GENETIC DETERMINATION OF PHRAGMITES AND SMALL MAMMALS USE OF REMNANT PATCHES ALONG THE CENTRAL PLATTE RIVER, NEBRASKA

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By

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ABSTRACT

Invasive phragmites (*Phragmites australis*) has encroached on the central Platte River in recent years potentially out-competing native stands of phragmites. Invasive stands are thought to have an overall negative impact on ecosystems, but do provide ecological benefits to some species as food or shelter. Little research has been conducted on its impacts on small mammals. The goals of this project were to identify potential native and invasive stands along the central Platte River and determine small mammals use of invasive phragmites. I examined 35 phragmites samples along the central Platte River using restriction fragment length polymorphism. I used molecular sequencing and morphological features to identify stands. All samples were determined to be invasive. Four study sites along the Platte River were selected to evaluate phragmites use and potential impacts on small mammals. Each study site was in a wooded grassland area and consisted of three patches of invasive phragmites and three patches of wooded grassland vegetation. Study sites were sampled using Sherman live traps from April to October 2014. I found no significant difference in overall small mammal use between vegetation types and no seasonal difference between use of phragmites and wooded

grassland stands. I did, however, catch fewer individuals in both habitat types during August. Deer mice (*Peromyscus maniculatus)* selected native vegetation whereas the white-footed mouse (*Peromyscus leucopus*), meadow vole (*Microtus pennsylvanicus*) hispid cotton rat (*Sigmodon hispidus*) and meadow jumping mouse (*Zapus hudsonius*) selected phragmites. Capture of hispid cotton rat was the first documentation of this species in Dawson County, Nebraska. Differences in selection by these species could be attributed to their different life histories or habitat preferences. The focus of management for invasive phragmites may not need to focus on total eradication. Additional sampling would be required to document the spatial extent of native phragmites stands along the central Platte River.

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Chapter One

Phragmites and the Significance of Study

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History and Spread of *Phragmites australis* in North America

When species are introduced outside their historic range, significant changes and detrimental effects can occur on native ecosystems (Hoffman et al. 2008, Silliman and Bertness 2004). In North America, an invasive form of phragmites or common reed (*Phragmites australis australis* (Cav.) Trin. ex Steud) is a problematic plant that poses threats to native ecosystems. The rapid expansion of the invasive variety of common reed has been a recent occurrence (150-200 years ago) which has caused a reduction in native habitats and species diversity resulting in considerable economic impacts (Marks et al. 1993, Chambers et al. 1999, Saltonstall 2002, Meyer 2003, Hoffman et al. 2008, Mozdzer et al. 2013). Native and genetically distinct haplotypes of phragmites (*P. a. americanus*) have been identified in North America (Saltonstall 2003, Saltonstall et al. 2004). The native haplotypes have occurred in North America for at least 40,000 years and has been an important component of native ecosystems (Hansen 1978, Saltonstall 2002, Saltonstall 2003, Saltonstall et al. 2004, Kaul et al. 2006). Common reed was utilized by Native Americans to make mats and other woven items but has not previously occurred in the abundance that is currently seen in North America (Breternitz et al. 1986, Kane and Gross 1986, Marks et al. 1994, Saltonstall 2002). Hybridization can occur where native and non-native common reed stands co-exist. Some concern has arisen that hybridization may drive native haplotypes out of ecosystems (Saltonstall

2003, Kettenring et al. 2010, McCormick et al. 2010, Meyerson et al. 2010, Paul et al. 2010).

Anthropogenic disturbances have been increasingly more important for driving changes in ecosystems including encroachment of invasive common reed (Keller 2000). Disturbed areas provide suitable areas for invasive plants, such as common reed, to colonize (Pyšek and Prach 1994, Planty-Tabacchi et al. 1996, Hoffman et al. 2008). Disturbed habitats which decreased soil salinities and increased nitrogen availabilities provide common reed, which grow in these conditions, optimal conditions for encroachment (Silliman and Bertness 2004). Examples of such disturbances that increase soil attributes for common reed include woody vegetation removal, changes in river currents due to dams and flow regulation, and land use (Pyšek and Prach 1994, Silliman and Bertness 2004, Hoffman et al. 2008). Dredging, recreational use, sedimentation, nutrient and contaminant loading also contributed to the degradation of habitats where invasive phragmites has been known to invade (Knapton et al. 2000, Meyer 2003).

Biology of Phragmites

Invasive common reed typically thrives in brackish or freshwater environments associated with wetlands and river systems (Marks et al. 1994, Hudon et al. 2005). It is a perennial, colony-forming grass that has an extensive rhizome system (Waller and Lewis 1979, Mal and Narine 2004). Plants can reach 3.7 m in height with roots that penetrate 0.9 to 2.7 m in depth. Rhizomes can grow up to 3 m in length, with stolons potentially extending outward 24 m. Once established in an area, common reed generally spreads rapidly via rhizomes, which creates multiple clones of itself. Researchers previously thought common reed produced few viable seeds (Gervais et al. 1993), reproducing primarily by rhizomes; however, more recent studies suggest sexual reproduction plays a more significant role in its spread than previously thought (McKee and Richards 1996, Kettenring and Whigham 2009). Common reed can colonize new areas via seed dispersal by wind, water, and birds as well as by vegetative reproduction by rhizomes (Kiviat 1987, Marks et al. 1994, Meyer 2003, Kettenring et al. 2010). Plants flower annually from July to September (Marks et al. 1993, Knezevic et al. 2008). Once seeds have been dispersed in late fall, above-ground portions of plants die back (Haslam 1968). The combination of seed dispersal and clonal spread by rhizomes helps explain the invasive nature of common reed.

Identification of Native vs. Invasive Phragmites

A number of physical characteristics can be used to distinguish native and invasive common reed. First, native stands are not as dense when compared to invasive ones (Swearingen and Saltonstall 2010). Second, native common reed plants are generally a lighter color of green, have a thicker ligule, and will often have small dark spots on the culm. These spots are formed by fungi species that have not yet adapted to infect the invasive common reed (Farr, et al. 1989, Swearingen and Saltonstall 2010). Invasive common reed typically has a fuller inflorescence which is often purple or gold in color (Swearingen and Saltonstall 2010). Although these morphological characteristics can be used to distinguish between the two, genetic testing can help confirm native and invasive plants (Swearingen and Saltonstall 2010).

Impacts on Ecosystems and Economics

Invasive common reed causes ecological problems by reducing native plant diversity, which could alter the diversity of animal species that use the habitat (Keller 2000, Talley and Levin 2001, Able et al. 2003, Minchinton et al. 2006, Kettenring et al. 2010). Due to its dense mat of rhizomes, high stem density and height, and buildup of reed litter, common reed competes with native flora by inhibiting seed germination, as well as shading and crowding out other plants (Jones and Lehman 1987, Rice et al. 2000, Meyer 2003). Buildup of litter increases the potential of winter fires and creates a potential safety hazard (Reimer 1973). If conditions of an area are suitable for invasive common reed growth, it can suppress all other plants and create a monoculture (Keller 2000). Under certain conditions, such as low water tables and nutrient availability or in the presence of another strong competitor, it will instead co-exist with the other plant species (Haslam 1971, Keller 2000). Invasive common reed has been documented to threaten rare and endangered plants (Marks et al. 1993). In wetland settings where common reed replaces native plants, food availability for waterfowl and other wetland species could be reduced (Benoit and Askins 1999, Meyer 2003). Most birds are suspected to avoid interior regions of common reed stands because of the density of plants (Benoit and Askins 1999).

Although invasive common reed has negative consequences for ecosystems, it does provide some ecological benefits to some wildlife species. For example, marsh wrens (*Cistothorus palustris*), red-winged blackbirds (*Agelaius phoeniceus*), and swamp sparrows (*Melospiza georgiana*) have been observed using common reed stands as habitat (Benoit and Askins 1999). One study found that some amphibian species were not largely affected by stands of phragmites, although some amphibians may still be negatively impacted (Meyer 2003, Perez et al. 2013). In addition, some mammals, such as muskrat (*Ondontra zibethicus*) and white-tailed deer (*Odocoileus virginianus*) use common reed as forage and shelter (Kucera 1974, Daiber 1982). River otters (*Lontra canadensis*) have also been recorded to have den sites within common reed stands along the central Platte River (Jenkins 1982, Williams 2011). It has been suggested that common reed could potentially provide habitat for small mammals. One study observed more small mammals in large stands of phragmites compared to marsh meadow and cattail (*Typha* spp.) stands; suggesting mammals use common reed as protective cover by impeding larger predators (Meyer 2003). Large stands of phragmites may have high densities of some invertebrates (Angradi et al. 2001) which would provide food sources for insectivorous mammals such as the masked shrew (*Sorex cinereus*; Meyer 2003). However, only 4 species of small mammal were caught and the study was in a wetland ecosystem (Meyer 2003). Reed canary grass (*Phalaris arundinacea*) is another invasive grass which can establish a monoculture in wetland and riparian settings. Spyreas et al. (2010) suggested invasive reed canary grass benefited shrews and voles whereas mice species were negatively impacted by reed canary grass. It seems possible that invasive common reed may have a similar ecological impact on small mammals as reed canary grass.

Due to the negative impacts that common reed can cause, it has been placed on Nebraska's watch list of invasive species (Anonymous 2007). Various conservation groups have been using herbicide treatments in an attempt to control it along the central Platte River (Anonymous 2013). Aerial spraying is frequently used along the main channel of the Platte River. Spot spraying by airboat is then administered to control phragmites in areas that were missed. For example, thick trees can conceal patches of common reed in its understory. Airboats have been used to spray some common reed next to the trees; however, spraying does not occur within wooded areas because the herbicide would kill the trees as well. Thus, common reed patches in these wooded areas have persisted.

STUDY AREA

The Platte River flows from west to east through Nebraska and drains into the Missouri River. Historically, the central Platte River had high flows during late spring and early summer and low flows during late summer which resulted in a wide and shallow river bordered with prairies (Eschner et al. 1981, Simon and Associates 2000). During the twentieth century, much of the river was dammed for recreation and irrigation purposes, which altered the hydrology of the system (Sidle et al. 1989). Regulation of water flow allowed for woodland expansion throughout the system (Currier 1982, Johnson 1994). Much of the historic surrounding wetlands have been converted into agricultural lands (Sidle et al. 1989). Wooded vegetation also had been common along the central Platte River, being found on large islands, banks, and side channels where woody vegetation has been able to evade the flows of the river (Johnson 1994, Currier and Davis 2000, Johnson and Boettcher 2000). Some of the more common woody species included cottonwood (*Populus deltoids* Bartram ex Marsh), American elm (*Ulmus americana* Planch), and willow trees (*Salix* spp. L.; Johnson 1994). Mixed grass (*Andropogon, Bouteloua, Buchloe*) and tall grass (*Andropogon, Panicum, Sorghastrum*) species can be found along the Platte River (Kaul 1975). Eroded areas which lack the protective cover of tall dense plants will support annual plants such as barnyard grass [*Echinochloa crusgalli* (L.) Beauv.], lovegrass [*Eragrostis pectinacea* (Michx.) Ness], nut-sedge (*Cyperus odoratus* L.) and cocklebur (*Xanthium strumarium* L.; Johnson 1994).

The central Platte River occupies the area between the cities of Lexington and Chapman, Nebraska. This area provides important habitat for a number of threatened and endangered species including the whooping crane (*Grus americana*), interior least tern (*Sterna antillarum athalassos*), and piping plover (*Charadrius melodus*; United States Fish and Wildlife Service 2006). Many portions of the central Platte River have been managed to support these threatened and endangered species, as well as the sandhill crane (*Grus canadensis*; Sidle and Faanes 1997, Schneider et al. 2005). This stretch of the river has been invaded by common reed in recent years when drought stressed the river system and allowed it to spread rapidly throughout our study area (Brei and Bishop 2008).

OBJECTIVES

The first objective of this study was to identify and document whether native and non-native stands of common reed occurred along the central Platte River. I expected to find both varieties within my study area as native stands have been documented (D. Simon and L. Reichart, personal communication). The second objective was to determine the effects of invasive common reed occurring in wooded areas on small mammal diversity and abundance. The null hypothesis was that I would observe no difference in small mammal use of patches with and without phragmites.

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Chapter Two

Identification of Native and Invasive Stands of Phragmites along the Central Platte River

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ABSTRACT

An invasive form of phragmites (*Phragmites australis australis*) or common reed has encroached on the central Platte River in recent years, potentially out-competing the native form of phragmites. Although morphological characteristics can be used to distinguish between the two, molecular methods can help confirm identification. I examined 35 phragmites samples along the central Platte River using RFLP and all samples were determined to be the invasive form of phragmites. My study demonstrated the invasive phragmites variety dominates stands along the Platte River, likely due to its inherent invasive qualities. Amore intensive sampling effort would be required to document the existance of stands of native phragmites along the central Platte River. Once identified, native stands could be protected from removal techniques used to control invasive phragmites to allow expansion of native phragmites.

KEY WORDS common reed, Nebraska, RFLP, Phragmites

A native form of common reed (*Phragmites australis americanus*; hereafter referred to as native phragmites) has occurred in North America for at least 40,000 years (Hansen 1978) and was thought to have been an important component in native ecosystems (Breternitz et al. 1986, Kane and Gross 1986, Marks et al. 1994, Saltonstall 2003, Saltonstall et al. 2004, Kaul et al. 2006). In recent years, an invasive lineage of common reed (*Phragmites australis australis* (Cav.) Trin. ex Steud; hereafter, referred to as invasive phragmites) has caused considerable economic impact and reduced native habitat and species diversity (Chambers et al. 1999, Mozdzer et al. 2013, Knezevic et al. 2008). Native and invasive phragmites are similar in appearance, but morphological features can often be used to distinguish between the two (Swearingen and Saltonstall 2010). However, genetic testing can be used as an additional tool in identification of native and invasive phragmites (Swearingen and Saltonstall 2010).

The earliest documented invasive phragmites in Nebraska was collected in 1973 in Lancaster County, Nebraska which is 264 km east of this study area (Larson et al. 2011). Invasive phragmites has also been more recently documented along the North Platte River, which empties into the central Platte River (Hoffman et al. 2008). The central Platte River occupies the area between Lexington and Chapman in Nebraska, has become invaded by invasive phragmites (Brei and Bishop 2008). Due to the detrimental effects phragmites can cause, herbicide treatments have been used in an attempt to control it along the Platte River (Knezevic et al. 2008, Platte Valley Weed Management Area and West Central Weed Management Area 2013). Although spraying has been effective, small patches of phragmites still occur along the river channel. The objective of this study was to use restriction fragment length polymorphism (RFLP) methods developed by Saltonstall (2003) to determine whether remnant patches were represented by native or invasive stands of phragmites in the central region of the Platte River.

METHODS

A total of 35 phragmites stands were systematically sampled between J2 Power Return (40.691630, -99.681836) and Chapman, Nebraska (40.985324, -98.144283) along the main channel of the Platte River in mid-July 2014. Twelve of these stands were at sites that were also part of my concurrent small mammal use study (three separate stands at 40.681894N -99.560466E, three separate stands at 40.688930N -99.357632E, three separate stands at 40.685041N -99.331926E and three separate stands at 40.748933N -98.588615E). Localities and site characteristics for each phragmites sample can be found in Table 1. Approximately 1.0 g of leaf from a single plant was collected from each patch and was placed in a 50 mL Falcon tube that contained enough silica gel tablets to cover the leaf while it was transported to the lab to be processed.

DNA was extracted using QIAGEN® DNeasy Plant kit following the manufacturer's instructions. RFLP analysis was used to determine whether the samples were native or invasive plants. PCR conditions were as follows: 45 sec at 94˚, 35 cycles of 45 sec at 94˚, 45 sec at 52˚, 1 min at 72˚, and a final cycle for 2 mins at 72˚. Restriction enzymes *Rsa*I and *Hha*I and primers for two noncoding regions in two different mitochondria loci. *Rsa*I restriction enzymes are used with the primer set *trnL* and *trnL*bR, whereas *Hha*I is used with the *rbcL*, and *rbcL*3R primer set (Taberlet et al. 1991, Saltonstall 2001, Saltonstall 2002, Saltonstall 2003). Ten µl of PCR product was mixed with 1.5 µl digestion buffer, 0.5 µl of either *Rsa*I or *Hha*I enzyme, and 3 µl H_2O to equal 15 µl total volume. The restriction enzymes were then incubated at 37 ˚C between 1 and 2 hours. Products were analyzed on a 3% agarose gel stained with ethidium bromide using gel electrophoresis following the protocol established by Saltonstall (2003). Controls from known native and invasive phragmites samples used in a different study were included on the gel as a reference along with one lane with a 100bp ladder. The restriction enzyme *Rsa*I cuts native haplotypes at 282 bp, but will not cut the invasive haplotype within the *trnL*b region. The restriction enzyme *Hha*I only cuts the introduced haplotype at the *rbcL* region at 14 bp, whereas it will not cut any native haplotype (Salsonstall 2003). *Rsa*I and *Hha*I bands were observed on separate gels. Bands on each gel were compared to both the native and invasive controls to determine lineage of each sample. An example gel for each enzyme is on Fig 1.

RESULTS

All 35 samples were determined to be invasive with both enzymes. My results suggest invasive phragmites occurs throughout the main channel of the Platter River from the J2 Power return to Chapman.

DISCUSSION

Although native stands of phragmites were not detected in this study, they may still occur in small numbers along the main channel, further inland, side channels, or in the Platte River watershed. Native phragmites has been documented in this area in 2012 (D. Simon and L. Reichart personal communication). However, as shown by this study invasive phragmites is the dominant form currently found along the central Platte River. If native stands occur in the main channel, a larger scale sampling effort will be needed to identify the stands (Larson et al. 2011).

Multiple potential reasons exist for the lack of native phragmites. One is the rapid spread and competitive nature of the invasive lineage. The competitive advantage of the invasive lineage is its extensive rhizome system as well as shading chokes out competing native vegetation (Waller and Lewis 1979, Mal and Narine 2004). Invasive phragmites may also proliferate in disturbed areas where soil is disturbed (Pyšek and Prach 1994, Silliman and Bertness 2004, Hoffman et al. 2008). Any area along the Platte River where disturbance occurs could provide opportunity for invasive phragmites to encroach. Drought has also contributed to the expansion of phragmites into the Platte River (Brei and Bishop 2008). Chemical spraying which has been used in attempts to eradicate invasive phragmites may have also contributed to the disappearance of native stands. Due to the similar morphological features between native and invasive plants, native stands could have been misidentified and sprayed. It is likely that other areas in

North America with similar drought conditions, vegetation removal, or eradication techniques could experience a similar loss of native phragmites stands and encroachment of invasive stands.

Managers trying to eradicate invasive stands likely do not need to be overly concerned about misidentifying common reed patches because native stands are not common along the central Platte River in Nebraska. However, a more intense monitoring protocol could be implemented to identify and track any expansions of native patches back into the main channel from any locations which have been previously been known to have native patches. This could then allow expansion of native patches which could be protected from removal techniques used to control invasive phragmites patches. Visual observation of phragmites shows it has been mostly eradicated from the main river channel of the Platte River from aerial application of glyphosate. However, because aerial spraying could potentially kill trees, spraying was not conducted near tree stands, and phragmites continues to grow in wooded areas.

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Table 1. Locations and site characteristics where phragmites samples were collected. Unless otherwise indicated, tree species associated with phragmites include cottonwoods.

Sample Number	Latitude	Longitude	Location in Channel	Characteristics of Sample Site
1-3[a]	40.68893	-99.35763	Inland, North side of channel	Within trees
4-6[a]	40.68504	-99.33192	Inland, North side of channel	Within trees
7-9[a]	40.68189	-99.56046	Inland, North side of channel on Jeffery Island	Within trees
10-12[a]	40.74893	-98.58861	Inland, North side of island	Within trees
13	40.68500	-99.35941	South bank of island	No trees, high bank
14	40.68107	-99.38174	North bank of island	No trees, high bank
15	40.68222	-99.55765	North bank of channel	No trees, high bank
16	40.65956	-99.07525	South bank of channel	Low bank
17	40.68160	-99.31635	North bank of island	Low bank
18	40.66204	-99.18503	SW bank of island	Low bank
19	40.66052	-98.95752	North bank of channel	Trees, low bank
20	40.67819	-98.87325	North bank of channel	Locus tree, low bank
21	40.67575	-98.84936	East bank of island	Trees, low bank
22	40.66041	-99.02375	North bank of channel	Low bank
23	40.96131	-98.17334	North bank of channel	Low bank
24	40.93953	-98.08279	Covers island	Low bank
25	40.88907	-98.26565	North bank of island	Low bank
26	40.85313	-98.30088	North bank of channel	Low bank
27	40.82276	-98.33631	South bank of channel	Trees, low bank
28	40.81969	-98.34521	North bank of channel	Low bank
29	40.80237	-98.38749	North bank of channel	Low bank
30	40.76624	-98.62849	North bank of channel	Low bank
31	40.75896	-98.52433	North bank of island	High bank
32	40.75297	-98.54674	North bank of channel	High bank
33	40.74175	-98.61363	North bank of channel	Low bank
34	40.72276	-98.68143	North bank of island	High bank
35	40.70147	-98.78716	North bank of channel	High bank

[a] Series of sample numbers indicate samples taken from one locality. Latitude and longitude for these samples were taken at the center of phragmites patch.

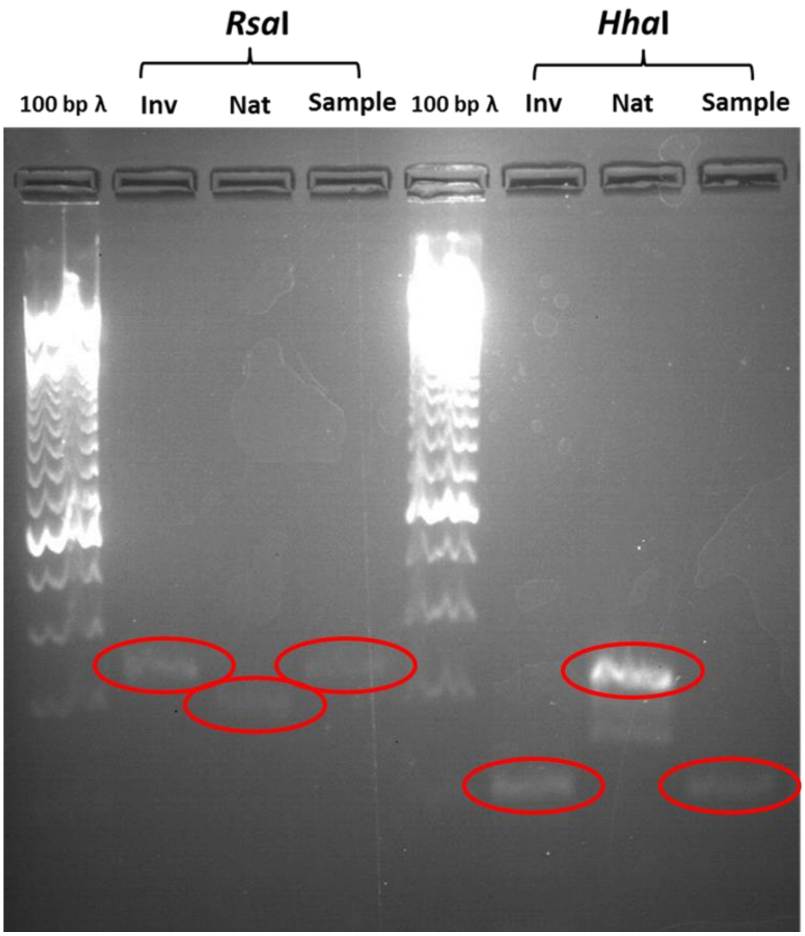

Figure 1. Sample gel showing bands for each enzyme cut with restriction enzymes *Rsa*I and *Hha*I. A control invasive (Inv) native (Nat) and a sample are shown for each along with a 100 bp ladder (λ) for size. Bands are circled in red

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Chapter Three

Small Mammal use of Phragmites along the Central Platte River

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ABSTRACT

Common reed, or phragmites (*Phragmites australis*), has become a problematic invasive plant that has resulted in loss of habitat and reduction in native species diversity in North America. Although the plant provides ecological benefits such as food or shelter for some species, phragmites overall has been shown to negatively impact ecosystems by supporting lower vertebrate diversity. However, little research has been conducted on the impacts of phragmites on small mammals. The goal of this project was to determine the impacts of the invasive form of phragmites on small mammal diversity and abundance in central Nebraska. Four study sites along the Platte River were sampled from April to October 2014 using Sherman live traps. Each study site was in a wooded area and consisted of three separate patches of phragmites and three patches of wooded grassland vegetation. I was unable to detect a difference in overall small mammal captures between vegetation types and no seasonal differences occurred other than catching fewer individuals in both habitat types during August. I observed significantly more deer mouse (*Peromyscus maniculatus)* in native vegetation than phragmites. However, I observed significantly more white-footed mouse (*Peromyscus leucopus*), meadow vole (*Microtus pennsylvanicus*), hispid cotton rat (*Sigmodon hispidus*) and meadow jumping mouse (*Zapus hudsonius*) in phragmites than wooded grassland. Total eradication of phragmites might actually reduce the diversity of habitat and small mammal species in an area as some small mammals use this novel, exotic habitat. However, for maintenance of other ecological functions along the Platte River, reducing the abundance of this exotic plant species likely outweighs the diversity in plant structure and small mammal use.

KEY WORDS: common reed, invasive species, phragmites, Platte River, Nebraska, small mammals,

Small mammals play important ecological roles which can impact ecosystems. Burrowing activities by small mammals provides shelter for invertebrates, influences diversity of plant cover, and affects mineral cycling (Golley et al. 1975). Small mammals also aid in dispersal of seeds affecting the distribution of plants in ecosystems (Reed et al. 2005; Beck and Vander Wall 2010; Muñoz and Bonal 2011, Barga and Vander Wall 2013). A diversity and abundance of small mammals provides food sources for larger vertebrates (Korschgen 1957, Korschgen and Stuart 1972) and influences other species within a system. For example, microtine species' abundance has been reported to reduce mule deer (*Odocoileus hemionus*) fawn mortality by coyotes (*Canis latrans*; Hamlin et al. 1984). Based on these examples, it is clear that changes in diversity or abundance of small mammals could alter the ecosystem and services it provides.

Common reed (*Phragmites australis* (Cav.) Trin. ex Steud; hereafter referred to as phragmites) thrives in brackish and freshwater environments in wetlands or along river systems (Marks et al. 1994, Hudon et al. 2005). A native form of phragmites has been present in North America for 40,000 years; however, it has not occurred in the large numbers and density that have been observed in the last 100 years (Kaul et al. 2006, Saltonstall 2003a, Saltonstall et al. 2004). It was shown that this is due to the invasive form of phragmites, which has become a problematic plant which has reduced native habitat and species diversity resulting in large patches (Chambers et al. 1999, Mozdzer et al. 2013). Invasive phragmites out competes other plants by inhibiting seed germination and shading and crowding out others with extensive rhizome structures and litter buildup, thus creating a monoculture (Jones and Lehman 1987, Keller 2000, Rice et al. 2000).

Overall, invasive phragmites has been shown to negatively impact some ecosystems and support lower vertebrate diversity (Jones and Lehman 1987, Benoit and Askins 1999, Meyer 2003). By replacing native plants, food and habitat availability is reduced for waterfowl and other birds (Benoit and Askins 1999, Meyer 2003). Some amphibians such as the Fowler's toad (*Anaxyrus fowleri*) and Northern leopard frogs (*Lithobates pipiens*) avoid or limit their use of phragmites (Meyer 2003).

Although phragmites has negative impacts for some species, it provides benefits for other vertebrate species. Various bird species have been observed using phragmites stands as habitat (Benoit and Askins 1999). Mammals such as muskrat (*Ondontra zibethicus*) and white-tailed deer (*Odocoileus virginianus*) have been recorded using phragmites as forage and shelter (Kucera 1974, Daiber 1982). It was originally thought that river otters (*Lontra canadensis*) were negatively impacted by phragmites, however, they use phragmites stands as den sites along the central Platte River (Jenkins 1982, Williams 2011). However, relatively few studies have examined the impacts of invasive phragmites on vertebrates.

The impacts of invasive phragmites on small mammals are largely unknown. Rodents can be negatively impacted by plant litter that prevents them from extracting seeds (Reed et al. 2004, Reed et al. 2006). Small mammals may avoid phragmites patches due to litter buildup which makes foraging for seeds difficult. Large stands of invasive phragmites could provide habitat or a source of invertebrate food for some small mammal species (Meyer 2003). However, seasonal use of phragmites by small mammals throughout the growing season has not been studied. Spyreas et al. (2010) looked at another invasive grass species, reed canary grass (*Phalaris arundinacea*), and observed shrews and voles were more abundant than mice species in reed canary grass. Herein, I investigated small mammal use of invasive phragmites throughout the growing season along the central Platte River in Nebraska.

STUDY SITES

Four study sites were identified along the central Platte River in Nebraska, USA (Fig. 1). The sites identified for this study were areas where separate patches of invasive phragmites and native grasses occurred in wooded areas. The western-most study site was the Jeffery Island site located on the eastern tip of Jeffery Island just west of the 444 Road-Platte River Bridge near Overton, Nebraska (40.681894N -99.560466E). The Blue Hole and Bartels sites were both located east of the Highway 183-Platte River Bridge near Elm Creek, Nebraska (40.688930N -99.357632E and 40.685041N -99.331926E, respectively). Finally, the Leaman-Burr site was located west of the Highway 11-Platte River Bridge near Wood River, Nebraska (40.748933N, -98.588615E). Coordinates were taken from the center of each study site.

Each study site consisted of an area with phragmites patches in a wooded area and a wooded grassland area with no phragmites to use as a control. Three patches of phragmites and three patches of wooded grassland vegetation were sampled at each study site (Figs. 2, 3, 4, and 5). Plants found in the wooded grassland areas consisted of plains cottonwoods (*Populus deltiodes*), eastern red cedar (*Juniperus virginiana*), smooth brome (*Bromus inermis*), prairie cordgrass (*Spartina pectinata*) and a variety of forbs (annual ragweed (*Ambrosia artemisiifolia)*, *Polygonum* spp.).

METHODS

Phragmites patches were mapped with ArcGIS and confirmed as invasive stands using morphological features and RLFP (Saltonstall 2003b; see also Chapter 2). Sample areas of native vegetation were mapped with ArcGIS to ensure equal sized sample areas.

Trapping for small mammals began in late April 2014 and was concluded by early October 2014. Trapping, handling, and euthanasia procedure complied with the Institutional Animal Care and Use Committee (#042011 to J. T. Springer). Each study site was trapped for four consecutive days in a week and then I rotated onto the next study site the following week. A combination of Sherman live traps (Sherman Traps Inc., Tallahassee, FL) and pitfall traps made of 12-oz (355-mL) plastic cups dug into the ground were used in this study. Due to pitfall traps popping out of the ground from raising water tables and being pulled out by wildlife, I ended the use of pitfall traps in mid-July and continued trapping solely with Sherman traps. Pitfall trap nights and captures were included in the data analysis. Traps were placed 1-2 m into phragmites patches from the edge, and traps were placed approximately 10 m apart. The reasoning for setting traps along the edges of phragmites stands was to minimize the trails I might have left behind which could influence movement of animals into, out of, and within the phragmites patches. Traps set in wooded grassland vegetation patches were equal in number and set up in a similar pattern to the phragmites patches in an area of similar elevation and proximity to the river. An example of trap set up between a phragmites patch and corresponding wooded vegetation trap set up can be found on Figure 6. Traps were set in late afternoon and baited with wild bird seed (Royal Wing Premium Mix Wild Bird Food, Tractor Supply Company Kearney, Nebraska). Traps were checked shortly after sunrise each morning.

For each animal captured, I recorded species, age (adult or juvenile), sex, weight, and reproductive status (scrotal, pregnant, or lactating). Each individual was marked by attaching an ear tag (Kent Scientific Corporation, Torrington, Connecticut) to their right ear.

RESULTS

A total of 5906 trap-nights were accrued throughout this study. Fewer trap nights occurred during June and July due to flooding events which limited the number of traps I was able to set or that remained set. In total, 480 individuals were caught consisting of (from most to least common) white-footed mouse (*Peromyscus leucopus*), deer mouse (*P. maniculatus*), meadow vole (*Microtus pennsylvanicus*), western harvest mouse (*Reithrodontomys megalotis*), northern short-tailed shrew (*Blarina brevicauda*), masked shrew (*Sorex cinereus*), hispid cotton rat (*Sigmodon hispidus*), and meadow jumping mouse (*Zapus hudsonius*). Except for the hispid cotton rat and meadow jumping mouse, all species were captured in both phragmites and native vegetation. All captures of the meadow jumping mouse (n = 6) occurred in phragmites at the Jeffery Island study site during the May trapping session. All captures of the hispid cotton rat (n = 9) occurred in phragmites at the Jeffery Island study site during the September trapping session. Incidental captures of two song sparrows (*Melospiza melodia*) and single captures of an eastern towhee (*Pipilo erythrophthalmus*) and white crowned sparrow (*Zonotrichia leuocophrys*) also occurred in traps set in phragmites.

When all species are combined, I did not detect a difference between total numbers of captures in phragmites (n = 262) and wooded grassland vegetation (n = 218; *t*-0.87, df = 38, *P* = 0.19). ANOVA tests show August captures for both habitat types was significantly lower compared to April/May, June, and July (Fig. 7). There were no significant differences in the numbers of captures or individuals between phragmites patches within or between study sites [F (4,14) = 1.79, *P* = 0.18]. When looking at individual captures using a chi-square test, the deer mouse was captured more frequently in native vegetation compared to phragmites patches whereas the white-footed mouse, meadow vole, hispid cotton rat and meadow jumping mouse were captured more frequently in phragmites patches over native vegetation (Table 1). The Shannon Weiner species index for native vegetation was 1.36 (evenness 0.76). Shannon Weiner species index for phragmites was 1.49 (evenness 0.72).

A total of 395 individuals were marked, 207 initially were marked in phragmites and 188 were initially marked in native vegetation. A total of 355 recapture events occurred throughout the trapping season with 198 in phragmites and 157 in native vegetation. We observed no difference in the number of recaptures between the two habitat types (χ^2 = 1.62, df = 1 , *P*=0.20). Twenty seven individuals (17 white-footed mice, 8 deer mice, and 2 western harvest mice) were recaptured in both phragmites and native vegetation throughout the trapping season. All other recaptured individuals were consistently caught in the same vegetation type.

DISCUSSION

When looking at all species combined, I observed no significant difference in small mammal captured between remnant phragmites patches and wooded grassland vegetation. When looking at individual species, the western harvest mouse, northern short-tailed shrew, and masked shrew did not select either vegetation type over the other. Although captured in small numbers and only during one part of the trapping season, the hispid cotton rat and meadow jumping mouse were found exclusively in phragmites and the white-footed mouse and meadow vole were found most often in phragmites. The deer mouse was found more commonly in wooded grassland vegetation. There does not seem to be a seasonality preference for either vegetation type, but August captures in both vegetation types were significantly lower compared to April/May, June and July. Overall, it seemed individuals stayed within the same vegetation type; however, some individuals of white-footed mouse, deer mouse, and western harvest mouse were found to move between vegetation types.

A number of factors could explain why some small mammal species use phragmites patches. The accumulation of dead plant material and extensive rhizomes systems of this perennial plant could provide nesting material and cover (Haslam 1968, Waller and Lewis 1979, Mal and Narine 2004). Macroinvertebrates can be found in higher densities in phragmites (Angradi et al. 2001, Yozzo and Osgood 2013), which would allow food availability for insectivorous mammals such as short-tailed shrews, masked shrews, or other omnivorous species such as white-footed mice (Whitaker 1966, Meyer 2003, Bowers et al. 2007). Seeds and shoots of phragmites could provide food for meadow voles (Lindroth and Batzli 1984, Kurta 1995), white-footed mice, and other species (Bowers et al. 2007). Phragmites could also provide protective cover from predators where small mammals could hide under the accumulated plant litter. The high stem density and plant height of phragmites could also make it difficult for large predators to move and search for small prey (Jones and Lehman 1987, Wywialowski 1987, Benoit and Askins 1999, Meyerson et al. 2000, Rice et al. 2000).

Predator avoidance and habitat preferences could also explain the use of phragmites patches by the hispid cotton rat and meadow jumping mouse in our study. Only six meadow jumping mice were captured at the Jeffery Island site during the April/May trapping session. The meadow jumping mouse selects more open habitat (Quimby 1951) and during our May trapping session, the stems of the phragmites were more open compared to the rest of the year. Due to the lack of captures during the rest of the year, this population could also have moved into another area which would be suitable for its habitat needs. The nine hispid cotton rats in this study were not caught until the final trapping session in September, suggesting a new population of this species may have just inhabited the Jeffery Island site (see Chapter 4). Phragmites may provide cover for a pioneering species such as the hispid cotton rat that they otherwise may not find.

The deer mouse was captured significantly more in native vegetation which may be attributed to their behavior and habitat preferences. Deer mice activity primarily revolves around their nest and seed caches (Baker 1983). The high density of rhizomes and litter buildup of phragmites (Jones and Lehman 1987, Keller 2000, Rice et al. 2000) may make it difficult for the deer mouse to create and later find their seed caches, which would result in more activity of this species outside of phragmites patches. Other invasive plant species do not appear to have impacted the deer mouse and it has been considered a generalist species (Bateman and Ostoja 2012, Longland 2012). Although phragmites was not the selected habitat, I had individuals which were recaptured in both habitat types. They may not primarily use phragmites, but may enter into patches to forage for food if patches occur on the edges of their territories.

Phragmites use by small mammals may also be linked to water levels (Meyer 2003). Phragmites typically thrives in brackish or freshwater environments typically associated with wetlands and river systems (Marks et al. 1994, Hudon et al. 2005). Both of these environments, including the Platte River, may be prone to flooding events or changes in water levels (Johnson 1994). Although a species may utilize phragmites patches, changes in water levels may temporarily make patches undesirable. One note to keep in mind, a flood-prone area in which phragmites has taken over would otherwise have native vegetation that is also prone to flooding. Regardless of vegetation available in these areas, flooding events would make the area undesirable to some species. In late June and July of this study, a flooding event made portions of each study area inaccessible. This flooding event may have washed away cover, food, or burrows which pushed small mammals out of the area. Macroinvertebrate density may be influenced by surface hydrology, and terrestrial invertebrates could drown in a flooding event (Bedford and Powell 2005, Yozzo and Osgood 2013). Any species that feeds on these invertebrates may vacate the area due to lack of food and not return until that food source recovers. This could explain the lower numbers of captured individuals during the August trapping session which followed the flooding event. Small mammals were either starting to move back or avoiding those areas due to depleted resources.

Invasive plants may not directly impact small mammals, but instead alter the surrounding habitats which influence what species will be present. Small mammal species such as deer mice and Merriams and Ords Kangaroo rats (*Dipodomys merriami* and *D. ordii*) have been documented using invasive plants such as saltcedar (Ellis et al. 1997, Longland 2012). Although some species were documented in saltcedar stands, other species such as the western harvest mouse and montane vole (*Microtus montanus*) were more likely to be found in native vegetation (Ellis et al. 1997, Longland 2012). Deer mice and white-footed mice have been named generalist species, meaning they can be found in many habitat types (Adler and Wilson 1987, Cummings and Vessey

1994, Longland 2012, Bateman and Osteja 2012). Generalist species may be less likely to be impacted by invasive plant stands (Longland 2012, Bateman and Osteja 2012). Due to this status, both the deer mouse and white-footed mouse will likely be found in most habitats regardless of vegetation types present. Small mammals may be more likely to select areas based on cover, food availability, and land disturbances rather than the presence or absence of an invasive species (Wywialowski 1987, Semere and Slater 2007). These examples show small mammals utilize areas which have become encroached by invasive plants however the composition of species present may change.

MANAGEMENT IMPLICATIONS

It may be difficult to completely eradicate an invasive species such as phragmites. Based on this study, overall phragmites does not seem to largely affect small mammal communities. When looking at individual species, the white-footed mouse, meadow vole, hispid cotton rat and meadow jumping mouse were captured more frequently in phragmites whereas the deer mouse were captured more frequently in native vegetation. Remnant patches of phragmites may not pose a threat to most small mammal species, as they are able to utilize the patches for habitat. However, there may be potential negative effects for some species if phragmites dominates an area or creates habitat fragmentation of native vegetation. Due to different requirements by various species, phragmites appears to both benefit some species where negatively impact others. Small mammal use of these areas may be more closely linked to changes in water levels than to the presence or absence of phragmites. The focus of management for phragmites may not need to focus on total eradication because some species do utilize it, but rather keeping it under control to ensure native vegetation exists for species which could be negatively affected by it. However, dependent on management goals, reducing the abundance of this exotic plant species likely outweighs the diversity in plant structure and small mammal use.

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Table 1. Total number of captures by species comparing phragmites to wooded grassland vegetation. Number of individuals are found in parenthesis.

Species	Total Captured in Phragmites	Total Captured in Wooded Grasslands
White-footed mouse**	281 (134)	185 (89)
Deer mouse*	82 (46)	130 (74)
Western harvest mouse	24 (17)	37 (28)
Meadow vole*	37 (32)	21 (18)
Northern short-tailed shrew	13 (13)	5 (5)
Masked shrew	5 (5)	4 (4)
Hispid cotton rat**	9 (9)	0 (0)
Meadow jumping mouse*	6 (6)	0 (0)

* Significant ($P < 0.05$)
**Significant ($P < 0.01$)

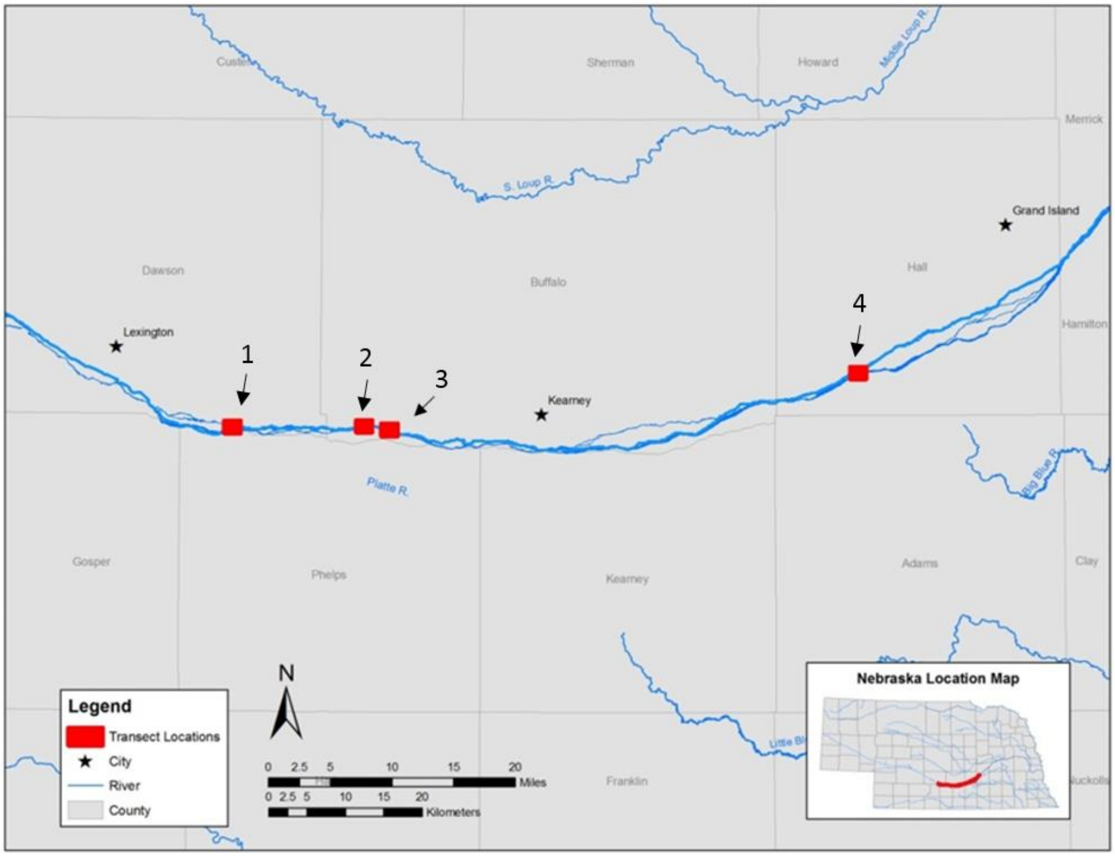

Figure 1. Study site locations for trapping small mammals in *Phragmites australis* stands located in wooded areas along the central Platte River. Study site labeled 1 is the Jeffery Island site, 2 is the Blue Hole site, 3 is the Bartels site, and 4 is the Leaman-Burr site.

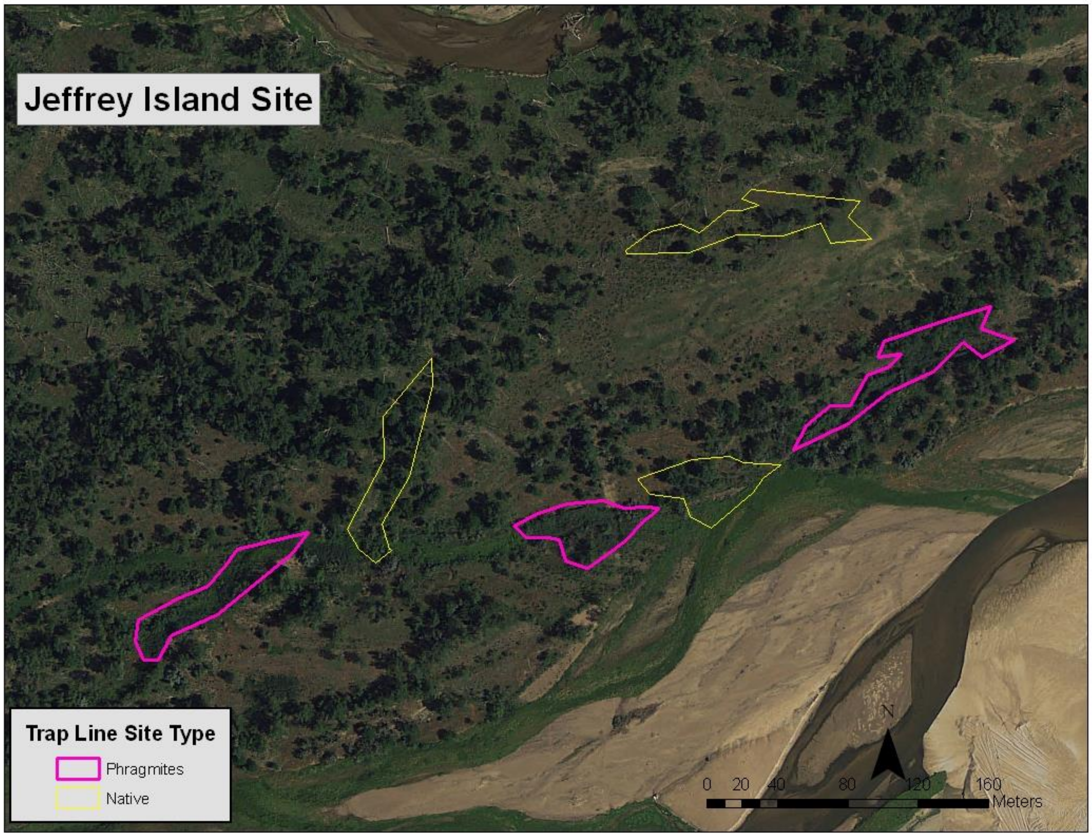

Figure 2. Jeffery Island site map. Purple polygons indicate phragmites patches and yellow polygons indicate the corresponding non-phragmites control patches.

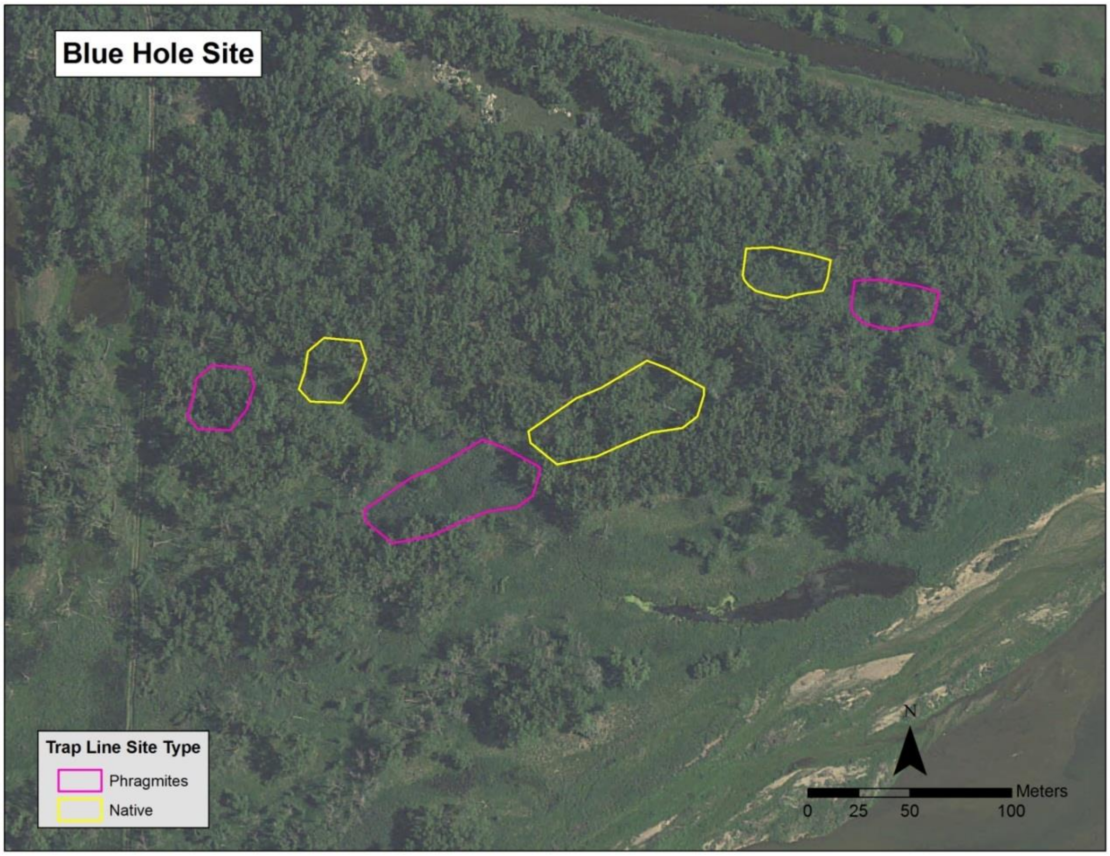

Figure 3. Blue Hole site map. Purple polygons indicate phragmites patches and yellow polygons indicate the corresponding non-phragmites control patches.

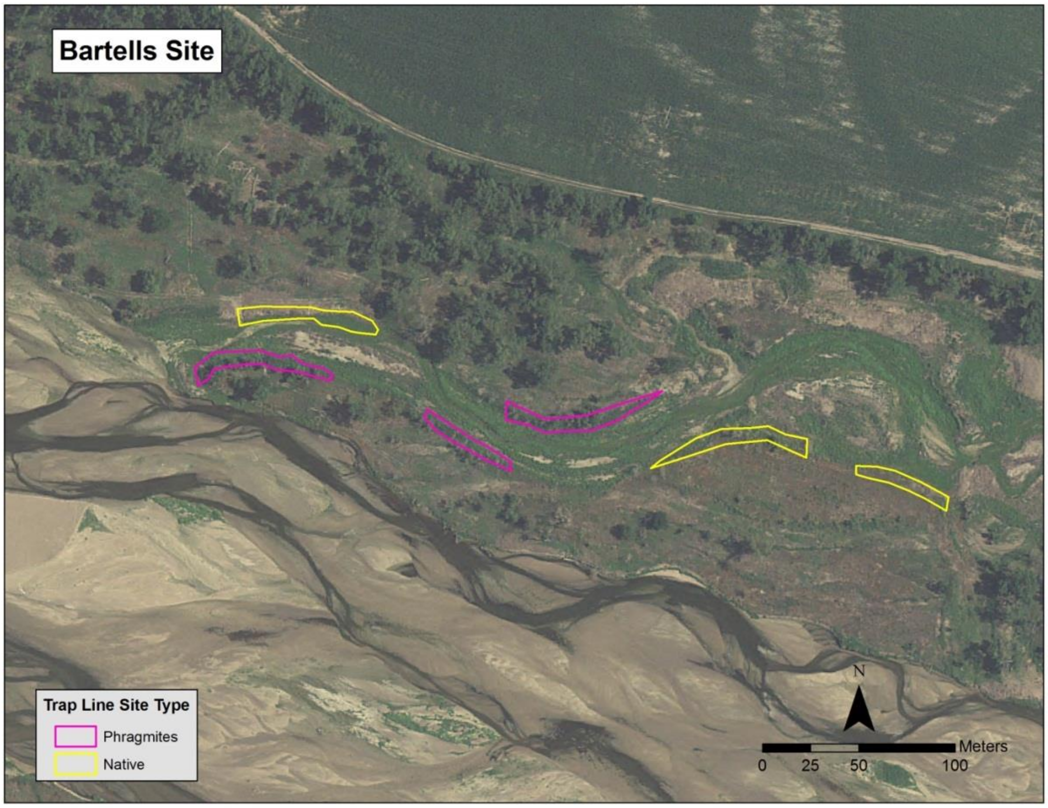

Figure 4. Bartells site map. Purple polygons indicate phragmites patches and yellow polygons indicate the corresponding non-phragmites control patch.

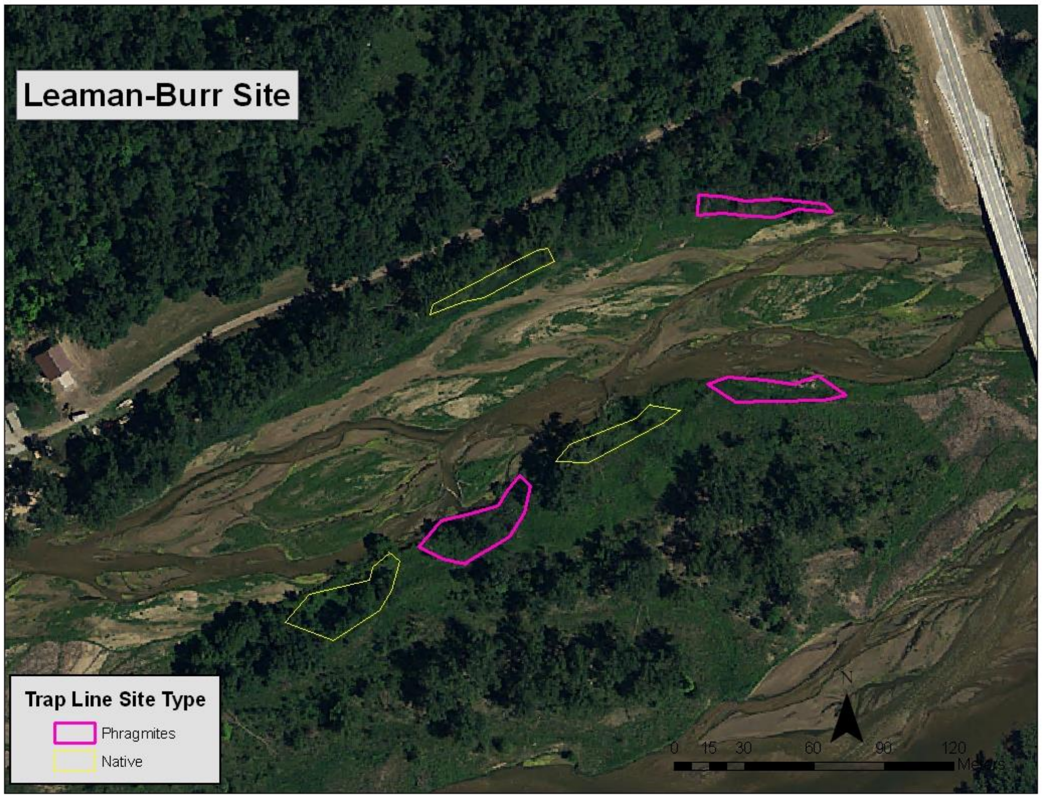

Figure 5. Leaman-Burr site map. Purple polygons indicate phragmites patches and yellow polygons indicate the corresponding non-phragmites control patch.

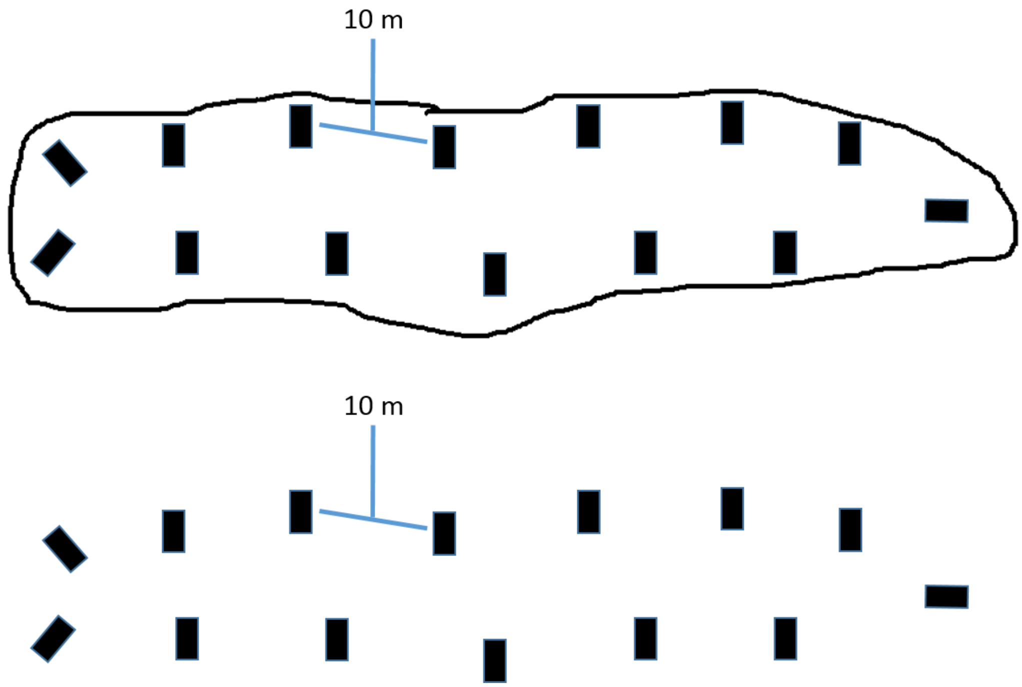

Figure 6. Hypothetical example of trap set up within a phragmites patch and a corresponding wooded grassland vegetation trap set up. The top polygon would be the example of a phragmites patch. Black rectangles indicate a trap. 10 m separated each trap from each other.

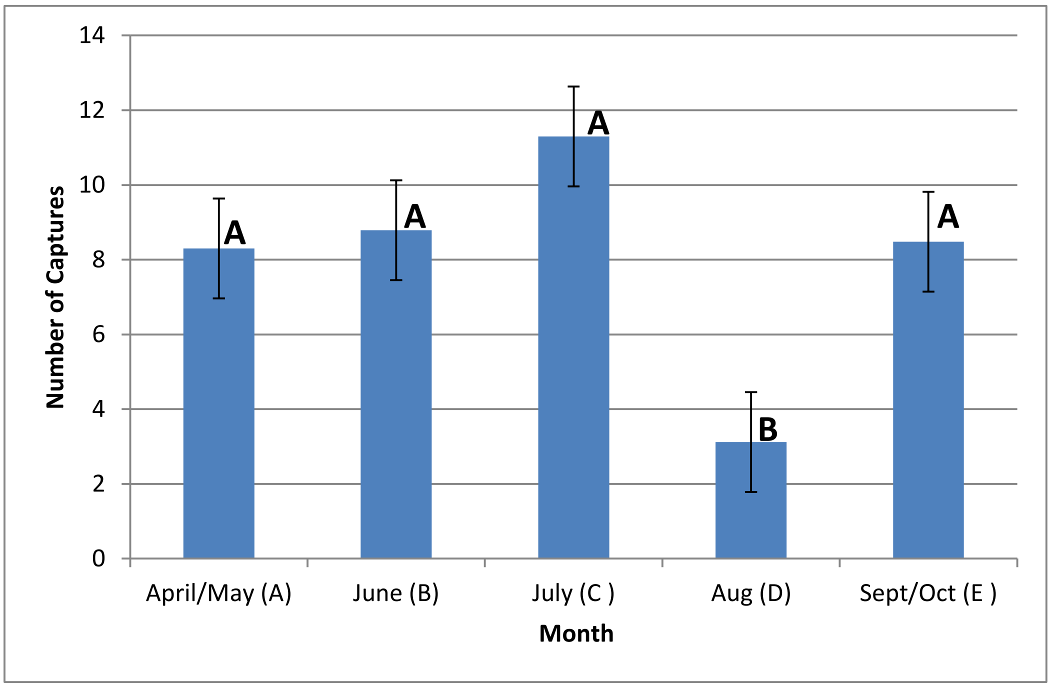

Figure 7. Total small mammals captured by month combining wooded grassland vegetation and phragmites per 100 trap nights. Similar letters above bars indicate no significant difference between those sampling months, different letters indicate significant difference. Standard error bars are found on each bar.

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Chapter Four

Distributional Expansion of the Hispid Cotton Rat into Dawson County, Nebraska

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ABSTRACT

In the last century, the hispid cotton rat (*Sigmodon hispidus*) has moved northward in central parts of the United States, reaching Nebraska in the late 1950s. Recent surveys demonstrate cotton rats inhabit counties across southern parts of the state south of the Platte River. Herein, we report on captures of *S. hispidus* from a new county (Dawson County), its seasonal occurrence at our study site, and the first known record of cotton rats north of Platte River channels. Our captures represent one of the northern-most records in the state.

KEY WORDS: Dawson County, hispid cotton rat, Nebraska, Platte River, *Sigmodon hispidus*

The hispid cotton rat (*Sigmodon hispisdus*) is wide-ranging across the United States from California to Virginia, and from southern Nebraska to northern parts of South America (Hall 1981). In the United States, distribution of hispid cotton rats have expanded northward in the Great Plains during the last century (Cockrum 1948), with the first documented specimens from Nebraska in Richardson County (Jones 1960). By 1965, the species expanded farther northward to Adams County (Genoways and Schlitter 1967), and by 1975, the cotton rat had expanded to six counties in Nebraska with its northern-most location from Kearney County (Farney 1975).

From 1948 to 1965, *S. hispidus* was estimated to move at 8.9 km per year in southern Nebraska (Genoways and Schlitter 1967), but Benedict et al. (2000) did not report a change in distribution for *S. hispidus* since the 1970s in the state. More recent surveys in southern Nebraska, however, demonstrate westward movements rather than continued northward movements in the state, as hispid cotton rats now are known from Chase, Gosper, Hayes, Phelps, and Red Willow counties (2011 Wright et al. 2010, Wills et al.). One hypothesis for the lack of further northward movements is that the Platte River formed a barrier to northward dispersal (Thompson and Finck 2013). Herein we report on the additional northward movement of *S. hispidus* in south-central Nebraska. We documented the species from a new county in the state, report on seasonal occurrence at our trapping site, and documented individuals north of channels along the Platte River.

METHODS

During a study to examine use of invasive common reed (*Phragmites australis*) by small mammals in central Nebraska, we set Sherman live traps (H.B. Sherman, Inc., Tallahassee, Florida) at four sites along the Platte River in south-central Nebraska. We set traps in Buffalo, Dawson, and Hall counties on one property managed by Central Nebraska Public Power and Irrigation District (CNPPID; 40.681894°N, 99.560466°W), one property managed by Nebraska Public Power District (NPPD; 40.688930°N, 99.357632°W), and two properties managed by the Platte River Recovery Implementation Program (PRRIP; 40.685041°N, 99.331926°W and 40.748933°N, 98.588615°W). Coordinates represent the center of study sites. At each site, we set 96 Sherman live traps divided equally between areas containing almost exclusively common reed and areas containing native vegetation. Dominant plants in native areas consisted of plains cottonwoods (*Populus deltiodes*), eastern redcedar (*Juniperus virginiana*), smooth brome (*Bromus inermis*), and a variety of forbs (annual ragweed (*Ambrosia artemisiifolia)*, *Polygonum* spp.). We set traps weekly, rotating between locations every week from late April to late August 2014, with one additional trapping session in late September into early October.

We baited traps with a mixture of mixed bird seed (Royal Wing Premium Mix Wild Bird Food, Tractor Supply Company Kearney, Nebraska). We set traps in late afternoon and checked them shortly after sunrise. Notes were recorded on species, sex, weight, reproductive status (scrotal, pregnant, or lactating), and age (adult and juvenile) for each individual captured in traps. Each individual was marked with a uniquely numbered 1 cm etched ear tag (Kent Scientific Corporation, Torrington, Connecticut). Most individuals were released shortly after processing, but a few individuals were kept as voucher specimens and deposited in the natural history collection at the University of Nebraska at Kearney (UNK). Trapping, handling, and euthanasia procedures were approved by the Institutional Animal Care and Use Committee at UNK (protocol #042011 to J. T. Springer).

From April to October 2014, we accrued 5,906 trap nights and captured eight species of small mammals. From most to least common, we captured white-footed deermouse (*Peromyscus leucopus*), North American deer mouse (*P. maniculatus*), meadow vole (*Microtus pennsylvanicus*), western harvest mouse (*Reithrodontomys megalotis*), northern short-tailed shrew (*Blarina brevicauda*), masked shrew (*Sorex cinereus*), hispid cotton rat, and meadow jumping mouse (*Zapus hudsonius*).

RESULTS/DISCUSSION

On 30 September, 2014, we captured our first hispid cotton rat during the entire study on the eastern portion of Jeffery Island (40.682027°N, 99.566324°W, WGS 84) within a patch of common reed that measured 0.16 hectares. The wooded area that surrounded the phragmites patch contained plains cottonwoods, eastern redcedar, and smooth brome. The first individual captured was an adult male that weighed 75.1 g (UNK 4498). Eight additional cotton rats (3 male, 4 female, 1 unknown sex) were captured during the same trapping session from 30 September to 3 October at the same site in patches of common reed. Average weight was 89.5 g (range 48.2-152.3 g), thus representing subadults and adults based on ages presented in Wright et al. (2010). We did not observe any reproductively active females based on visual observations. Our captures represent the first records of hispid cotton rats in Dawson County and the first record north of channels of the Platte River in Nebraska. The closest published record to our site is located in Phelps County at the High State Wildlife Management Area 14.3 km to the southeast (Wills et al. 2011).

A number of hypotheses have been proposed as limiting factors restricting the continued northward movements of S. *hispidus* in Nebraska, including climatic limitations, predator interactions, and physical barriers (Farney 1975, Benedict et al. 2000, Thomson and Finck 2013). The Platte River has been proposed as a limiting physical barrier in the northward expansion in Nebraska, similar to the northern limits of cotton rats along the Missouri River in western Missouri (Thomson and Finck 2013). In 2014, the Platte River had increased flows June-early July that likely would have prevented individuals from crossing the river during this period. However, during late July and August, portions of the river were dry or shallow, likely enabling some cotton rats to cross such a barrier and establish a population at the study site. We did not detect their presence until late September, which suggests cotton rats were not previously there, were present in limited abundance, or had not yet reached and crossed the river channel from more southerly populations. Our study suggests that during times of limited or no flows, the Platte River is not a barrier for cotton rats. If other conditions are appropriate, such as climatic factors, we predict populations will continue to increase and expand north of the Platte River in Nebraska. A combination of warmer climatic conditions in recent years allowing populations to persist nearby as well as a period of limited flows at the study site likely enabled this barrier to be crossed. Other vertebrate species in the region also have been documented responding to usually warm climatic conditions in recent years along the Platte River (Geluso et al. 2014, Wright et al. 2014).

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