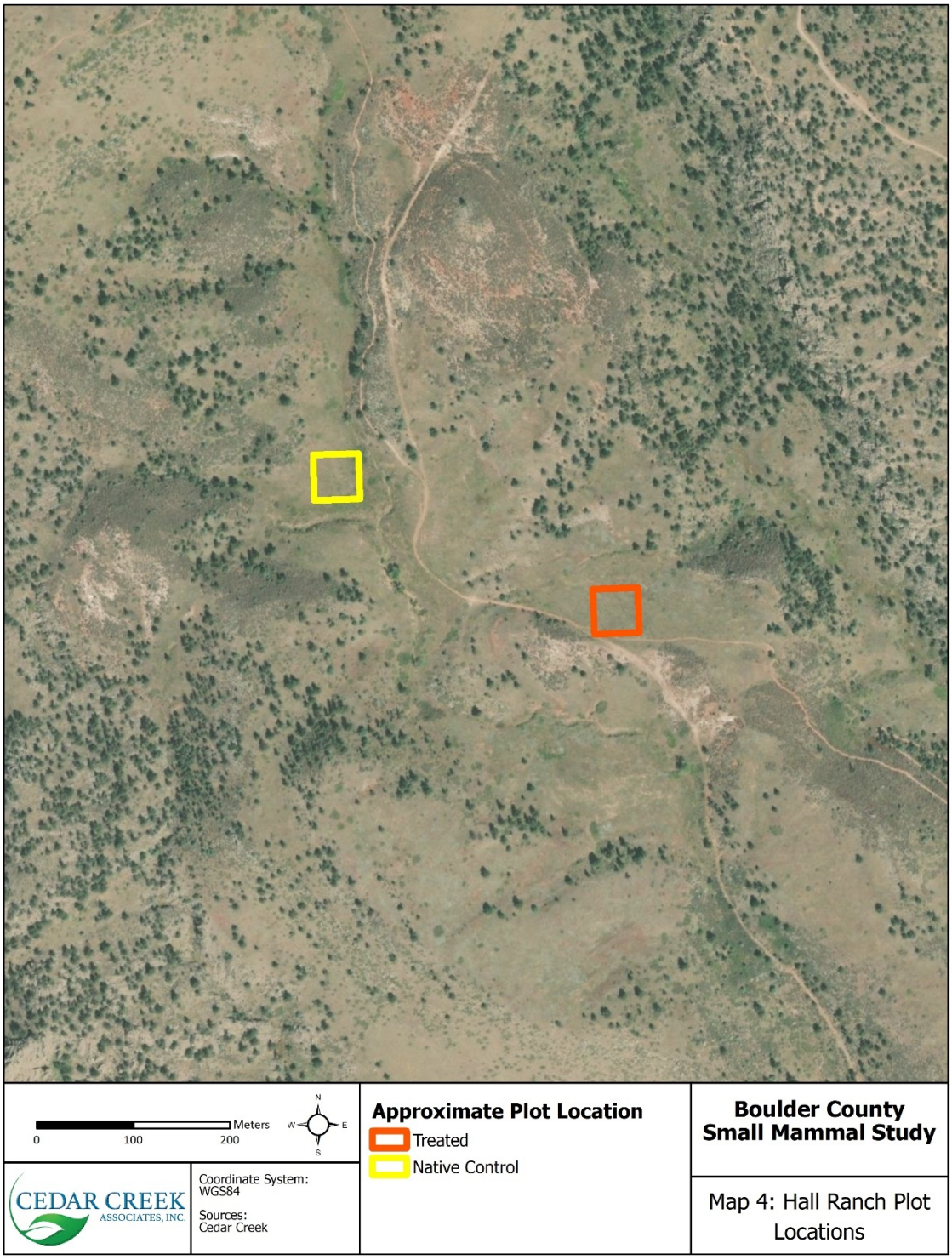
Map 2. Ron Stewart Preserve at Rabbit Mountain Plot Locations



Map 3. Trevarton Plot Locations



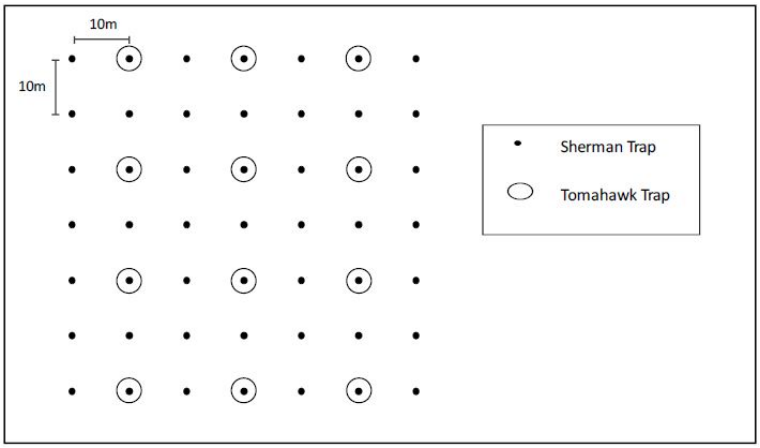
Map 4. Hall Ranch Plot Locations



3.2 Methods

Three trapping efforts occurred on BCPOS properties throughout the study: Rabbit Mountain June 22-24th, Trevarton July 29-31st, and Hall Ranch August 25-27th. Each study site consisted of a 60-meter x 60-meter trapping array with a Sherman trap (3 x 3 x 9”) spaced at 10-meter intervals (n_{trap}=49) (Figure 1). A Tomahawk trap (5 x 5 x 16”) was placed at 20-meter intervals (n_{trap}=12). One trap night was defined as a single trap set for one night. Each array consisted of 61 trap nights per night. Trapping occurred for three consecutive nights at each site. All traps were baited with a mixture of rolled oats, peanut butter, molasses, and mixed seeds. Additionally, wool batting was added to Sherman and Tomahawk traps in cold temperatures to provide insulation to mitigate potential exposure mortalities. Traps were not set during heavy rain or when temperatures are at or below 0°C (32°F). All trapped animals were processed at the site, marked with non-toxic nail polish to verify subsequent recapture, and released at their exact capture location. If captured individuals had been previously marked with nail polish, they were classified as recaptures. Traps were baited and opened just before sunset and checked the following morning at sunrise. All trapped animals were released within two hours of sunrise to avoid extended exposures. One paired sampled site was evaluated during each trapping effort. Data collected included species, age (adult or juvenile), sex, breeding status, and weight. Measurements such as body, tail, hind foot, and ear length were also collected for species identification.

Figure 1. Trapping Array



3.3 Analysis

Species richness and Shannon-Wiener diversity indices were calculated, and a Hutcheson t-test was used to compare species diversity indices between treated and native sites (Hutcheson 1970). The Hutcheson t-test was developed as a method to compare the diversity of two samples using the Shannon-Weiner diversity index. The Hutcheson t-test and Shannon-Weiner diversity value is calculated using the following equation:

Hutcheson t-test

$$t = \frac{H_a - H_b}{\sqrt{s^2_{H_a} + s^2_{H_b}}}$$

H = Shannon diversity index from two communities (subscript *a, b*);
s^2 = variance of the Shannon diversity index;

Shannon-Weiner Diversity Index

$$H' = \sum_{i=1}^{s} (p_i)(\ln p_i)$$

p = proportion of total sample represented by species;
s = number of species;

4.0 RESULTS

Sites treated with Rejuvra™ displayed no statistically significant impacts on small mammal diversity while demonstrating an observable effect on species richness (Table 1, Table 2, Table 4). Across all sites, a total of six small mammal species and 46 individuals were captured over 1,098 trap nights during the study. Within treated sites, 26 individuals were captured, while 20 individuals were captured in the native sites. Both sites showed similarities in species composition and total captures (Table 3). For treated sites, the most common species captured was Mexican woodrat (*Neotoma Mexicana*) with 10 individuals captured, while for native sites deer mouse (*Peromyscus maniculatus*) was the dominant species with 12 individuals captured. (Table 1, Table 2). No mortalities were recorded during the study. The Trevarton sites had the most individuals trapped with 27, accounting for 59% of the study's total catch. The Hall Ranch and Rabbit Mountain sites had lower productivity with 11 and eight individuals trapped accounting for 24% and 17% of the study's total catch, respectively (Table 3).

Table 1. Native Control Demographics

Common name	Scientific name	Individuals Captured	Age			Sex		Breeding Status			Average Weight (g)
			Juvenile	Sub-Adult	Adult	Male	Female	non-breeding	testes enlarged	lactating	
Deer mouse	*Peromyscus maniculatus*	12	2	1	9	5	7	10	-	2	17.3
Mexican woodrat	*Neotoma mexicana*	6	2	-	4	5	1	4	1	1	100+
Olive-backed pocket mouse	*Perognathus fasciatus*	1	-	-	1		1	1	-	-	23.0
Western harvest mouse	*Reithrodontomys megalotis*	1	-	-	1	1		1	-	-	12.0
Total (# of individuals)		**20**	**4**	**1**	**15**	**11**	**9**	**16**	**1**	**3**	**-**
Percent (% of total)		**-**	**20%**	**5%**	**75%**	**55%**	**45%**	**80%**	**5%**	**15%**	**-**

* Calculated on first capture only.

Table 2. Treated Demographics

Common name	Scientific name	Individuals Captured	Age			Sex		Breeding Status			Average Weight (g)
			Juvenile	Sub-Adult	Adult	Male	Female	non-breeding	testes enlarged	lactating	
Deer mouse	*Peromyscus maniculatus*	9	1	2	6	6	3	7	1	1	17.9
Hispid pocket mouse	*Chaetodipus hispidus*	2	-	-	2	2	-	2	-	-	68.3
Long-tailed vole	*Microtus longicaudus*	1	-	-	1	-	1	-	-	1	32.5
Mexican woodrat	*Neotoma mexicana*	10	2	-	8	5	5	5	2	3	100+
Olive-backed pocket mouse	*Perognathus fasciatus*	4	-	-	4	1	3	4	-	-	34.5
Total (# of individuals)		**26**	**3**	**2**	**21**	**14**	**12**	**18**	**3**	**5**	**-**
Percent (% of total)		**-**	**12%**	**8%**	**81%**	**54%**	**46%**	**69%**	**12%**	**19%**	**-**

* Calculated on first capture only.

Table 3. Trapping Summary

Common name	Scientific name	Rabbit Mountain (6/22-6/24)				Trevarton (7/29-7/31)				Hall Ranch (8/25-8/27)			
		Treated		Native Control		Treated		Native Control		Treated		Native Control	
		Captures	Recaptures	Captures	Recaptures	Captures	Recaptures	Captures	Recaptures	Captures	Recaptures	Captures	Recaptures
Deer mouse	*Peromyscus maniculatus*	3	1	2	-	4	4	4	6	2	-	6	4
Hispid pocket mouse	*Chaetodipus hispidus*	1	1	-	-	1	-	-	-	-	-	-	-
Long-tailed vole	*Microtus longicaudus*	1	-	-	-	-	-	-	-	-	-	-	-
Mexican woodrat	*Neotoma mexicana*	1	-	-	-	8	5	5	4	1	-	1	-
Olive-backed pocket mouse	*Perognathus fasciatus*	-	-	-	-	4	1	1	-	-	-	-	-
Western harvest mouse	*Reithrodontomys megalotis*	-	-	-	-	-	-	-	-	-	-	1	-
Species Diversity (# of species observed)		**4**		**1**		**4**		**3**		**2**		**3**	
Total Captures		6	2	2	0	17	10	10	10	3	0	8	4
Total Trapped (Captures and Recaptures)		**8**		**2**		**27**		**20**		**3**		**12**	

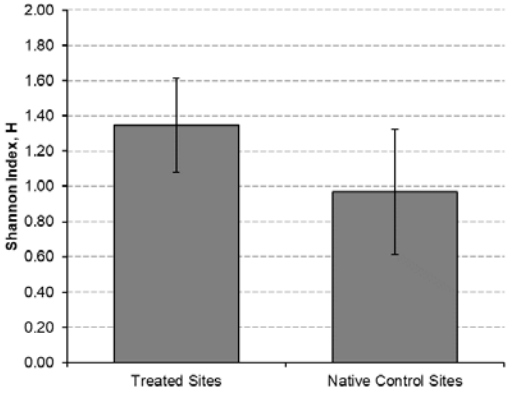
4.1 Species Richness

Species richness for treated and native sites was five and four species, respectively (Table 1, Table 2). A direct comparison of species richness reveals treated sites contained one more species than the native sites. Both treated and native sites supported deer mouse, Mexican woodrat, and olive-backed pocket mouse (*Perognathus fasciatus*) species. The hispid pocket mouse (*Chaetodipus hispidus*) and long-tailed vole (*Microtus longicaudus*) were only captured in the treated sites, while Western harvest mouse (*Reithrodontomys megalotis*) was only captured in the native sites (Table 1, Table 2).

4.2 Shannon-Wiener Diversity Index

The Shannon-Wiener diversity index for the treated sites was calculated at 1.35 compared with 0.97 for the native control sites (Figure 2). This number is a unitless, quantitative measure that reflects the number of different species and how evenly the individuals are distributed among those species within a sample. The value of a diversity index increases when the number of species increases and the evenness increases. Therefore, the treated sites exhibited a marginally higher diversity index than the native control. Additionally, treated sites diversity was more even than native control sites. The most common species within the treated sites accounted for 38% of the total individuals captured while the most common species captured in the native sites accounted for 60% of the total individuals (Table 1, Table 2)

Figure 2. Shannon-Wiener Diversity Index



4.3 Hutcheson t-test

After the calculation of the diversity indices, the Hutcheson t-test was used to assess statistical significance of the findings. Table 4 displays the results of the Hutcheson t-test:

Table 4. Hutcheson t-test Results

Hutcheson t-test	
t=	1.705249201
Degrees of Freedom=	39.31865136
Critical Value=	2.02269092
t<Crit	Fail to reject Null

The t-value calculated with the Hutcheson test does not exceed the critical value, thus confirming the results are not statistically significant. This failure to reject the null hypothesis indicates the data did

not provide sufficient evidence to show weed treatment with Rejuvra® had significant impact on the diversity of small mammal populations.

5.0 DISCUSSION

Overall, our findings suggest that the incorporation of targeted action Rejuvra® within BCPOS properties does not have a significant impact on small mammal diversity. There was a greater species richness and evenness observed within treated sites, however our sampling was not robust enough to demonstrate this statistically. Additionally, both treated and native sites had similar percentages of non-adult individuals (20%, 25%, respectively) (Table 1, Table 2) suggesting Rejuvra® does not substantially impact the fecundity or fertility of breeding adults, however supplementary investigation is necessary.

6.0 LITERATURE REVIEWED

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