

MORPHOLOGICAL DIFFERENCES AND DIVERSITY OF SMALL MAMMAL SPECIES

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ABSTRACT

Small mammals play important roles in ecosystems, but are often overlooked due to their small size and plentiful abundance. Two studies evaluated the morphological differences and diversity of small mammal species based on how they react to different environmental conditions. The first study analyzed the morphological differences of skulls between and within two species of *Peromyscus* species due to differences in shape, size, and geographic position. This study also determined if landmark-based geometric morphometrics could reliably discriminate between the two species. The second study analyzed small mammal diversity and abundance due to differences in microhabitat conditions in an endangered ecosystem: tallgrass prairie. Small mammals are an important food source to other vertebrate and also help regulate vegetation composition in the tallgrass prairie ecosystem. Results of both studies could help future studies identify how different variables affect small mammal morphology and diversity.

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PREFACE

Small mammals play an important role in ecosystems. In the upper mid-west they are a major food source for other species, such as the red fox (*Vulpes vulpes*), the red-tailed hawk (*Buteo jamaicensis*), and the great-horned owl (*Bubo virginianus*). They also alter vegetation by consuming plant matter and dispersing seeds throughout the habitat. But small mammals are often overlooked, due to their small size and vast abundance in number. There is a lot that can be learned by studying mammals from not only an individual species perspective but also small mammal community dynamics. Within this research project, both species and community perspectives were studied.

The first study examined differences between two very similar species. The white-footed mouse (*Peromyscus leucopus*) and the deer mouse (*Peromyscus maniculatus*) are two of the most widespread members of the genus *Peromyscus* in North America. *P. leucopus* and *P. maniculatus* are difficult to distinguish from one another because of their remarkable similarities in appearance. Thus, the goals of this study were to 1) describe how skull shape morphology of *P. maniculatus* and *P. leucopus* in the upper-Midwest of the United States is influenced by differences in species, sex, location, and size 2) describe how skull size is influenced by differences in sex and location, and 3) determine if geometric morphometrics can reliably discriminate between *P. maniculatus* and *P. leucopus* species based on skull shape and size.

The second study focused on the community dynamics perspective of the small mammal community in an endangered ecosystem: tallgrass prairie. Population fluctuations within small mammal communities have been shown to affect energy levels and nutrient transfer through food webs, ultimately affecting the overall energy and population dynamics of an ecosystem (Wike et al. 2000). The purpose of this study was to survey small mammal populations in the Sheyenne

National Grasslands in order to better understand how small mammal diversity and abundance are impacted due to differences in local habitat and grazing in tallgrass prairie. The results could allow for a better understanding of small mammal presence and abundance in not only one of the world's most endangered ecosystems but also the largest publically owned tallgrass prairie reserve in the United States.

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CHAPTER 1. MORPHOLOGICAL VARIATION WITHIN AND BETWEEN PEROMYSCUS SPECIES

Abstract

The white-footed mouse (*Peromyscus leucopus*) and the deer mouse (*Peromyscus maniculatus*) are two of the most widespread members of the genus *Peromyscus* in North America. *P. leucopus* and *P. maniculatus* are difficult to distinguish from one another because of their remarkable similarities in appearance. We used landmark-based geometric morphometric techniques on species skulls to determine how skull shape morphology differs within and between the species due to differences in species, sex, location, and size. We analyzed 232 specimens that were collected from North Dakota and Minnesota that were a part of the skull collection at North Dakota State University. Our results demonstrated skull shape and size differences due to species, size, location, and sex. Shape change was most apparent along the maxilla, zygomatic arch, and braincase regions of the skulls, with our predictor variables describing only a small percentage of total shape change seen. *P. leucopus* exhibited larger skull sizes than *P. maniculatus* throughout the collection and skull sizes of both species increased with increases in latitude. The results of a discriminant function analysis also suggest that the use of geometric morphometrics to distinguish between *P. leucopus* and *P. maniculatus* was only mildly successful, with a total success rate of 81%. Landmark placements or misidentification in the field could have contributed to the less than 100% success rate. Overall, we were able to identify significant variation between and within species using geometric morphometrics but geometric morphometric measures were not sufficient for distinguishing between species.

Introduction

The white-footed mouse (*Peromyscus leucopus*) and deer mouse (*Peromyscus maniculatus*) are two of the most widespread members of the genus *Peromyscus* in North America (Robbins et al. 1983). According to fossil data, *P. leucopus* and *P. maniculatus* diverged around the mid Pleistocene, approximately 500,000 years ago (Hibbard 1968) with molecular data suggesting that divergence occurred ~2.5 million years ago (Platt et al. 2015). While morphologically similar, these species have not been shown to interbreed (Dice 1933) but both are primarily nocturnal species and contribute to ecosystems in similar ways by serving as a food source to predators and also as consumers and dispersers of plant seeds. *P. leucopus* and *P. maniculatus* are also very similar in appearance and are commonly confused with one another, especially when attempting to identify in the field (Bruseo et al. 1999).

There have been many attempts to identify the two species based on external measurement and coloring characteristics (e.g. Feldhamer et al. 1983, Kamler et al. 1998, Bruseo et al. 1999, Stephens et al. 2014), but due to geographical variation within species (Blair 1950, Choate 1973, Aquadro and Patton 1980) and overlap in external characteristics it is recommended to use electrophoresis of salivary amylase or DNA analysis to confirm species identification (Aquadro and Patton 1980). For example, from external measurements, Feldhamer et al. (1983) reported a 76.7% success rate based on tail length and head/body ratio and Rich et al. (1996) found that coloration and morphological measurements successfully identified specimens correctly in only 55% of cases. Lindquist et al. (2003) reported an 80% successful identification rate based off of 4 external measurements of ear, hind-foot, tail, and tail/head-body for adults in New York populations and stated that genetic analysis remained the only reliable field method for differentiation of the two species.

The two species can also be difficult to distinguish as preserved specimens. Choate (1973) and Rich et al. (1996) were among many who developed identification techniques based on traditional morphometric measurements of the skull. Both Choate (1973) and Rich et al. (1996) used discriminate function analysis of skull morphometric measurements to correctly categorize specimens, with 100% success in different populations in Northeast North America. However, these discriminate functions were not generalizable with Choate's (1973) function, correctly categorizing specimens as *P. leucopus* or *P. maniculatus* less than 66% of the time when applied to a separate set of specimens (Rich et al. 1996).

The use of geometric morphometric techniques to differentiate species of preserved *Peromyscus* specimens is a less explored topic and may reveal additional information about species differences. Geometric morphometrics differs from traditional morphometric measurements—like those used by Choate (1973) and Rich et al. (1996)—in that it quantifies the shape of an object after the effects of nonshape variation (position, orientation, and scale) have been mathematically held constant (Rohlf and Slice 1990). This technique focuses on the coordinates of landmarks and the geometric information about their relative position to describe shape (Adams et al. 2004). Unlike traditional morphometrics, which focus on linear distance measurements, geometric morphometrics are preferable because they retain the geometry of shape throughout the analysis while providing independent shape variables not related to size (Rohlf and Marcus 1993). Although size is mathematically held constant in geometric morphometrics, it is still recorded for each specimen before shape is analyzed. The use of geometric morphometrics may improve phenotypic discrimination of *Peromyscus* due to this ability to independently quantify shape and size. For example, Breno et al. (2011) found geometric morphometrics to be more sensitive than traditional morphometric methods in

detecting variation in skull morphology within a population of *Mastomys natalensis*. Likewise, Maderbacher et al. (2008) found geometric morphometrics to be more powerful than traditional morphometrics in identifying population differences in cichlids (*Tropheus moorii*).

Geometric morphometrics has also been shown to be sufficiently powerful to solve complex taxonomic problems within species (e.g. (Villemant et al. 2007, Dehon et al. 2014). For example, Dehon et al. (2014) used geometric morphometrics to assess the similarity of forewing shape between extant and fossil taxa and Villemant et al. (2007) applied geometric morphometrics to wing venation to assess a complex case of sibling species in the genus *Eubazus* (Hymenoptera, Braconidae).

Rodent cranial variation has also been intensively explored using geometric morphometrics (Samuels 2009, Scalici and Panchetti 2011, Yazdi et al. 2012, Yazdi and Adriaens 2013). Cranial morphology is particularly important as skulls have been shown to be sensitive to environmental demands and to respond to environmental change and selective pressures in rodents (Pergams and Ashley 2001, Pergams and Lawler 2009). For example, Yazdi and Adriaens (2013) were able to use geometric morphometrics to find species specific morphological variation due to shape and size differences among four closely related and sympatrically occurring species of jirds (genus *Meriones*). These jirds exhibited large variation in the size of the tympanic bulla which it was suggested reflected differences in hearing performance among species. Similarly, Samuels (2009) was able to use geometric morphometrics to interpret variation in skull shape morphology amongst rodents due to dietary preferences, showing that living rodents with similar diets display convergent morphology, despite their independent evolutionary histories.

Understanding how shape morphology changes between the two *Peromyscus* species might help not only to discriminate between the two species, but also help clarify ecological and evolutionary differences within and between populations and species. Thus, the goals of this study were to 1) describe how skull shape morphology of *P. maniculatus* and *P. leucopus* in the upper-Midwest of the United States is influenced by differences in species, sex, location, and size 2) describe how skull size is influenced by differences in sex and location, and 3) determine if geometric morphometrics can reliably discriminate between *P. maniculatus* and *P. leucopus* species based on skull shape and size.

Methods

For this study 193 specimens collected from North Dakota (USA) and 39 collected from Minnesota (USA) for a total of 232 specimens were analyzed. Specimens were obtained from the North Dakota State University collection and were collected from 1948 to 1991 throughout North Dakota and Minnesota. The *Peromyscus* specimens used in this study were categorized as 179 *P. maniculatus* and 53 *P. leucopus*, with species assignment occurring at the time of capture. Specimen labels did not include subspecies identification. Geographic distribution of samples was biased, with most specimens being from Cass (ND), Clay (MN), and Ransom (ND) counties (Figure 1.1).

Landmark-based geometric morphometric techniques were used to determine shape variation within and between the *Peromyscus* species. For each skull 2 images were recorded: one from the ventral view of the skull and another from the lateral view (left side of skull). A Nikon Coolpix P510 camera mounted to a fixed platform was used to capture the skull images. Error was minimized by placing the skulls on a custom made clay mount while taking the pictures to help ensure that all skulls positions were as consistently placed as possible.

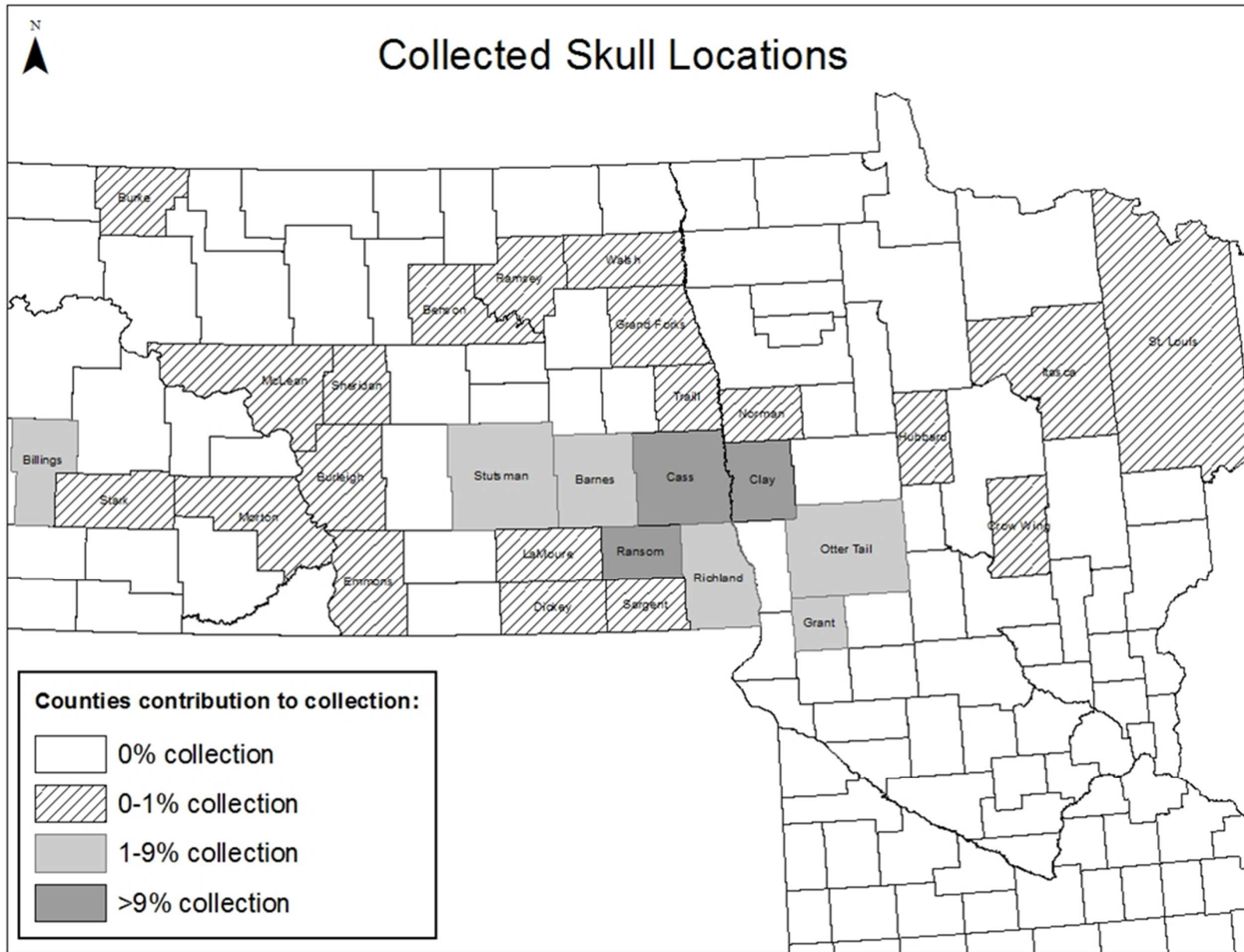


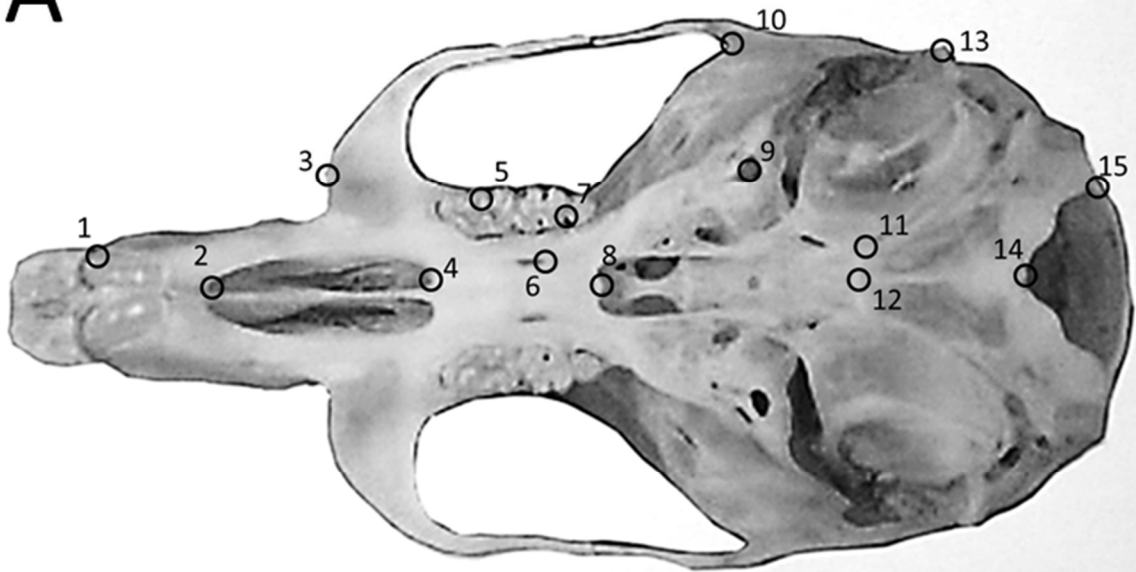
Figure 1.1. Original locations of skull specimens from North Dakota and Minnesota that were used in this study.

Once the images were captured landmarks were placed on the skulls using tpsDIG2.12 (Rohlf 2004). Fifteen landmark positions were chosen for the ventral view along with sixteen for the lateral view (Figure 1.2, Table 1.1). Placements of landmarks on the skulls were based on previous studies with *Peromyscus* (Myers et al. 1996, McPhee 2004, Grieco and Rizk 2010), and based on easily identifiable structures on the skull that were found on every specimen (Figure 1.2). Descriptions of landmark locations are presented in Table 1.1. Once landmarks were placed there were 232 individual landmark configuration pairs (ventral and lateral).

After the landmark configurations were complete configurations were analyzed by the use of General Procrustes Analysis (GPA) using tpsRelw (Rohlf 2007) to calculate relative-warp (RW) values and centroid size measurements for each skull configuration. RWs are principal components of partial warp scores and here were weighted to emphasize bending energy, while centroid size is the size measure used in geometric morphometrics that is uncorrelated with shape and controls for allometry (Zelditch et al. 2012). Three separate GPA's were performed in this study: one for the entire data set (GPA1), one for only *P. maniculatus* (GPA2), and one for only *P. leucopus* (GPA3). The morphological shape variation of the skull described by each RW was visually assessed via thin-plate splines generated with tpsRelw.

Products of landmark-based geometric morphometric analyses of skull shape and size were analyzed using multivariate analysis of (co)variance (MANCOVA), and subsequent analysis of (co)variance (ANCOVA) and discriminate function analysis (DFA) (Quinn and Keough 2002). Separate MANCOVAs were performed for each view (2) to correspond to each of the three GPA's performed to test for the impacts of our predictor variables (species, sex, latitude, longitude, and centroid size) on skull shape (RWs). Statistical significance in MANCOVAs was determined based on F values and P values. Subsequent ANCOVAs were

A



B

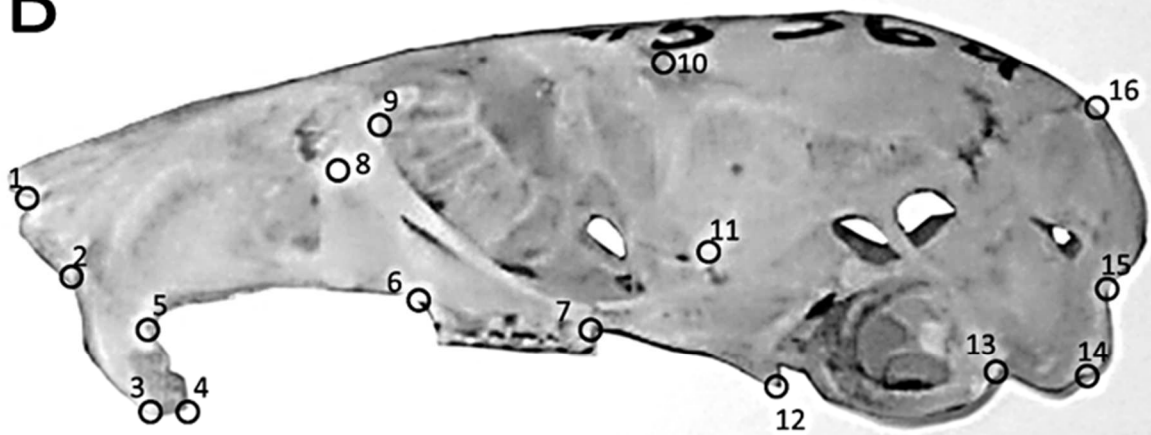


Figure 1.2. Landmark placements on specimen skulls for ventral (A) and lateral (B) views. Locations were chosen based off previous studies and also the availability of consistent identification by the author across each specimen.

Table 1.1. Landmark position descriptions for ventral and lateral views used in this study.

Ventral view	Landmark Description Source
1. Junction of anterior lateral margin of incisor with premaxillary	Greico and Rizk 2010
2. Anterior end of incisive foramen	Greico and Rizk 2010
3. Anterior end zygomatic arch anteriormost curve	This paper
4. Posterior end of incisive foramen	Greico and Rizk 2010
5. Tip of paracone of first molar (if worn, roughly in the center of cusp outline)	Greico and Rizk 2010
6. Posterior palatine foramen	Greico and Rizk 2010
7. Anterolingual margin of third molar where it meets right second molar	Greico and Rizk 2010
8. Anteriormost point of mesopterygoid fossa	Greico and Rizk 2010
9. Middle of foramen ovale	McPhee 2004
10. Interior corner formed by intersection of zygomatic arch with braincase	Greico and Rizk 2010
11. Point formed by intersection of bulla with suture between basioccipital and basisphenoid	Greico and Rizk 2010
12. Midpoint of suture between basioccipital and basisphenoid	Greico and Rizk 2010
13. Outermost edge of auditory bulla (tympanic bone)	This paper
14. Midpoint of suture between basioccipital and basisphenoid	McPhee 2004
15. Interior corner at the posterior intersection of the foramen magnum and basioccipital	McPhee 2004

Table 1.1. Landmark position descriptions for ventral and lateral views used in this study (continued).

Lateral view	
1. Antermost point of premaxillary	Greico and Rizk 2010
2. Point at which anterior face of incisor emerges from premaxillary	Greico and Rizk 2010
3. Tip of incisor	Greico and Rizk 2010
4. Point at which posterior face of incisor emerges from premaxillary	Greico and Rizk 2010
5. Dorsal extent of cutting edge of incisor	Greico and Rizk 2010
6. Anterior edge of first molar where it emerges from maxillary	Greico and Rizk 2010
7. Posterior edge of third molar where it emerges from maxillary	Greico and Rizk 2010
8. Point at which ventral margin of zygomatic arch meets zygomatic plate	Greico and Rizk 2010
01 9. Innermost anterior point of zygomatic arch below meeting of zygomatic arch and frontomaxillary portion of cranium	This paper
10. Foramen located in frontal along dorsal margin of orbit	Myers et al. 1996
11. Postermost extension of temporal fossa	Greico and Rizk 2010
12. Postermost tip of pterygoid process	Greico and Rizk 2010
13. Antermost point of curve in between auditory bulla (tympanic bone) and occipital condyle	This paper
14. Upper most point on curve of occipital condyle	This paper
15. Indentation in posterior outline of braincase above the basioccipital	McPhee 2004
16. Outer most point of curve behind where Midsagittal point on interparietal/occipital suture	This paper

Descriptions based on those found in: Greico and Rizk 2010; McPhee 2004; and Myers et al. 1996.

used on individual RWs when significant findings were identified by MANCOVA to determine impact of predictor variables on specific skull features. Only the first RW for which a significant variable was identified by ANCOVA was used to describe morphological change. The first RW identified was focused on as it captured the most morphological variation. MANCOVAs and ANCOVAs were similarly used to examine the effects of our predictor variables on skull size using centroid size estimates.

DFA's were used to discriminate species based on shape and size products provided from the study. DFA was performed on the entire data set, for which sample sizes were uneven (*P. maniculatus* 179; *P. leucopus* 53). To test for potential bias due to uneven sample sizes, 1000 DFA simulations were performed with equal sample sizes for the two species. Each simulation randomly selected 53 *P. maniculatus* from the 179 available. Mean and median scores from those simulations were then examined to determine impact of uneven sample sizes.

Results

Shape

The first five RWs explained a combined 60% of the total ventral and lateral shape variation within each GPA (Table 1.2), while subsequent RW's explained very little shape variation (~0-6%). Morphological variation captured by each RW was interpreted based on visual examination of thin-plate splines, with morphological change being recorded along each RW for each of the five RWs analyzed.

Morphological Descriptions of Relative Warps

Morphological variation associated with the relative warps were similar for all three GPAs (Table 1.2): For the ventral view RW1 corresponded to an overall broadening/narrowing of the total skull width in all three GPAs. Likewise, in the lateral view, RW1 corresponded to an

Table 1.2. Percent variance explained by each RW, and the corresponding morphological change, for each of the three GPA 's.

GPA Morphological Results				
GPA	View	RW	Variance %	Description
Combined	Ventral	RW1	19.30%	broadening/narrowing of the total skull width
		RW2	14.20%	shift in the position of the anterior portion of the jugal and also an expansion/contraction of the incisive foramen length
		RW3	10.20%	shift in the position of the anterior portion of the jugal and also an expansion/contraction of the incisive foramen length
		RW4	8.90%	a lateral shift in the palatine region
		RW5	7.60%	broadening/narrowing of the maxilla
	Lateral	RW1	23.10%	expansion/contraction of the braincase region of the skull, causing skull to shift from concave to convex appearance
		RW2	15.80%	expansion/contraction of the braincase length while correspondingly contraction/expansion of the maxilla width
		RW3	10.30%	vertical shift in position of the zygomatic process of squamosal
		RW4	6.90%	lateral shift in position of the zygomatic process of squamosal
		RW5	6.60%	vertical shift in the position of the zygomatic process of squamosal and broadening/narrowing of the width of the maxilla region
<i>P.maniculatus</i>	Ventral	RW1	18.21%	broadening/narrowing of the total skull width
		RW2	14.62%	shift in the position of the anterior portion of the jugal and also an expansion/contraction of the incisive foramen length
		RW3	10.01%	shift in the position of the anterior portion of the jugal and also an expansion/contraction of the incisive foramen length
		RW4	9.15%	a lateral shift in the palatine region
		RW5	7.70%	broadening/narrowing of the maxilla
	Lateral	RW1	14.96%	expansion/contraction of the braincase region of the skull, causing skull to shift from concave to convex appearance
		RW2	11.58%	expansion/contraction of the braincase length while correspondingly contraction/expansion of the maxilla width
		RW3	10.01%	vertical shift in position of the zygomatic process of squamosal
		RW4	8.47%	lateral shift in position of the zygomatic process of squamosal
		RW5	7.82%	vertical shift in the position of the zygomatic process of squamosal and broadening/narrowing of the width of the maxilla region
<i>P.leucopus</i>	Ventral	RW1	21.51%	overall broadening/narrowing of the skull
		RW2	13.78%	variation in the position of the anterior portion of the zygomatic arch and also an contraction of the incisive foramen
		RW3	9.54%	represents an anterior/posterior shift in the palatine region with maxilla expansion/contraction
		RW4	8.83%	contraction of the palatine region
		RW5	6.84%	slight expansion/contraction of braincase region
	Lateral	RW1	20.98%	contraction/expansion of the braincase region, skull to shift from convex to concave appearance
		RW2	15.58%	expansion/contraction of the braincase while correspondingly contraction/expansion of the rostrum
		RW3	11.54%	vertical shift in position of the zygomatic process of squamosal
		RW4	8.91%	lateral shift in position of the maxilla and frontal regions
		RW5	8.49%	diagonal shift in the position of the zygomatic process of squamosal and broadening/narrowing of the width of the maxilla region

expansion/contraction of the braincase region of the skull, causing skull to shift from concave to convex appearance. GPA3 differed from the other analyses in both views for relative warps 2-5, with *P. leucopus* skulls varying primarily in zygomatic characteristics whereas the combined and *P. maniculatus* skull GPAs varied primarily in incisive foramen and brain case length. Results for all GPAs, relative warps, and views can be found in Table 1.2.

Effects On Shape-GP1 (P. maniculatus and P. leucopus combined)

MANCOVA analysis on the ventral view for GP1 identified significant relationships in the data set with species ($F_{5,221}=15.248$; $p<<0.001$), latitude ($F_{5,221}=3.441$; $p=0.005$), and centroid size ($F_{5,221}=42.863$; $p<<0.001$). Neither longitude ($F_{5,221}=1.519$; $p=0.185$) nor sex ($F_{5,221}=1.715$; $p=0.132$) were significantly related to shape for GPA1. Based on ANCOVA, the three significant variables were significantly related to RW1. *P. maniculatus* displayed higher RW1 values than *P. leucopus* ($t_{225}=2.687$; $p=0.008$) (Figure 1.3), as latitude increased RW1 values decreased ($t_{225}=-2.230$; $p=0.027$), and as centroid size increased RW1 values decreased ($t_{225}=-7.281$; $p<<0.001$) (Figure 1.4). These results suggest that *P. maniculatus* on average have broader skulls than *P. leucopus*, specimens at higher latitudes have narrower skulls, and specimens with larger centroid size also have relatively narrower skulls (Figure 1.5)(Table 1.2).

MANCOVA results for the lateral view of GP1 identified significant shape differences due to species ($F_{5,221}=19.675$; $p<<0.001$), sex ($F_{5,221}=2.556$; $p=0.028$), latitude ($F_{5,221}=3.495$; $p=0.005$), longitude ($F_{5,221}=3.567$; $p=0.004$), and centroid size ($F_{5,221}=65.848$; $p<<0.001$). Based on ANCOVA results species, sex, and centroid size were related to RW1. *P. maniculatus* had higher RW1 values than *P. leucopus* ($t_{225}=2.741$; $p=0.007$) (Figure 1.3), males had higher RW1 values than females ($t_{225}=2.066$; $p=0.040$), and as centroid size increased RW1 values

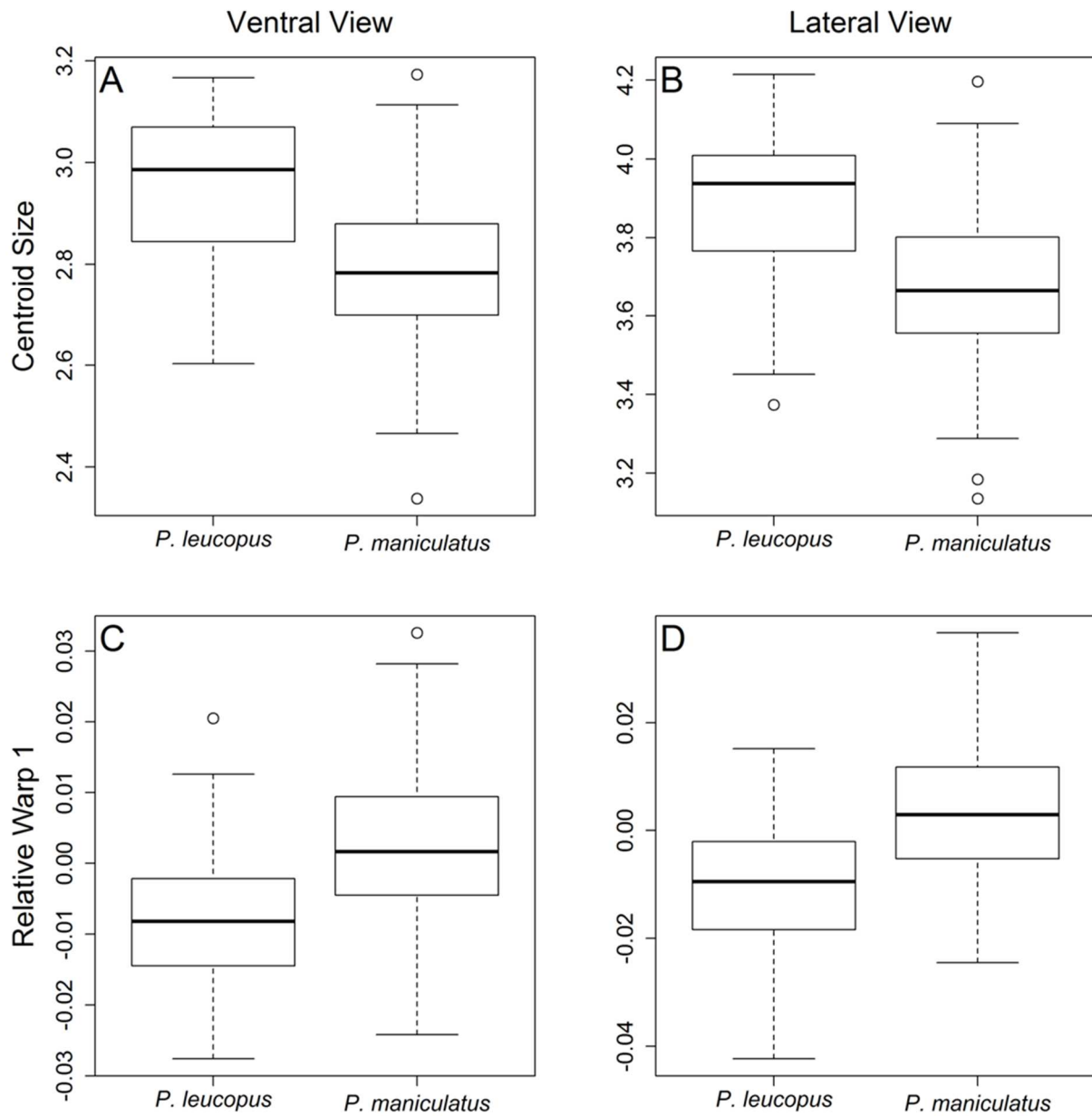


Figure 1.3. Centroid size comparisons between *Peromyscus* species for ventral (A) and lateral (B) views; RW1 comparisons for *Peromyscus* species for ventral (C) and lateral (D) views.

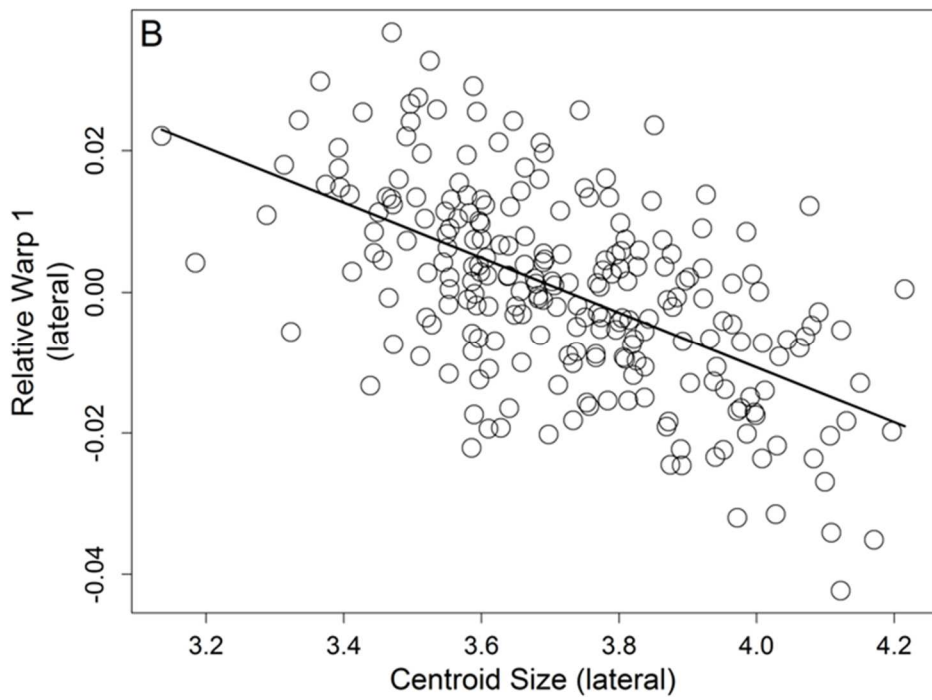
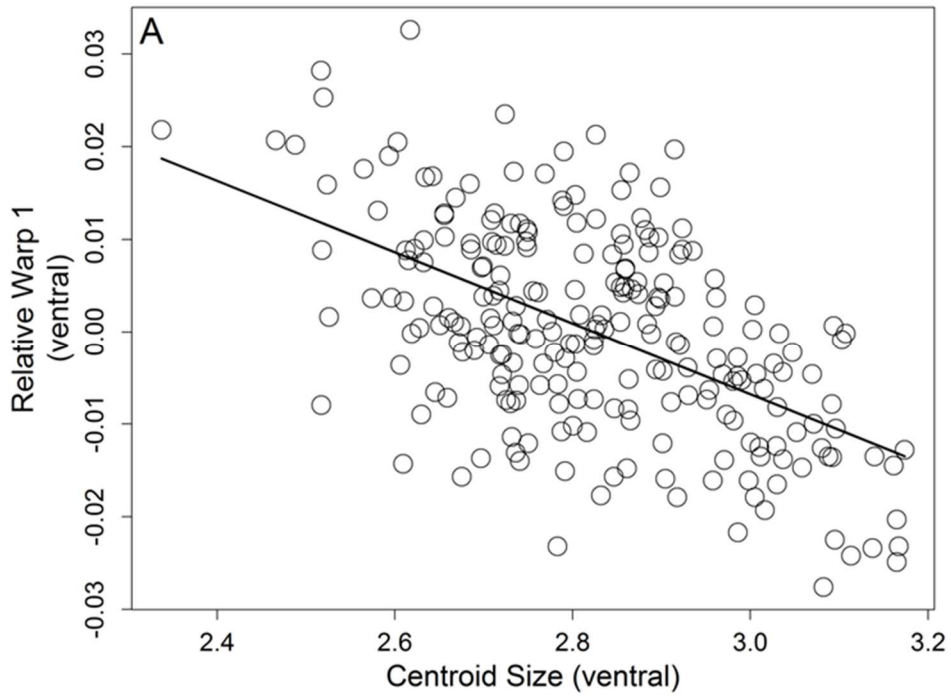


Figure 1.4. Relationship between RW1 values and centroid size for ventral (A) and lateral (B) views for GPA1.

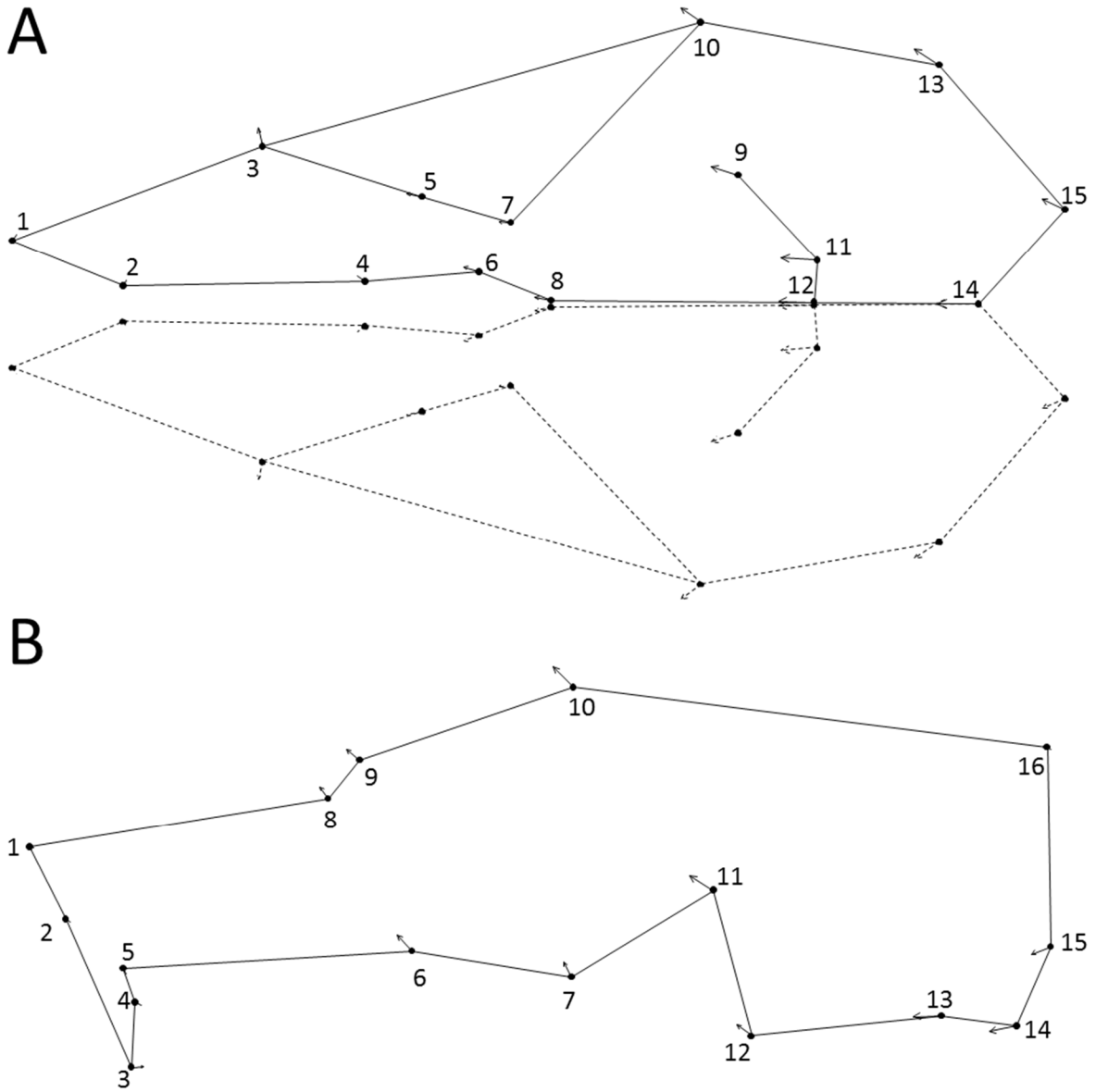


Figure 1.5. Thin-plate splines for positive RW1 for ventral (A) and lateral (B) views of GPA1; vector arrows represent morphological shift along RW1 axis.

decreased ($t_{225}=-8.653$; $p<<0.001$)(Figure 1.4). These results suggest that *P. maniculatus* have relatively more expanded braincases than *P. leucopus*, males have relatively more expanded braincases than females, and specimens with larger centroid sizes have reduced relative braincase sizes compared to specimens with smaller centroid sizes (Figure 1.5, Table 1.2). ANCOVA results suggested that latitude was significantly related to RW3: as latitude increased RW3 values decreased ($t_{225}=-2.064$; $p=0.040$), suggesting that specimens at higher latitudes display a higher position of the zygomatic process of squamosal than those at lower latitudes. Longitude was significantly related to RW5: as longitude increased RW5 values decreased ($t_{225}=-2.855$; $p=0.004$), suggesting that specimens at higher longitudes display a lower position of the zygomatic process of squamosal and narrower width of the maxilla region. For complete ANOVA by RW results, see supplemental information.

Effects On Shape-GPA2 (P. maniculatus)

MANCOVA analysis on the ventral view of GPA2 identified significant relationships in the data set in shape with latitude ($F_{5,169}=2.471$; $p=0.034$) and centroid size ($F_{5,169}=34.165$; $p<<0.001$). Based on ANCOVA results, latitude was significantly related to RW1 ($t_{173}=-2.044$; $p=0.043$). As latitude increased RW1 values increased, suggesting that specimens from higher latitudes display broader width skulls than specimens from lower latitudes. Based on ANCOVA, centroid size was significantly related to was RW2 ($t_{173}=-10.990$; $p<<0.001$). As centroid size increased RW2 values decreased, suggesting that specimens with larger centroid sizes displayed a lower position of the anterior portion of the jugal and also a contraction of the incisive foramen length.

MANCOVA results for the lateral view of GPA2 included significant effects on shape from longitude ($F_{5,169}=3.991$; $p=0.002$) and centroid size ($F_{5,169}=47.982$; $p\ll 0.001$). Based on ANCOVA results centroid size was significantly related to RW1 ($t_{173}=-5.099$; $p\ll 0.001$). As centroid size increased RW1 values decreased, suggesting that specimens with larger centroid sizes displayed shorter braincase length than specimens with lower centroid sizes. Longitude was significantly related to RW2 ($t_{173}=1.986$; $p=0.048$). As longitude increased, RW2 values increased, suggesting that specimens from higher longitudes displayed larger braincase length (relative to other parts of the skull) and also shorter length in maxilla region. For complete RW-ANCOVA results see supplemental information.

Effects On Shape-GPA3 (*P. leucopus*)

MANCOVA results for GP3-ventral view included significant effects of sex ($F_{5,43}=2.492$; $p=0.046$) and centroid size ($F_{5,43}=9.377$; $p\ll 0.001$). Even though sex was reported significant in the MANCOVA, no subsequent ANCOVAs on relative warps included sex as a significant effect. Based on ANCOVA, centroid size was significantly related to RW1 ($t_{47}=3.057$; $p=0.004$). As centroid size increased, so did RW1 values, suggesting that specimens with larger centroid sizes display narrower skull widths than specimens with larger centroid sizes.

MANCOVA results for GP3-lateral view identified significant effects of latitude ($F_{5,43}=2.561$; $p=0.041$), longitude ($F_{5,43}=5.336$, $p=0.001$), and centroid size ($F_{5,43}=11.741$; $p\ll 0.001$). Based on ANCOVA, latitude was significantly related to was RW4 ($t_{47}=2.538$; $p=0.145$), longitude was significantly related to RW3 ($t_{47}=2.017$; $p=0.049$) and centroid size was significantly related to RW1 ($t_{47}=3.773$; $p<0.001$). These results suggest that as latitude increased RW4 values increased; that is, specimens at higher latitudes displayed a posterior shift in position of the maxilla and frontal regions than specimens with lower latitudes. Also, as

longitude increased, RW3 values increased, suggesting that specimens from higher longitudes displayed a lower shift in position of the zygomatic process of squamosal than specimens at lower longitudes. Finally, as centroid size increased, RW1 values increased, suggesting that specimens with larger centroid sizes displayed shorter relative braincase length than specimens with lower centroid sizes. For complete RW-ANCOVA results see supplemental information.

Size

MANCOVA analysis demonstrated significant skull size differences in the collection due to species ($F_{2,225}=29.4832$; $p<<0.001$) and latitude ($F_{2,225}=3.4672$; $p=0.033$). *P. leucopus* was consistently larger than *P. maniculatus* in the ventral view ($t_{226}=-7.285$; $p<<0.001$) and lateral view ($t_{226}=-6.939$; $p<<0.001$) (Figure 1.3). As latitude increased so did specimen centroid size in the ventral view ($t_{226}=2.807$; $p=0.005$) and lateral view ($t_{226}=2.775$; $p=0.006$).

When analyzed individually, *P. maniculatus* showed significant skull size differences due to latitude ($F_{2,173}=5.013$; $p=0.008$). As latitude increased so did specimen centroid size in the ventral view ($t_{174}=3.467$; $p=0.001$) and lateral view ($t_{174}=3.399$; $p=0.001$). No significant relationships were found for *P. leucopus* alone. For full centroid size results, see supplemental information.

Species Discrimination

Discriminant function analysis successfully identified 45% of *P. leucopus* (24 of 53) and 92% of *P. maniculatus* (165 of 179). Total percent correct of all samples was 81%. To correct for uneven samples of each species, we ran DFA analyses with all 53 *P. leucopus* and a random set of 53 *P. maniculatus*. In 1000 simulations of even sample sized specimens, *P. leucopus* was successfully identified on average 73% of the time, and *P. maniculatus* 73% of the time, for an average of 73% when data sets were even (53).

Discussion

Shape

Using geometric morphometrics, we found skull shape differences within and between *Peromyscus* species. Shape change was most apparent along the maxilla, zygomatic arch, and braincase regions of the skulls (Table 1.2), with our predictor variables describing small percentages of total shape change seen. In the ventral view, on average, *P. maniculatus* displayed wider skulls with a larger relative braincase region than *P. leucopus*. In the lateral view similar differences were displayed with *P. maniculatus* having a more extended braincase than *P. leucopus*. However, *P. leucopus* displayed larger skull sizes than *P. maniculatus*. Therefore it might be the case that as individual *Peromyscus spp.* become larger, their skull shape becomes relatively more shallow (Figure 1.4). GPA1 and GPA2 displayed similar shape change along their respective RW axis's, which is likely due to the majority of GPA1 specimens also being analyzed in GPA2 (77%).

While we were able to detect significant shape variation in *Peromyscus* species skulls based on predictor variables such as species, location, and size; no individual predictor variable could explain more than 9% of the total skull shape variation seen. Further, no single relative warp was able to distinctly explain more than 23% of the total variation. Taken together this could mean that although there was significant shape variation within this dataset, this variation was likely the accumulation of many smaller differences rather than fewer, more drastic differences. These results also suggest that the majority of the observed variation is attributable to other unmeasured environmental variables.

Despite explaining a low amount of shape variation overall, shape differences due to species were statistically significant. This is possibly consistent with findings by Kamler et al.

(1998) wherein morphological characteristics of both *Peromyscus* species under allotropic and syntopic conditions differed due to competition between and within *Peromyscus* species for preferred habitats. Specifically, *P. maniculatus* and *P. leucopus* were morphologically distinct under allotropic conditions, but under syntopic conditions individuals of each species displayed intermediate external measurements when compared to the allotropic populations and could not easily be assigned to correct species (Kamler et al. 1998).

Size

Our chief findings in regards to size were that *P. leucopus* skulls were consistently larger than *P. maniculatus* (Figure 1.3) and that the size of skulls increased with an increase in latitude in both species. This finding that skull size increased with latitude in *Peromyscus* is consistent with Bergmann's Rule which states that individuals living in colder climates tend to be larger than individuals of that same species living in a warmer climate (Mayr 1963). Bergmann's Rule arises due to benefits of large size for thermoregulation (McNab 1971) and latitude is a common proxy for temperature, with more northern populations assumed to experience lower temperatures (Ashton et al. 2000). However, there has been controversy regarding the evidence for Bergmann's Rule and whether other factors such as increases in food availability and quality might give rise to similar patterns (Ashton et al. 2000, Blackburn and Hawkins 2004).

The results in this study are consistent with Bergmann's Rule in that the sizes of skulls increase with an increase in latitude. However, the range of latitudes across which specimens were collected is narrow (from 45.93 to 48.79), with little difference in the average temperatures across that range (4°C to 5.8°C). The fact that our results were consistent with Bergmann's Rule across this small latitudinal range suggests that mechanisms other than thermoregulation might underlie Bergmann's Rule, or at least this particular expression of Bergmann's Rule.

Species Discrimination

The results of our discriminant function analysis suggest that the use of geometric morphometrics to distinguish between *P. maniculatus* from *P. leucopus* was only somewhat successful. When the entire data set was analyzed (with uneven sample sizes) the total success rate was 81%, with *P. maniculatus* more successfully identified (~92%) compared to *P. leucopus* (~45%). When even sample sizes were analyzed *P. maniculatus* success rate decreased (73%) while *P. leucopus* increased (73%) for an overall success rate of 73%. These results stand in stark contrast to the high success other researchers have observed for species assignment of *Peromyscus* based on discriminate function analysis (Choate 1973; Feldhamer et al. 1983; Rich et al. 1996; Lindquist et al. 2003).

Our relatively low success to categorize specimens might be due to some combination of at least three factors: First, in contrast to other uses of discriminate function analyses to distinguish *Peromyscus*, we were using a geometric morphometrics approach. Despite the purported advantages of geometric versus traditional morphometrics, the approach may obscure species differences. Second, landmark placement might have contributed to failure to discriminate between the species with a high percentage of success. The majority of landmarks in this study were based on previous studies (Myers et al. 1996, McPhee 2004, Grieco and Rizk 2010), with some based on the authors' ability to consistently pick identify skull features throughout the collection. Different landmarks may better capture skull shape as it differs between species. Third, and in our opinion most likely, the low success may be due to the fact that specimens were identified in the field and their species designation could be incorrect. The specimens in the collection that we analyzed were collected from 1964-1991, when salivary amylase and DNA sequencing to help confirm species identification were not largely in use. Our

samples therefore run the risk of not being properly identified to species. Despite this possibility, significant shape and size differences were observed between the species. Presumably, misidentification of specimens would have the effect of reducing differences between species, suggesting that actual species differences are likely greater than reported here.

Conclusion

This study identified significant morphological skull shape and size variation between and within *Peromyscus* species in the upper Mid-West. Although initial species identification may have been incorrect, *Peromyscus* species displayed significant skull shape and size variation throughout the collection. Factors influencing skull variation were species, size, location, and sex. Considerable variation remained unexplained by these factors. Additional influences on skull variation potentially include factors such as niche partitioning, population density, or possibly climate change. Future research could help expand on the reason for morphological differences between and within *Peromyscus* species in this region.

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CHAPTER 2. DIVERSITY AND ABUNDANCE OF SMALL MAMMAL SPECIES IN TALLGRASS PRAIRIE

Abstract

Small mammals are a vital component to an ecosystem but are often overlooked due to their abundance and small size. Small mammals are an important food source to other species and also help alter vegetation by consuming and dispersing seeds to increase species diversity. Tallgrass prairie is a very endangered ecosystem in North America and knowing how small mammals are reacting to different habitat alterations and management techniques could help improve management and restoration efforts. In this study we trapped small mammals in the Sheyenne National Grasslands for two consecutive trapping seasons to research impacts on the small mammal community due to factors such as habitat fragmentation, habitat disturbance, and management techniques. We found that woody encroachment and grazing significantly impact the small mammal community in the Sheyenne National Grasslands, and that management for small mammal biomass and diversity will constantly be at odds when utilizing our results.

Introduction

Small mammal diversity and abundance can be very important to the health of ecosystems. Small mammals serve an important role in many ecosystem functions by serving as a food source for other species and by altering vegetation composition (Sieg 1987) and population fluctuations within small mammal communities have been shown to affect the energy and nutrient transfer through the food web, ultimately affecting the overall energy and population dynamics of the ecosystem (Wike et al. 2000). In prairie ecosystems small mammals selectively consume plants, distribute seeds and spores, mix and aerate soil and detritus, and serve as prey for larger predators (Falout and Nelson 1997). Manipulating small mammal populations and

densities might also be used as important management tools to assess the stability and health of remaining grassland ecosystems because changes in species densities and biodiversity can have strong trickle-down effects on predatory species such as raptors, coyotes, etc. (Bueno et al. 2011). Small mammals are also indicators of ecosystem health because their short life spans and rapid reproduction rate rates allow for effects to be studied across multiple generations while observing rapid responses of population composition to environmental pressures (Bueno et al. 2011). Unfortunately, small mammals are often overlooked because they are typically small and abundant in number. By understanding variables that affect small mammal presence and abundance we might be able to more efficiently manage ecosystems as a whole, including threatened ecosystems such as tallgrass prairie.

Tallgrass prairie was once the most dominant ecosystem in the continental United States, but has been reduced by over 95% of its historic range in North America (Samson and Knopf 1994). Currently it is considered the “most endangered” major ecosystem in North America, largely because of the expansion of agriculture and urban development (Noss et al. 1995, Howe and Brown 1999). While little remains, tallgrass prairie still is generally characterized by high levels of biodiversity, and in North Dakota, is an important habitat for many endangered or threatened species in the state such as the greater prairie chicken (*Tympanuchus cupido*), the Dakota skipper (*Hesperia dacotae*), and Regal Fritillary (*Speyeria idalia*). The Sheyenne National Grasslands in North Dakota-the largest remaining tallgrass prairie remnant in the United States- also contains one of largest populations of the western prairie fringed orchid, which has been placed on the list of Threatened Species by the U.S. Fish and Wildlife Service (IUCN 2015).

Small mammal responses to tallgrass management techniques, for example grazing and burning, have been measured in some populations (Yarnell et al. 2007, Bock et al. 2011, Bueno et al. 2011). Bock et al. (2011) found that small mammals in the family Heteromyidae increased in areas following fire-caused reductions in grass cover, while Cricetidae species were slower to return to pre-fire population levels. Yarnell et al. (2007) found that low level grazing and burning helped maintain small mammal biodiversity, if conducted under appropriate rainfall levels, and that high levels of grazing combined with fire conducted under drought conditions can have a negative impact on small mammal biodiversity. Bueno et al. (2011) described higher population densities of deer mice (*Peromyscus maniculatus*) in grazed plots than ungrazed plots, and demonstrated that meadow voles (*Microtus pennsylvanicus*) displayed a strong avoidance to areas of grazing even at low intensity.

Small mammals have also been demonstrated to affect tallgrass prairie restoration and succession (Falout and Nelson 1997, Kezar and Jenks 2004, Richardson 2010). Falout and Nelson (1997) found a positive correlation between the diversity of plants and small mammals, with early successional prairies having highest plant and mammal diversity. Kezar and Jenks (2004) reported that restored prairie habitats provide adequate components to support viable small mammal communities, with some species being more abundant in native prairie and others being more abundant in restored prairie. Richardson (2010) reported small mammal species richness was relatively low in grasslands that were recently burned and highest in older successional grasslands. Woody encroachment, an indicator of succession in tallgrass prairie, has been shown to alter abundance, biomass, species richness, and composition of small mammal communities even at low levels (Matlack et al. 2008). Roadways have also shown to impact small mammal communities and densities due to differences between right-of-way habitat and

adjacent habitat, and by also inhibiting small mammal movement (Oxley et al. 1974, Adams and Geis 1983). Agriculture crops impact small mammal communities with some species, such as the northern grasshopper mouse (*Onychomys leucogaster*), preferring habitat near agricultural practices (Seabloom et al. 2011).

Despite knowledge of these general effects, little research has been done in the Sheyenne National Grasslands, which is the largest publically owned tallgrass prairie reserve in the United States (Martin and Svingen 2003). Specifically targeting this large representation of habitat could help predict small mammal impacts on other tallgrass areas. The Sheyenne National Grasslands contains both woody encroachment and also burning and grazing as management techniques. Given the importance of small mammal species and the roles they perform for ecosystems, it would be helpful to be able to better understand how small mammals in the Sheyenne National Grasslands react to these conditions, along with also impacts of agriculture, roads, and possibly the influence of soil characteristics. The purpose of this study was to survey small mammal populations in the Sheyenne National Grasslands in order to better understand how small mammal diversity and abundance are impacted due to differences in local habitat and grazing in tallgrass prairie. The results could allow for a better understanding of small mammal presence and abundance in not only one of the world's most endangered ecosystems but also the largest publically owned tallgrass prairie reserve in the United States.

Methods

This research took place in the Sheyenne National Grasslands located in Ransom and Richland counties in North Dakota during the summers of 2014 (June 16-Sept. 12) and 2015 (May 25-Aug. 14). 12 trapping sites were set up in the grasslands (Figure 2.1), with sites being chosen based on previously monitored sites by Sweitzer (2003), adequate coverage of the

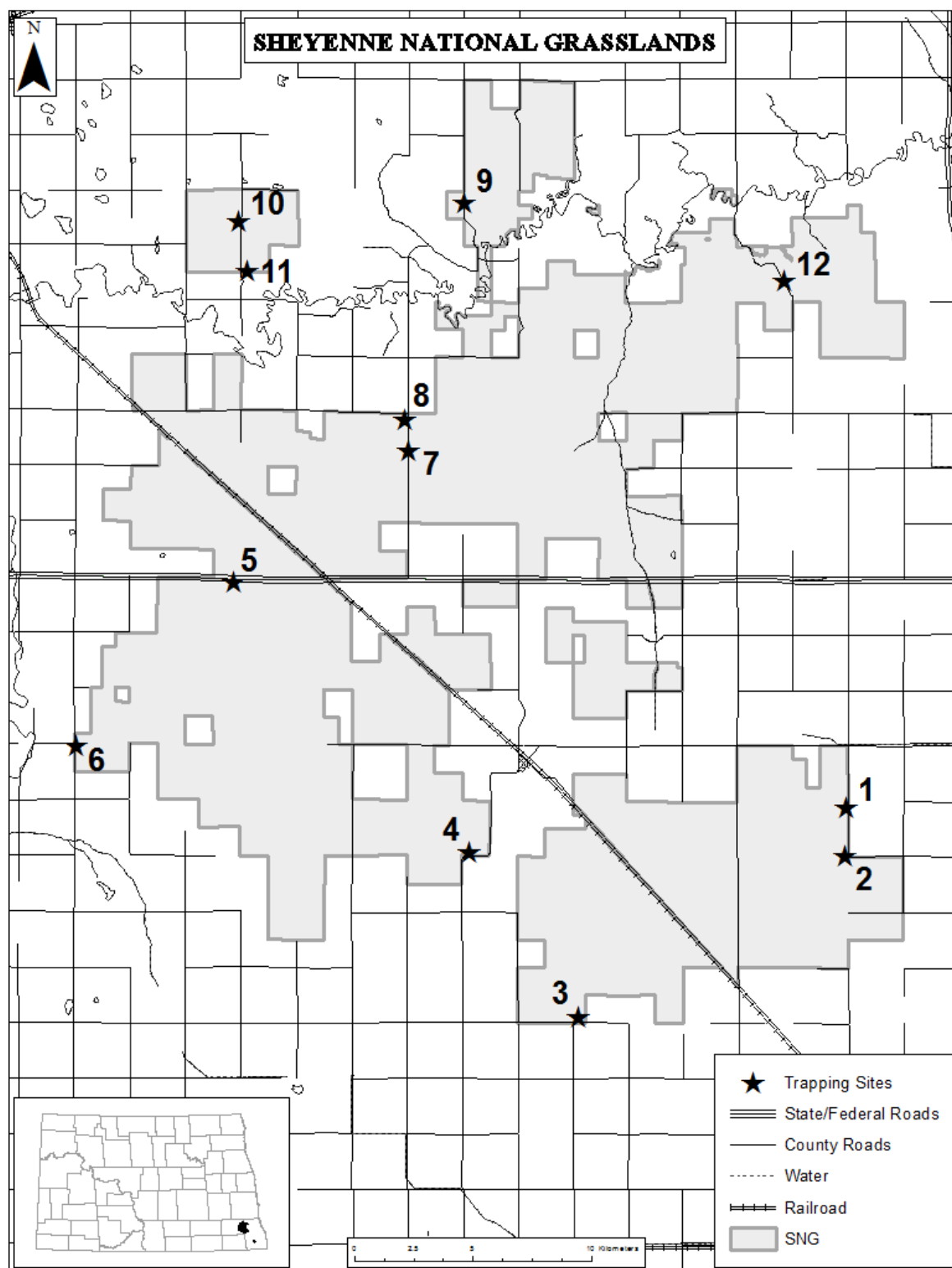


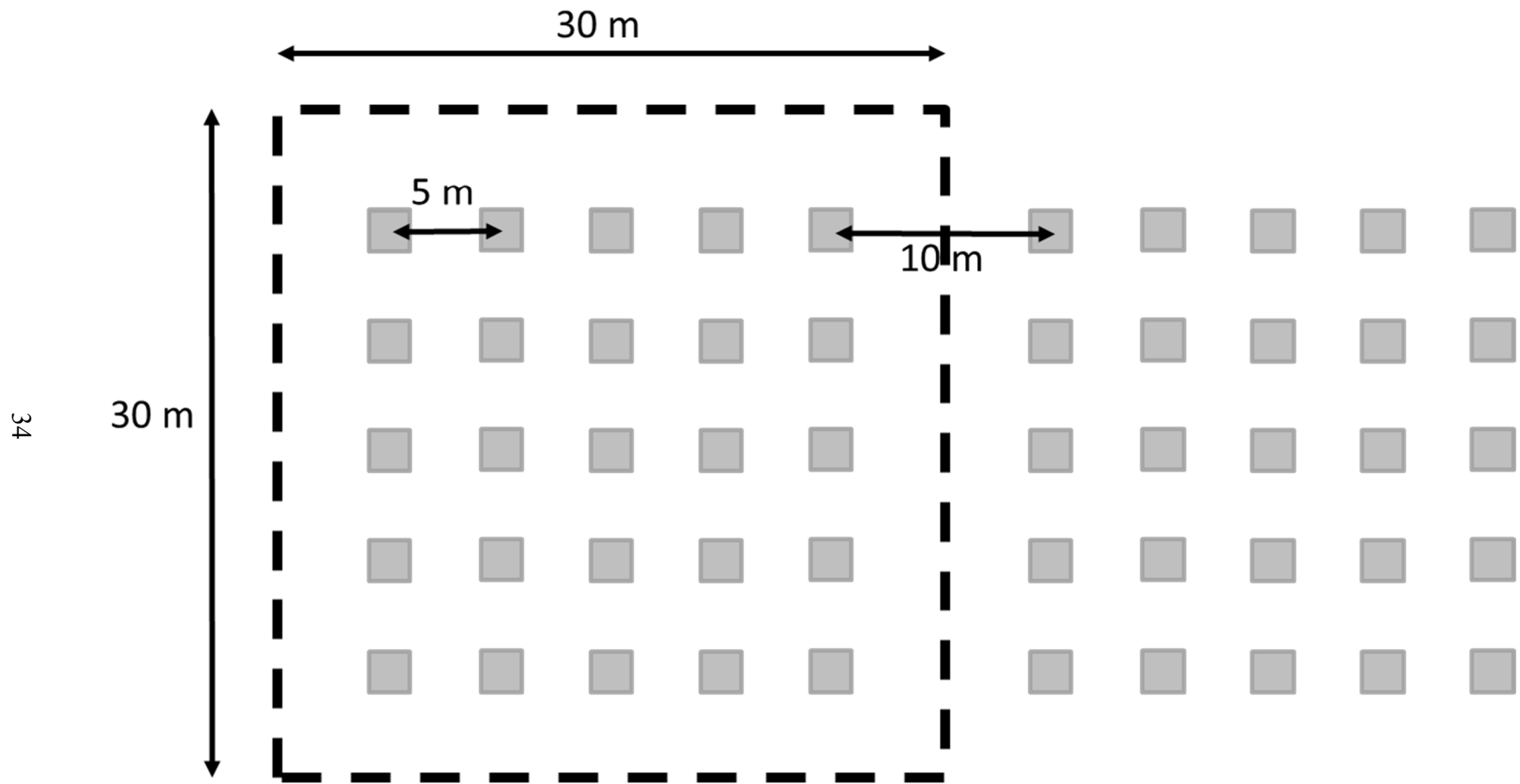
Figure 2.1. Map of trapping sites located in the Sheyenne National Grasslands, located in Ransom and Richland counties in North Dakota.

majority of the grasslands, and also choosing areas that were easily accessible by foot. The research reported here was also done in collaboration with another thesis study (Preston 2015), which compared small mammal community dynamics in response to short term grazing application.

Trapping

Each site consisted of two 30 x 30 meter paired trapping grids that were located adjacent to each other (Figure 2.2). Inside those trapping grids were Sherman live traps that were set up in a 5 x 5 layout with 5 meters of separation between each trap. Traps were baited with bird seed and peanut oil, with one grid inside an electrical fence enclosure and the other outside of the enclosure. One of the grids at each site was within an electric fence that excluded access to cattle and which was set up at the beginning of the trapping period each season, while the adjacent site was open for cattle grazing (Figure 2.2). By having 2 paired grids at each site we provided a difference in habitat availability to the small mammals in the area of each site while also testing the short term response of small mammals to the varying grazing pressure that occurred throughout the trapping season.

Trapping occurred for 4 consecutive nights with 3 sites being actively trapped at a time. We were able to cycle through all 12 sites 3 periods each field season. Traps were checked/closed early in the morning and then reset each afternoon/evening. All small mammals captured were identified to species, had their sex and mass recorded, and were marked with the use of ear tags or PIT tags to identify previously captured individuals. Only the mass of individuals at first capture was used in subsequent analyses. For individuals that were not weighed, their mass in subsequent analysis were estimated as the average mass of all weighed



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Figure 2.2. Trapping grid set up at each site.

individuals of the same species. Whether the individual was captured in the enclosure or not was also recorded for each individual.

Based on a previous report (Sweitzer 2003) and a previous pilot trapping in the fall of 2013 at the Sheyenne National Grasslands, it was expected that the most common species that were likely to be caught in the Sheyenne National Grasslands were deer mice (*Peromyscus spp.*), meadow voles (*Microtus pennsylvanicus*) and thirteen-lined ground squirrels (*Ictydomys tridencelineatus*). The main species of *Peromyscus* present in the grasslands are the white-footed mouse (*Peromyscus leucopus*) and the deer mouse (*Peromyscus maniculatus*). These two species are often misidentified in the field due to their remarkable similarities in appearance, and therefore were grouped together as *Peromyscus spp.* for this study.

Habitat Assessment

Vegetation composition, vegetation height, and surrounding habitat for each site was recorded, along with weather conditions for each trapping day. Cattle presence and grazing pressure was determined based off declines in vegetation height throughout the trapping periods at each site. Soil drainage classifications for each site were determined based on the soil type present at each site. Distance from roads, distance from agriculture, soil type, and woody encroachment were determined by spatial analysis of satellite imagery with the help of ArcMap GIS software. A 250 meter buffer, which was determined based on containing all the home range sizes of the likely species to be caught (Table 2.1), was placed around each site to help determine level of woody encroachment at each site. Woody encroachment was then classified by identifying how many trees were within the home range size buffer and how spread out they were from one another (Table 2.2).

Table 2.1. Typical home range sizes for common small mammal species predicted to be caught in the Shyenenne National Grasslands.

Species	Home Range (ha)
Franklin's Ground Squirrel	9-25
Thirteen-lined ground squirrel	2-5
Peromyscus species	0.01-1.2
Meadow Voles	0.016-0.34
Meadow Jumping Mice	0.1-1
Masked Shrew	0.5
Northern Grasshopper Mice	2.3
Northern Short-tailed Shrew	2.5
Plains Pocket Mice	0.02-0.04

Table 2.2. Predictor and response variable descriptions used in this study

Type	Variables	Descriptions
Response	Biomass	Mass of living biological organisms in a given area or ecosystem at a given time
	Simpson's Index	Measure of diversity which takes into account the number of species present, as well as the relative abundance of each species. As species richness and evenness increase, so diversity increases.
Predictor	Woody Vegetation	Low=little/no trees within 250 meter radius Moderate=few trees spread out within 250 meter radius High=many trees clumped within 250 meter radius
	Distance From Roads	Distance in meters from site to nearest road
	Distance From Ag.	Distance in meters from site to nearest agriculture
	Soil Type	Classification of soil at site
	Drainage Type	Based on site soil characteristics
	Vegetation Height Ave.	Changes in vegetation height due to grazing throughout the trapping seasons
	Grass	Percent grass of total trapping grid area
	Forbs	Percent forbs of total trapping grid area
	Bare Ground	Percent bare ground of total trapping grid area
	Grazing Treatment	Inside or outside of electric fence enclosure

Data Analysis

Total biomass for each site and Simpson's Index of small mammal diversity were calculated for each site. Biomass was determined for each site by determining average weight of each species caught and then multiplying those values by the number of unique individuals caught at each site for each species. The relationships between the predictor variables (woody encroachment, vegetation height, drainage, distance from roads, distance from agriculture, year, grazing treatment, percent forbs, percent grass, percent bare ground) and the response variables (biomass and Simpson's Index) (Table 2.2) were determined using linear mixed models with Program R. Linear mixed models were used to control for pseudoreplication based on repeated measurement of sites across years by including site as a random factor. Minimum Known Alive values were determined based on the number of unique individual's that were recorded throughout the trapping seasons. Despite individuals being uniquely tagged, formal mark-recapture analysis was not used because most sites had fewer than 10 individuals captured of each species (Table 2.3). Simpson's index and biomass were analyzed at both the landscape level and for effects of treatment. The landscape analysis was based on combined captures in both the fenced and unfenced grids and examined the effects of woody encroachment, soil drainage, distance from roads and agriculture, and average vegetation height. The treatment analysis examined the effects of grazing treatment (fenced or unfenced) and percent forbs, grass, or bare ground at a site.

Results

A total of 540 captures of small mammals occurred in 2014 and 832 in 2015. In 2014 there were 254 unique individuals caught, with the majority of those consisting of thirteen-lined ground squirrels (186) (Table 2.3). Other species that were caught included deer mice (38),

Table 2.3. Minimum known number of individuals caught at each site in 2014 and 2015.

2014													
Species/Site	1	2	3	4	5	6	7	8	9	10	11	12	Totals
Thirteen-lined Ground Squirrels	21	5	1	38	29	9	33	19	14	9	2	6	186
Franklin's Ground Squirrels	0	0	0	0	1	0	0	0	0	0	0	1	2
<i>Peromyscus</i> Species	0	0	7	2	3	0	4	5	6	1	10	0	38
Northern Grasshopper Mice	0	0	0	0	0	0	0	0	0	0	0	0	0
Plains Pocket Mice	0	2	0	0	0	0	0	0	0	0	0	0	2
Meadow Jumping Mice	0	0	0	0	0	0	0	0	0	0	0	0	0
Masked Shrew	0	0	0	0	0	0	0	0	0	0	1	0	1
Meadow Voles	0	0	2	0	0	9	1	0	0	0	1	4	17
Northern Short-tailed Shrews	2	0	0	0	1	3	0	0	0	2	0	0	8
Totals	23	7	10	40	34	21	38	24	20	12	14	11	254

2015													
Species/Site	1	2	3	4	5	6	7	8	9	10	11	12	Totals
Thirteen-lined Ground Squirrels	20	13	2	54	41	24	26	18	21	12	2	19	252
Franklin's Ground Squirrels	0	0	0	0	0	0	1	1	0	4	0	2	8
<i>Peromyscus</i> Species	4	3	2	1	3	0	0	4	1	1	3	0	22
Northern Grasshopper Mice	3	0	0	0	0	0	0	0	0	0	0	0	3
Plains Pocket Mice	0	1	0	0	0	0	0	0	0	0	0	0	1
Meadow Jumping Mice	1	0	0	0	0	0	0	0	0	0	0	0	1
Masked Shrew	0	0	0	0	0	0	0	0	0	0	0	0	0
Meadow Voles	0	1	6	0	0	3	0	0	0	0	0	2	12
Northern Short-tailed Shrews	0	0	0	0	0	1	0	0	0	0	0	0	1
Totals	28	18	10	55	44	28	27	23	22	17	5	23	300

meadow voles (17), northern short-tailed shrews (*Blarina brevicauda*) (8), Franklin's ground squirrels (*Poliocitellus franklinii*) (2), pocket mice (*Perognathus flavescens*) (2) and a masked shrew (*Sorex cinereus*) (1). In 2015 there were 300 unique individuals to have been caught, with the majority of those again consisting of thirteen-lined ground squirrels (252). Other species that were caught included deer mice (22), meadow voles (12), Franklin's ground squirrels (8), grasshopper mice (3), pocket mice (1), meadow jumping mice (1), and a northern short-tailed shrew (1). There were a total of 9 different small mammal species caught throughout the duration of the study, but only thirteen-lined ground squirrels were common enough to be independently analyzed.

The thirteen-lined ground squirrel accounted for 91% (22,768 g) of total biomass in 2014 while also accounting for 90% (30,337 g) in 2015. The number of thirteen-lined ground squirrels and biomass were significantly correlated ($r=0.97$; $p<0.001$) in this study, suggesting that biomass values for each site were significantly driven by the number of thirteen-lined ground squirrels present. The number of thirteen-lined ground squirrels and Simpson's Index were also significantly and negatively correlated ($r=-0.85$; $p<0.001$) in this study, suggesting that Simpson's Index values for each site were significantly driven by the number of thirteen-lined ground squirrels present. Deer mice were the second most abundant species each year, but only accounted for 2.9% of biomass in 2014 and 1.4% in 2015. Simpson's Index and biomass were significantly correlated ($r=-0.83$; $p<0.001$); as Simpson's Index increased, biomass decreased (Figure 2.3).

Out of the 540 total captures in 2014, 56% (300) came within the fenced trapping grid, while 44% (240) were from outside of the enclosure. Out of the 832 total captures in 2015, 61.9% (515) came within the fenced trapping grid, while 38.1% (317) were from outside of the

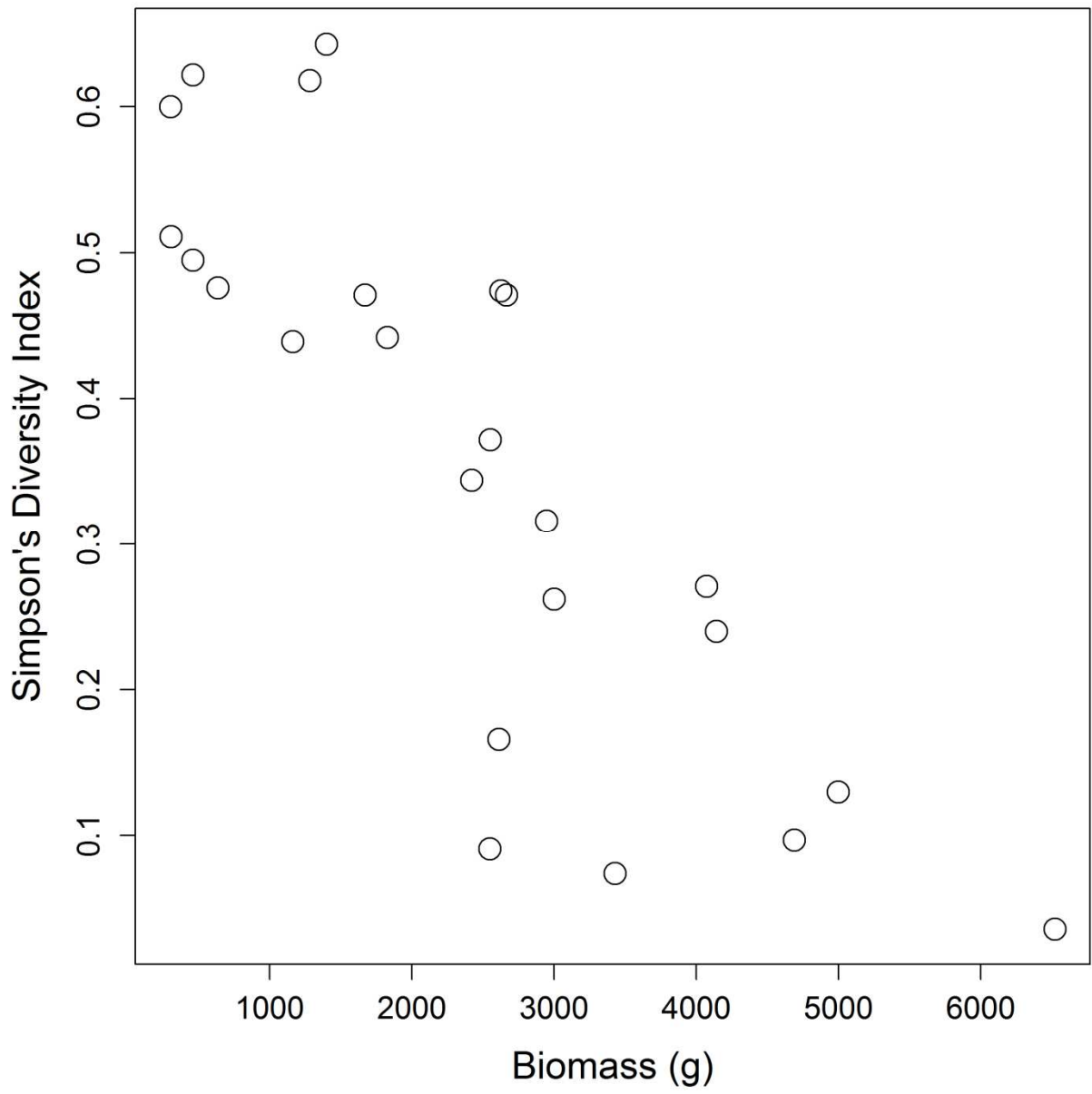


Figure 2.3. Correlation between Simpson's Index and biomass in our study ($r=-0.83$; $p<0.001$).

enclosure. The number of thirteen-lined ground squirrels and total captures were significantly correlated ($r=0.91$; $p<0.001$), suggesting that the number of captures at each site was driven by the number of thirteen-lined ground squirrel individuals present the site.

Landscape Response

When comparing trapping results between years without controlling for site, results demonstrated that distance from agriculture was significantly related to Simpson's Index ($F_{1,15}=11.949$; $p<0.004$) (Figure 2.4). No other variables were significantly related to Simpson's Index (Table 2.4).

When analyzing the impact on biomass, distance to agriculture ($F_{1,15}=24.710$; $p<0.001$), woody encroachment ($F_{2,15}=10.170$; $p=0.002$), and drainage type ($F_{2,15}=4.189$; $p=0.036$) were all significantly related to biomass. Distance to roads, average vegetation height, and year were not significantly related to biomass (Table 2.4).

When comparing trapping results between years and controlling for site, woody encroachment was significantly related to Simpson's Index ($F_{2,15}=4.000$; $p=0.041$) (Figure 2.5). As woody encroachment decreased, Simpson's Index values also decreased ($t_{2,15}=-2.718$; $p=0.016$), suggesting that as trees in the area decrease so does small mammal species diversity. No other variables were significantly related to Simpson's Index (Table 2.4). The predictor variables explained 50% of the variance seen when including and also excluding random effects ($R^2=0.50$).

When analyzing impact on biomass, woody encroachment remained significantly related to biomass ($F_{2,15}=6.778$; $p=0.034$) (Figure 2.5) after controlling for site, with biomass increasing with a decrease in woody encroachment ($t_{2,15}=3.674$; $p=0.015$), suggesting that as trees in the area decrease, small mammal biomass increases. No other variables were significantly related to

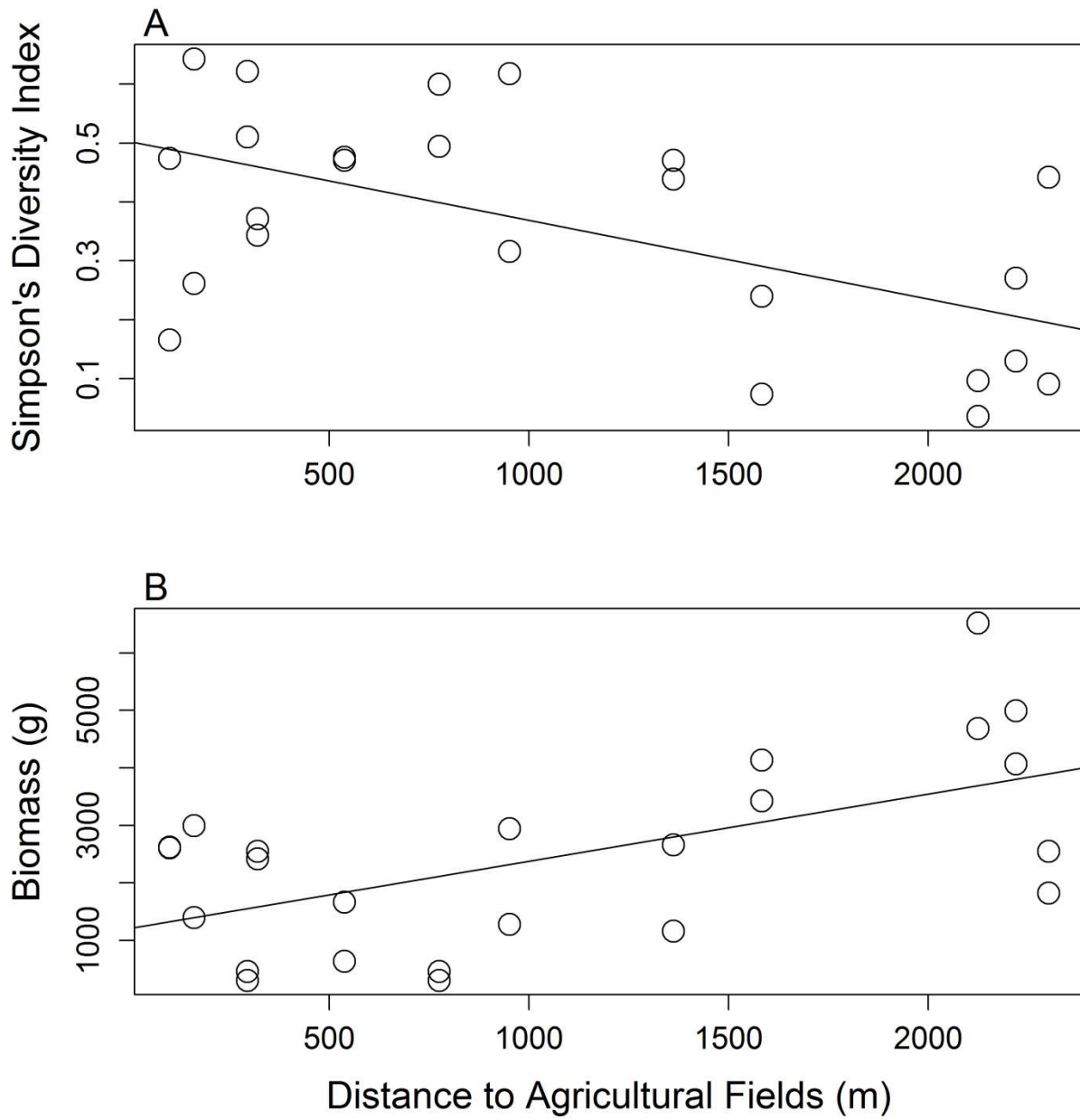


Figure 2.4. Impact of distance to agriculture on biomass in our study ($F_{1,15}=24.710$; $p<0.001$).

Table 2.4. ANOVA and mixed model results for our predictor variables and their corresponding response variables.

	Landscape Response	Simpson's Index				Biomass			
	ANOVA	Df	MS	F	P	Df	MS	F	P
A	Roads	1	0.017783	0.8038	0.384137	1	1328156	1.6162	0.2229833
	Agriculture	1	0.264364	11.9487	0.003523	1	20306380	24.7103	0.0001675
	Woody Habitat	2	0.047363	2.1407	0.152104	2	8357497	10.1700	0.0016169
	Drainage Type	2	0.039784	1.7982	0.199519	2	3442782	4.1894	0.0358516
	Vegetation Height Ave	1	0.000084	0.0038	0.951796	1	1045106	1.2718	0.2771525
	Year	1	0.042663	1.9283	0.185223	1	2126237	2.5874	0.1285597
	Residuals	15	0.022125			15	821778		
B	Landscape Response	Simpson's Index				Biomass			
	MIXED MODEL	Df	MS	F	P	Df	MS	F	P
	Roads	1/15	0.063949	2.8904	0.10975	1/15	1485616	3.9488	0.10398
	Agriculture	1/15	0.004949	0.2237	0.64305	1/15	98530	0.2619	0.62982
	Woody Habitat	2/15	0.088508	4.0004	0.04052	2/15	2549835	6.7776	0.03379
	Drainage Type	2/15	0.041729	1.8861	0.18592	2/15	740243	1.9676	0.23345
	Vegetation Height Ave	1/15	0.014525	0.6565	0.43047	1/15	16641	0.0442	0.83739
	Year	1/15	0.042663	1.9283	0.18522	1/15	1587132	4.2187	0.06706
C	Treatment Response	Simpson's Index				Biomass			
	MIXED MODEL	Df	MS	F	P	Df	MS	F	P
	Treatment	1/42	0.077023	0.90483	0.3485	1/42	2226107	7.6889	0.009386
	Bare	1/42	0.065099	0.76476	0.3868	1/42	11624	0.0401	0.842347
	Forb	1/42	0.68962	0.81014	0.3732	1/42	50654	0.175	0.67832
	Grass	1/42	0.073298	0.86107	0.3587	1/42	21882	0.0756	0.784997
	Year	1/42	0.031848	0.37413	0.5445	1/42	141439	0.4885	0.489647

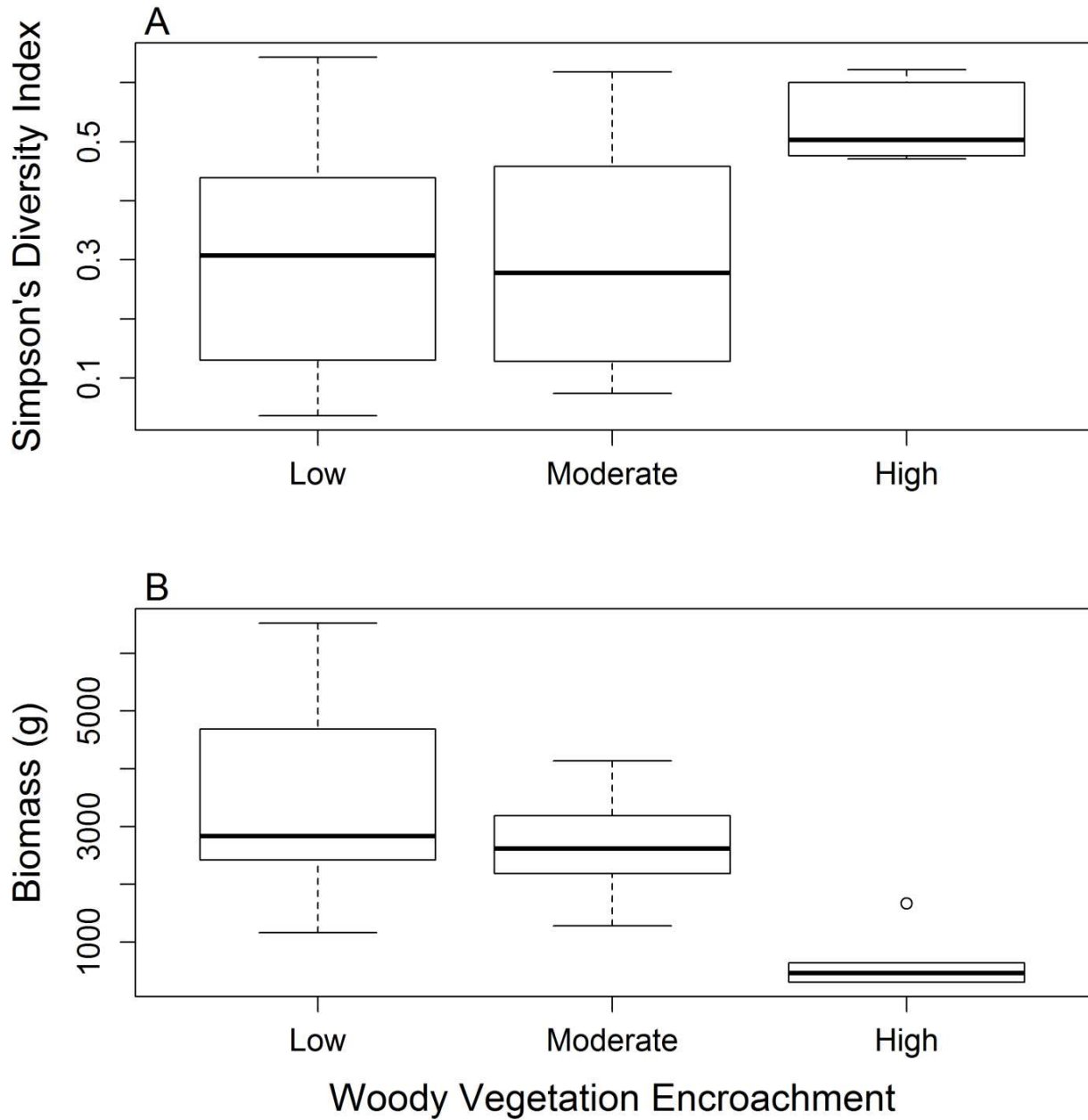


Figure 2.5. Impact of woody encroachment on Simpson's Index ($F_{2,15}=4.000$; $p=0.041$) and biomass ($F_{2,15}=6.778$; $p=0.034$) in our study.

biomass (Table 2.4). The predictor variables explained 88% of the variance seen when including the random effects ($R^2=0.88$), and 67% when excluding the random effects ($R^2=0.67$).

Treatment Response

To test for impacts of short-term grazing pressure in tallgrass prairie at each site we were limited to variables that were unique to each trapping grid, therefore only treatment type, year, and percentage of grasses, forbs, and bare ground at each site were able to be uniquely analyzed on a treatment level.

When analyzing between years and individual sites, there were no significant variables that affected Simpson's Index (Table 2.4). The predictor variables explained 20% of the variation seen when including random effects ($R^2=0.20$), while only describing 4% when excluding random effects ($R^2=0.04$). When analyzing impacts to biomass between years and among sites treatment type had a significant impact on biomass, with biomass decreasing in grids outside of the enclosure compared to inside ($F_{1,42}=7.689$; $p=0.009$) (Figure 2.6). No other variables were significantly related to biomass (Table 2.4). The predictor variables explained 66% of the variation seen when including random effects ($R^2=0.66$), while also describing 14% when excluding random effects ($R^2=0.14$).

Discussion

Tallgrass prairies are known to typically support low small mammal densities (Clark et al. 1987, Kaufman et al. 1988), here we did not catch large numbers of any species other than thirteen-lined ground squirrels. It is possible that winter conditions (The North Dakota Agriculture Weather Network reported below average snowfall from 2013-2015) between the field seasons contributed to the low number of deer mice and meadow voles, which are two

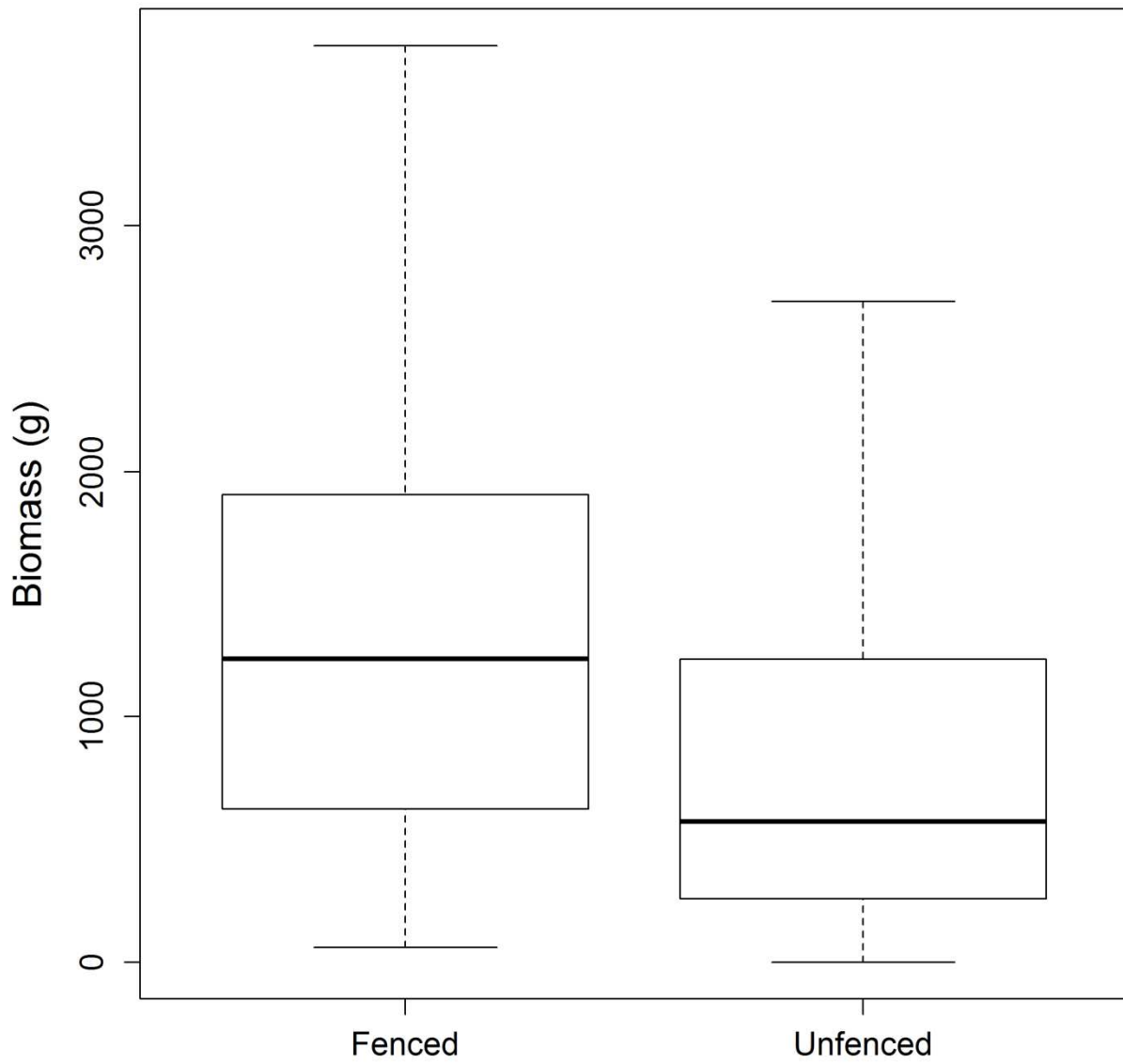


Figure 2.6. Comparison between biomass inside vs outside of the cattle enclosure ($F_{1,42}=7.689$; $p=0.009$).

species that are usually very abundant in the Sheyenne National Grasslands (Sweitzer 2003). Small mammal species inhabiting the subnivean environment during the winter are sensitive to cold or freezing temperatures and need snow to act as insulation to help make it through the winter (Merritt 1987). The lack of snowfall could have exposed the smaller species to worse conditions, causing population decreases. Alternatively, trapping could have been biased to favor the capture of thirteen-lined ground squirrels while also limiting capture of other species because traps were set during the afternoon each day and thirteen-lined ground squirrels are a diurnal species. Therefore, thirteen-lined ground squirrels had first and longer availability to occupy traps and thus decreased the number of traps available to be occupied by species that are more nocturnal in their habits (deer mice).

Grazing significantly affected small mammal biomass when controlling for site and year, with increases in biomass being seen inside of the exclosures compared to outside. Biomass was also significantly correlated with captures in this study, and thus it was not surprising that there were more captures inside the exclosures compared to outside the exclosures. Specifically the higher biomass and captures inside fenced areas could have been due to the fact that there was higher vegetation height on average inside the exclosures than outside due to the cattle grazing, which could have provided more cover from predators inside the exclosure compared to outside the exclosure. However, average vegetation height did not significantly affect diversity or biomass, suggesting that there could have been other factors contributing to the higher capture rate and biomass inside the exclosure. Traps outside of the exclosure were more prone to disturbance from cattle, and thus there could have been more traps available to be occupied inside the fence compared to outside the fence.

Woody encroachment did significantly affect small mammals in this study. When controlling for both year and site, woody encroachment was determined to be the only predictor variable that significantly affected small mammal presence in our study. It affected both Simpson's Index and biomass, with Simpson's index increasing and biomass decreasing with an increase of woody encroachment. This could be viewed as support for Richardson (2010), who found that there was higher species richness in later successional stages of prairie, although high richness does not guarantee high diversity. It would seem that sites that had some presence of trees invited not only open habitat species but also woody habitat species, thus increasing species richness. Because thirteen-lined ground squirrels tend to prefer open habitat, woody encroachment could decrease the abundance of thirteen-lined ground squirrels in the area. As reported earlier, thirteen-lined ground squirrels and biomass were also positively correlated, which would also explain as to why there was a decrease in biomass as woody encroachment increased.

As distance to agriculture increased, Simpson's Index decreased when controlling for year. Distance to agriculture also significantly affected biomass, but the degree of the effect was not significantly distinct, and so we were not able to tell how the distances were impacting the variables. This suggests that other pressures might have been acting upon changes in biomass in addition to distance to agriculture. Although agriculture did not significantly impact Simpson's Index or biomass when controlling for site, it still could have impacted small mammal diversity. For example, our study found that the only site that northern grasshopper mice were captured at was the site that was closest to agriculture (100 meters). This finding is consistent with previous findings that northern grasshopper mice tend to be found near agriculture crops (Seabloom et al. 2011).

Drainage type of the soil also significantly affected biomass when controlling for year, with biomass decreasing with a decrease in drainage capability. This could suggest that small mammals (or just thirteen-lined ground squirrels) tend to prefer faster draining soils, which could fit into the open habitat description that thirteen-lined ground squirrels tend to prefer. Distance to roads did not show up as a significant factor to affect Simpson's Index or biomass in our study. It was thought that the edge effects caused by the roads could alter small mammal populations, but in this study such effects were not detected.

Conclusion

This study provided information regarding small mammal species presence in tallgrass prairie. If management was aimed at increasing small mammal diversity this study could suggest allowing woody encroachment to happen. If managing for small mammal abundance you would not want woody encroachment because thirteen-lined ground squirrels were the most numerous species caught and prefer open habitat. These two management approaches are at odds with each other, so management strategy chosen depends on the goals that wish to be accomplished.

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